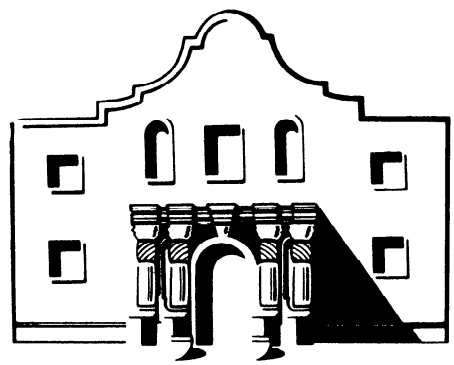
APS Conference

Interactions of the Endocrine and Cardiovascular Systems in Health and Disease

Marriott Rivercenter Hotel

San Antonio, Texas

September 29—October 3, 1991



13th Annual Meeting
IUPS
Commission on Gravitational Physiology

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INTERACTIONS OF THE ENDOCRINE AND CARDIOVASCULAR SYSTEMS IN HEALTH AND DISEASE
SEPT 29-OCT 3, SAN ANTONIO, TEXAS

SUNDAY, SEPTEMBER 29
2:00-6:00 **REGISTRATION**
2:00-4:00 **PANEL DISCUSSION** Issues on animal experimentation
6:00-7:30 **OPENING RECEPTION**

MONDAY, SEPTEMBER 30	
AM	**PM**
8:30-11:30 **GRAVITATIONAL SYMPOSIUM** Gravitational cell physiology	12:00 Noon-1:00 **MEET THE PROFESSOR**
8:30-11:30 **SYMPOSIUM** Hormome transport in blood: emerging concepts.	1:30-2:30 **LECTURE** Thyroid hormone and cardiac function.
8:30-11:30 **SYMPOSIUM** Mechanisms of endocrine hypertension.	2:30-4:30 **POSTERS**

TUESDAY, OCTOBER 1	
AM	**PM**
8:30-11:30 **GRAVITATIONAL SYMPOSIUM** Current concepts of gravitational physiology.	12:00 Noon-1:00 **MEET THE PROFESSOR**
	1:00-3:00 **POSTERS**
8:30-11:30 **SYMPOSIUM** Neurotransmitter release in brain nuclei controlling CV and pituitary function.	2:30-5:30 **SYMPOSIUM** Neuropeptides in blood flow regulation.
8:30-11:30 **SYMPOSIUM** Vascular specialization in endocrine organs.	2:30-5:30 **SYMPOSIUM** Regulation of angiogenesis.
	7:00-9:00 **TUTORIAL** Second messenger systems in vascular smooth muscle.
	7:00-9:00 **TUTORIAL** Methods of hormone measurement.

WEDNESDAY, OCTOBER 2	
AM	**PM**
8:30-11:30 **GRAVITATIONAL SYMPOSIUM** Physiology of high G loadings.	12:00 Noon-1:00 **MEET THE PROFESSOR**
8:30-11:30 **SYMPOSIUM** Atrial natriuretic factor and cardiovascular regulation.	1:30-2:30 **LECTURE** Cardiovascular and endocrine aspects of aging.
	2:30-4:30 **POSTERS**
8:30-11:30 **SYMPOSIUM** Identification and treatment of cardiovascular disease in diabetes.	7:00-9:00 **SOCIETY DINNER AND LECTURE** A physiologist's views of the animal rights movement

THURSDAY, OCTOBER 3	
AM	**PM**
8:30-11:30 **GRAVITATIONAL SYMPOSIUM** Mathematical modelling for gravitational physiology.	12:00 Noon-1:00 **MEET THE PROFESSOR**
	1:00-3:00 **POSTERS**
8:30-11:30 **SYMPOSIUM** Calciotropic hormones and cardiovascular function.	2:30-5:30 **SYMPOSIUM** Neurohumoral mechanisms in bulbospinal control of the circulation.
8:30-11:30 **SYMPOSIUM** Hormonal signal transduction and regulation of vascular smooth muscle.	2:30-5:30 **SYMPOSIUM** Interrelationship between insulin resistance and hypertension.

Sessions with Contributed Abstracts

Monday

Tuesday

Wednesday

Thursday

Interactions of Endocrine and Cardiovascular Systems in Health and Disease

Organizing Committee

Chairman
David Wasserman, Vanderbilt University

Ronald H. Freeman, University of Missouri
Joseph R. Haywood, University of Texas, San Antonio
George A. Hedge, West Virginia University
Hershel Raff, Medical College of Wisconsin

9.1

THE ENTRAINMENT BY LIGHT OF OSCILLATORY GRAVICURVATURE IN THE MAIZE PRIMARY ROOT. Dennis Fantin. University of California, Berkeley, CA 94720

Cv. Merit maize primary roots positioned transverse to gravity in the presence of incandescent light show a negative curvature phase following the initial descent. The magnitude of the rising phase is approximately one-third of the descent. The rising phase occurs irrespective of the light intensity or the light regimen. It is possible to stimulate secondary and tertiary cycles of root curvature by adjusting the schedule of light exposures. The effect can also occur in the dark although the wave amplitude is considerably reduced. In 12-hour and 16-hour-long trials the periodic light treatments (6sec/10min and 3min/hr) induced the effect in about a third of the roots. When the frequency of light pulses was diminished to 3min/2hr there was a substantial rise in the proportion of roots which exhibited nutational curvature. However, the power spectra for individual roots indicated a mixture of significant peaks suggesting that a true resonance effect was not achieved. By reducing the frequency to 3min/4hr the percentage of roots which manifested cyclic gravicurvature rose again to 86%. Of these, nearly all showed power spectrum spikes close to 4 hours. Finally, when a second light pulse was timed to correspond to the trough of the first wave it delayed the onset of the rising phase of the curve. These results are explained in light of a biophysical model (presented in a separate paper).

9.2

A BIOPHYSICAL MODEL FOR LIGHT-POTENTIATED GRAVITROPISM IN THE MAIZE PRIMARY ROOT. Dennis Fantin. University of California, Berkeley, CA 94720

A fluid-mechanical model is proposed which links the observed movements of cv. Merit maize primary roots exposed to incandescent light and transverse gravity. The observed root movements are explained in relation to the physical forces and molecular flows presumed to be involved. The mathematical expression of the model incorporates the following physiological features: Geosensors in the columella cells of the root cap sediment under the influence of gravity; An inhibitor of cell extension is activated under the joint influences of the sinking geosensor and a photo-convertible pigment molecule; The inhibitor diffuses along the bottom margin of the root from root cap to extension zone where growth retardation occurs leading to downward curvature; The inhibitor is transported by convection, circumferentially around the extension zone where it retards growth at all points in its path at a rate equivalent to its concentration (the concentration of inhibitor diminishes at an exponential rate from the time of its formation); When the concentration of inhibitor at the top of the extension zone exceeds that at the bottom, the root begins to bend back upwards. Model-generated curves are presented with experimental data as a measure of the theory's efficacy. An alternate model is also discussed which features transport of two different inhibitors from the root tip to the growth zone. Results from preliminary numerical testing of the alternate model are also presented.

9.3

RECEPTOR MEDIATED ENDOCYTOSIS IN OSTEOBLASTIC CELLS UNDER GRAVITATIONAL STRESS. A. Malouvier (1), X. Holy (1), E. Zerath (1), P.J. Marie (2), J.C. Caissard (1) & D.A. Schmitt (3). (1)=CERMA, Brétigny sur Orge, (2)=INSERM U18, Paris, (3)=INSERM U133, Toulouse, France.

It has previously been shown (COGOLI and coworkers) that cell activation might be impaired in microgravity. Cell activation is often induced by a receptor-ligand interaction and the receptor is then down-regulated by a receptor-mediated endocytosis (RME). This RME is an ubiquitous mechanism by which a cell internalizes a ligand fixed to its specific receptor. From a theoretical point of view, this mechanism could be impaired under microgravity conditions. We have previously shown in osteoblastic cells that the binding between the membrane receptor and its ligand is not modified by parabolic flight induced microgravity (SCHMITT D.A. et al., 1991), a result which is consistent with findings in other cell types (SCHAFFAR et al., 1991). In the present study, we used cultured rat osteosarcoma cells (ROS 17/2.8) and two ligands, insulin and transferrin, conjugated to 15 nm gold particles in order to visualized under transmission electron microscopy their intracytoplasmic internalisation. Cells were labelled with the ligands at 4°C 2 hours prior to an incubation at 37°C for various times, and then fixed. The same experiment was carried out with cells submitted to "gravitational stress" obtained during parabolic flight (1G, 1.8G, "0"G, 1.8G sequences). The cells were fixed after 18 minutes of flight, i.e. 5 parabols. The presence of gold particles was investigated in the different structures involved in RME, i.e. coated pits, coated vesicles, endosomes and secondary lysosomes. Our results show that the different steps of RME are not stopped at any time during the gravitational stress. It is now planned to carry out this experiment under "0"G conditions using a sounding rocket. *This work was supported by CNES.*

9.4

ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELL SUBPOPULATIONS FOLLOWING LONG DURATION SPACE SHUTTLE MISSIONS. Sams, C.F.*, Meehan, R.T.*, Kraus, E.T.*, and Cintron, N.M.* (SPON: S. Fortney) NASA/Johnson Space Center and KRUG Life Sciences, Houston, TX; University of Colorado Health Science Center, Denver, CO.

Alterations in various aspects of the immune system have been repeatedly observed following space flight. Changes in blastogenic response, lymphokine production and distribution of peripheral leukocyte populations have been reported after flights of varied duration. Previous results from our laboratory have shown changes in specific subpopulations of peripheral mononuclear cells (PMC) following Space Shuttle flights of 4-5 days. Monoclonal antibodies used to identify subpopulations included T helper, T inducer, T suppressor, T cytotoxic, B cells, NK cells, monocytes, insulin receptor (INSr) and insulin-like growth factor-I receptor (IGFr). Preliminary data from Shuttle missions of 9-10 days duration (n = 7 crewmembers) exhibit qualitatively similar subset alterations to those observed on the shorter missions. Subset analysis performed immediately postflight indicated significant ($p < 0.05$) increases in monocytes and T suppressor cells along with significant decreases in total T cell population, T inducer, and T cytotoxic cells. No changes were observed in T helper, B cells or NK cells immediately postflight. The increase in monocytes was greater following the long duration flights as compared to the short flights (200% increase over baseline vs 68% increase over baseline, respectively). The increase in monocytes was due to the appearance of INSr and IGFr negative monocytes in agreement with results from short term flights. In addition, monocyte expression of class II histocompatibility antigen (HLA-DR) was decreased postflight suggesting the release of immature monocytes into the circulation. These data support our previous observations and suggest the degree of subset changes may be a function of flight duration.

9.5

ULTRASTRUCTURAL ASPECTS OF CHONDROGENESIS IN "CELLS" FLIGHT HARDWARE M. Campbell*, E. Daane*, P.J. Duke, University of Texas Health Science Center, Dental Branch, Houston, TX 77225

Mammalian chondrocytes are known to be sensitive to gravitational changes (Δg) in vivo and in organ culture. To determine if these cells are sensitive to Δg in cell culture, micromass cultures will be flown aboard the shuttle Discovery in 1992. Flight hardware has been developed utilizing a two-welled chamber made of a collapsible, gas permeable, Silastic membrane. This hardware has been shown (by Alcian blue staining) to support chondrogenesis. Recent ultrastructural studies have confirmed these observations. Thick sections were used to locate cartilage nodules from which thin sections were taken. Nodules contained typical rounded hypertrophic chondrocytes as well as flatter, proliferative-type cells, both with abundant mitochondria, greatly expanded rough endoplasmic reticulum, and a high nuclear/cytoplasmic ratio. Matrix consisting of collagen fibrils and proteoglycan granules was seen between both cell types. Cells were in contact along broad expanses of their cell membranes with occasional formation of special junctional complexes between cells, and between the cell and the Cell-Tak (Collaborative Research Incorporated) coat on the coverslip support. Several cilia were seen as well as a number of degenerating cells.

Research supported by NASA Grant #NCC2-423

9.6

PROTEOGLYCANS IN MICROMASS CULTURES OF EMBRYONIC MOUSE LIMB MESENCHYMAL CELLS: PRELIMINARY STUDIES FOR THE "CELLS" SPACEFLIGHT EXPERIMENT. D. Montufar-Solis* and P.J. Duke. Univ. of Texas, HSC, Dental Branch, Houston TX 77225.

Histomorphometric studies of tibial epiphyseal growth plates of spaceflown rats have found significant changes in cartilage differentiation within the growth plate. Proteoglycan granule (PGG) sizes and density (#granules/mm^2) have been reported among the ultrastructural alterations. It is not known whether this is due to changes in PGG production and/or aggregation in microgravity. The effects of spaceflight on differentiation of cartilage is to be investigated in vitro, in an experiment to be flown on the shuttle mission IML-1. Preliminary 1g studies used indirect immunofluorescence staining and immunoperoxidase procedures to localize chondroitin sulfate, keratan sulfate, and 4-sulfated and 6-sulfated chondroitin sulfates in micromass cultures of embryonic mouse limb mesenchymal cells. Because steric hindrance may affect the results and because of the small number of in-flight samples, additional studies are underway using sections of micromass cultures grown on coverslips in flight hardware. Positive immunostaining appears to be concentrated in differentiated cartilage nodules. Regions between nodules also showed some positive staining.

9.7

IMMUNOLOGICAL RESPONSE OF JAPANESE QUAIL TO SHORT-TERM HYPODYNAMY IN TWO LINES WITH DIFFERENT RESISTANCE TO HYPODYNAMY. M. Juráni, P. Blažíček*, E. Somogyiová, D. Lamošová, P. Výboh, Ľ. Košťál, K. Boda. Inst. Biochem. Genet. SASci, Ivanka p.D., Czechoslovakia, *Milit. Hosp., Bratislava, Czechoslovakia.

In experiment the reaction of immune system to short-term (1/2, 1, 2, 4 h) hypodynamy was compared between quails from lines nonselected and selected for resistance to hypodynamy. The levels of immunoglobulines, histamine and calcium in plasma were observed. Hypodynamy induced the immunoglobulines decrease after 2 and 4 h of experiment in nonselected line, while the level of immunoglobulines was not changed in selected line during experiment. The basal level of histamine was higher in selected line. In both lines no changes in histamine content during the 4 h lasting hypodynamy were found. The concentration of calcium in nonselected line increased rapidly after 30 min of hypodynamy and remained elevated. In selected line the concentration of calcium was not changed significantly. Results demonstrated that selection for resistance to hypodynamy modified the immune response of Japanese quail.

9.8

GRAVITATIONAL EFFECTS ON MAMMALIAN CELLS. Atsushige Sato*, Yasuhiro Kumei*, Kazuko Sato* and Tohru Nakajima*. (SPON: H.Bjurstedt) Tokyo Medical and Dental Univ.,Tokyo 113, Japan

Mammalian cells in vitro are known to respond to hypergravity or microgravity, by which mechanism is unknown. The way of response to hypergravity and its difference among various types of cells were investigated. HeLa cells from human uterine cancer, osteoblastic MC3T3-E1 cells from mouse calvaria, and JTC-12 cells from monkey kidney tubules were cultured at 10-40 G, followed by measurement of cell growth rate and quantitation of prostaglandin E_2 (PGE_2) production and the c-myc gene expression. The growth of these cells was enhanced at 40 G: 134% in HeLa, 127% in MC3T3-E1, and 108% in JTC 12 cells, respectively, compared to 1 G-controls. The PGE_2 produced in the 40 G culture of MC3T3-E1 cells was 861 ± 317 pg/10^5 cells, which was greater than the control, 474 pg/10^5 cells. Any promotive effects of hypergravity on PGE_2 production in HeLa cells were not observed. In HeLa cells, 3-fold higher level of c-myc expression was induced by 30 min exposure to 35 G. The results showed, 1) responsiveness to hypergravity was different depending on the type of cells, 2) growth enhancement of MC3T3-E1 cells by hypergravity might be mediated by PGE_2, and 3) growth enhancement of HeLa cells by hypergravity might not be mediated by PGE_2 but through expression of cell growth-related genes.

This study was supported in part by The Fund from Science and Technology Agency and a grant-in-aid from Ministry of Education, Science and Culture, Japan.

9.9

MULTIUNIT ANALYSIS CONTROLLED ON REALISTIC COMPOUND PATTERNS. László Simon and István Csiszár.* 1st Dept. Anatomy, Semmelweis Univ. Medical School, 58.Tüzoltó-u., Budapest, Hungary. H-1450

Analytic computer program was developed for neurograms (i.e. multiunit extracellular records) with special reference to cerebellar cortical activity. Peaks and interpeak measures of triphase spikes were detected for a combined cluster-analysis. The separated single unit patterns served for a subsequent sequence-analysis based on the complex spike-generating mechanism (Simon, 1981) of cerebellar Purkinje cells(Pc). A relevant test -facility should be introduced for stages of improvement and also for the final efficacy (hits and false positives) of the analytic paradigm. For this reason test patterns were composed of realistic spike shapes gained by averaging from spontaneously firing Pc-s of the cat. First single unit sequences were randomly generated performing the realistic interspike interval statistics of a Pc. 2-6 of sequences were prepared with different spike shapes and amplitudes, corresponding to the cells at different orientation and distance around electrode tip in the modelled record. These patterns were then linearly summated and completed with definite level of white noise. The time sequences of ingredients were recorded, thus when controlling the efficacy of multiunit analyzing program schedules, every failure could be observed and utilized for adjusting the parameters of identification, classification and clustering of spikes. Compound test patterns also help to realise the non-avoidable limitations of the multiunit analysis in correlation to the number of units to be distincted and to different noise levels.

9.10

RESPONSES OF GRAVITY LEVEL VARIATIONS ON THE NASA/JSC BIOREACTOR SYSTEM. Y.D. Tsao, D.A. Wolf, and G.F. Spaulding. (SPON: Suzanne Fortney). Johnson Space Center, Houston, TX 77058

This study presents a numerical simulation for the flow field and the trajectories of particles in the Johnson Space Center (JSC) Couette flow bioreactor. The forces on a particle are assumed to be drag from the fluid's circulation, buoyancy from gravitational force, and centrifugal force from rotation of the vessel. Results of the study indicate that the trajectories in unit gravity and microgravity were very similar except for small spatial deviations on the fast time scale in unit gravity. The total force per unit cross-sectional area on a particle in microgravity, however, was significantly smaller than the corresponding value in unit gravity, which was also smaller than anticipated. Hence, this study indicates that using this bioreactor with optimal rates of rotation can provide a good environment for culturing cells in microgravity. In order to project a cell culture effect from unit gravity to microgravity, it is necessary to understand bioreactor-induced shear stress under different operating parameters. For modeling purposes it was assumed that inclusion beads could be treated as solid spheres. There was no interaction between beads, and a bead's motion did not affect the flow of culture medium. Acting on a microcarrier bead are Stokes drag and gravitational buoyancy force, as well as a centripetal force that depends on the rotating rate of the bioreactor. In our study, one continuous fluid phase will always be present with particles of different size suspended within the fluid. The computational approach to this problem is to solve the system of partial differential equations for the flow field of fluid, and then to solve the system of ordinary differential equations to track representative particles. This determination of the particle's trajectory is based on the dynamics of the individual particle as influenced by the drag exerted by the surrounding fluid phase and other forces.

9.11

DELIVERY OF RECOMBINANT HUMAN GROWTH HORMONE (rhGH) TO RATS DURING EXPOSURE TO MICROGRAVITY ON NASA SPACE SHUTTLE DISCOVERY STS-41. M. Cronin, J. Battersby*, W. Hancock*, R. Schwall and R. Clark.* Genentech, Inc., So. S.F., CA 94080.

Growth hormone secretion is reported to be impaired in rats exposed to microgravity. Therefore, we treated weanling male rats with rhGH (Nutropin) or excipient via osmotic minipumps. However, there was no experience either with delivering a protein to research animals or with osmotic pumps on earth orbit. There was also concern that the increased radiation could damage the protein. Pumps were implanted subcutaneously into anesthetized rats 1 d prior to launch. After the 4 d flight, the rats were judged healthy by 2 veterinarians, and the pumps were removed from the rats 4-6 h later. The amount of rhGH remaining in the pumps helped prove that they performed to specifications. Serum levels of rhGH at landing were 36.1 $\pm$ 5.6 ng/ml (n = 8) in the flown and 39.3 $\pm$ 5.0 (n = 11) in the nonflown animals that received rhGH containing pumps (mean $\pm$ SEM), whereas there was no rhGH in excipient treated rats. The quality of the rhGH remaining in the pumps was established by tryptic maps and reversed phase HPLC. There were no differences in these parameters when microgravity was compared to ground control samples. We conclude that rhGH can be preserved over 4 d and successfully delivered to rats in microgravity using osmotic minipumps. (We thank Astronauts W. Shepherd and B. Melnick, as well as Dr. T. Bowman, D. Mortensen and the Assay Services Group for their technical support.)

9.12

CENTRAL HYPOVOLEMIA, ANGIOTENSINE AND ALDOSTERON SECRETION. Petru Groza and Ioana Boros, Inst. of Physiology, Bucharest, Romania, 78159

Central hypovolemia, as in +gz, may be produced by hemorrage and clino-ortostatism, circumstances in witch plasma renine activity (PRA) and aldosterone plasma concentration (p.c.) increase, as results from literature, and also from our experiments in anesthesied dogs. It is generaly asserted that aldosterone hypersecretion in these circumstances results from the activation of a "renin-angiotensine-aldosterone" system. We do not found a clear relation between the increase in PRA and aldosterone p.c. Furthermore, in bilateraly nephrectomized dogs, despite of a drasstically decrease of PRA, aldosterone p.c. continued to increase. The stimulatory way of the aldosterone secretion in these circumstances are therefore not angiotensin. It is stimulated probably by an ACTH hypersecretion, because, as also results from our experiments, they are a good correlation between the increase of aldosterone p.c. and that of cortisol p.c. Another pathways for the aldosterone hypersecretion may be discussible.

10.1

AORTIC WAVE REFLECTION AND INPUT IMPEDANCE AS A FUNCTION OF GRAVITY STRESS IN A CHRONICALLY INSTRUMENTED PRIMATE. Ricky D. Latham [1], Cong Chi Tran* [2], John W. Fanton* [1], Curtis D. White* [1], Richard W. Owens* [1], David A. Self* [1]. Laboratory for Aerospace Cardiovascular Research, Armstrong Laboratory, Brooks AFB 78235, and USAARL, Ft Rucker, AL & [2] C.E.R.M.A., Bretigny-sur-Orge, France

Most data on central hemodynamics is known for the supine posture. Little data describes the systemic arterial tree, in hydraulic terms, for the upright posi tion, which is the most relevant to daily patient activities. We have instrumented mature male baboons (n=4) to study central hemodynamics in a conscious model for gravitational states ranging from 0G to +9Gz. The primates are exten sively trained to accept the study environments. Instrumentation, placed via left thoracotomy, includes LV, Ao, LA, RA pressure transducers, an Ao flow probe, LV dimension crystals, IVC occluder. Ao pressure and flows were recorded su pine (sup) and with 70° upright tilt (up). Fourier analysis allowed a determination of input and characteristic impedance (Zc). A 3-element Windkessel model was used to estimate compliance (C). Reflected pressure (Pb) was calculated from Zc, Ao pressure and flow. Mean Ao pressure and flow were used to calculate peripheral resistance Rp. Ao (mean± SD, mmHg) increased from 114± 7 sup to 121± 12 up with an expected increase in Rp (d*s*cm^{-5} 2813± 154 to 3822± 257). Zc changed little from sup (111± 12 d*s*cm^{-5} to 126± 7). C decreased minimally from 2.5± 1.2 cc/mmHg to 181± 6. Interestingly, Pb was also unchanged from 11.5 ± 3.6 mmHg supine to 12.2 ± 3.9 upright. In conclusion, even though Rp is increased when upright, Pb returning to the heart is unchanged for this model. Although C trends downward, previous work suggests proximal aortic C in creases when upright and may contribute to increased proximal attenuation of Pb.

10.2

VENTRICULAR/VASCULAR COUPLING UNDER HYPERGRAVITY IN A CHRONICALLY INSTRUMENTED CONSCIOUS PRIMATE MODEL. Cong Chi Tran* [1], Ricky D. Latham [3], John W. Fanton* [2], Curtis D. White* [2], Richard W. Owens* [2] and David A. Self* [2]. [1] C.E.R.M.A. Bretigny-sur-Orge, France, [2] Laboratory for Aerospace Cardiovascular Research, Armstrong Laboratory, Brooks AFB 78235, and [3] USAARL, Ft. Rucker, AL.

This study determines the change of peripheral resistances (Rp), aortic compliance (C) and left ventricular pulsatile power (Wp) under hypergravity in a chronically instrumented baboon model. Baboons were chronically instrumented to measure left ventricular antero-posterior dimension (ap-dim) from piezoelectric crystals, aortic electromagnetic flow velocity (Flo) from the ascending aorta, aortic and left ventricular pressures (Ao and LV), and the left and right atrial pressures (LA and RA). Input impedance Zo, Rp, C and Wp were obtained from Ao and Flo signals submitted to Fourier analysis. Head-to-foot hypergravity Gz was produced by centrifugation in the awake baboon using gradual onset rates with successive plateaus from 2, 3, ...to 9 Gz, separated with a rest plateau at 1.4 Gz. The initial results showed that Rp increased with Gz level. C tended to decrease with increasing Gz, whereas Zc appears to change little. Wp decreased when Gz-level increased whereas the ratio Wp/Wt (pulsatile power/total power) seemed to be unchanged, but at high Gz level Wp/Wt increased (4.68% vs 8%). Our results confirm that Rp increases under hypergravity, and our method can give beat to beat values. Results suggest that cardiovascular response depends on Gz-level. However the significant difference in results between low and high Gz may result from the low aortic flow velocity at high Gz. Thus Wp/Wt increases at higher Gz implying ventricular/vascular coupling is more efficient at lower Gz levels.

10.3

MODELING AND DIGITAL SIMULATION OF BLOOD FLOW IN A VESSEL. APPLICATION TO INFLIGHT LOSS OF CONSCIOUSNESS OF FIGHTER AIRCREW.

*D.Gaffié, **P.Quandieu, **Ph.Liébaert, **A.Guillaume: *ONERA 29, avenue de la division Leclerc 92320 Chatillon (France). **CERMA, Base d'Essais en vol 91228 Brétigny sur Orge. (France)

A theoretical and digital study of physical mechanisms involved in inflight LOC affecting fighter aircrew is presented as an asymptotic analysis of equations expressing blood flow in a deformable vessel. A one-dimensional formulation of the problem is finally obtained. It takes into account simultaneous actions of the heart and of the external field of disturbances resulting from aircraft motion. The digital calculations made in the very elementary case of a single vessel where certain physical characteristics are changing (resting cross-section, stiffness, source terms, etc...) show that two different blood flow rates may develop as a function of conditions i.e. a subcritical and a supercritical flow rate. The supercritical flow rate is generally unstable, and immediately preceded by a transition shock which causes upstream blood pooling. Inflight LOC observed in aircrew during rapid onset rate +Gz acceleration could therefore be caused by intracranial hypertension associated with increased mechanical stresses inside brain tissue.

10.4

MUSCULAR ADAPTATIONS INDUCED BY DOBUTAMINE AND THEIR INFLUENCE ON G-TOLERANCE IN MINIATURE SWINE. B. Girten, J. Cooper, W.M. Sherman, P. Niciforos and A.J. Merola (SPON: R.W. Gotshall). Wright State University School of Medicine, Dayton, OH 45435.

Chronic administration of dobutamine (DOB) has been shown to induce a variety of adaptive changes. Sixteen male miniature swine (pigs) were treated with DOB and saline (SAL) for 6 weeks to determine if muscular adaptations could be induced and if so, if these changes could influence the animals G-tolerance. Eight pigs receiving SAL were compared to a group of 8 pigs receiving DOB (24 mcg/kg/min). Animals were infused 2 hours/day, 5 days/week for 6 weeks. Approximately 48 hours after infusions were discontinued each pig was tested (RUN 1) on the Dynamic Environmental Simulator (DES) to determine +G_z tolerance. Thirty minutes following RUN 1 pigs were given a beta-blocker (Brevibloc) and tested a second time (RUN 2). The acceleration profile ranged from 3 to 9 G's with G-tolerance expressed as minutes before loss of consciousness (GLOC) during the 9 G exposure. Biochemical analyses of muscle tissue revealed that soleus citrate synthase, superoxide dismutase and catalase, as well as, percent of slow twitch muscle fiber in the gastrocnemius were all significantly greater ($P<0.05$) in the DOB group as compared to the SAL group. Heart weight/body weight ratio and ventricular citrate synthase were also significantly greater in the DOB treated group. G-tolerance between groups was not different for RUN 1 or 2, however the SAL animals demonstrated a significant decrease in time to GLOC between runs, while the DOB animals showed a slight increase. These data indicate that although DOB did not have an effect during the initial centrifuge exposure, it was able to effectively attenuate a significant decrease in G-tolerance during a second exposure which occured after beta-1 adrenergic blockade.

10.5

RAPID ONSET RATE ,G.LOSS OF CONSCIOUSNESS (ROR G-LOC): INTRACRANIAL HYPERTENSION. A NEW CONCEPT? *P.Quandieu, *A. Guillaume, **D.Gaffié, *Ph.Liébaert, *M.Briane, *J.C.Sarron, *D.Tran: CERMA, Base d'Essais en vol 91228 Brétigny sur Orge.** ONERA 29, avenue de la division Leclerc 92320 Chatillon (France).

Long steep turns generated HS.ROR+ Gz acceleration. A new clinical form of inflight LOC occured: rapid, with no visual warning, and no recollection of the accident. A strictly biomechanical explanation of sudden inflight LOC was proposed, based on practical facts: the pilot is suddenly placed into a high intensity volume forces field, the mechanical behavior of nerve structures is viscoelastic, the blood mass circulating inside the brain possesses properties of inertia. Brain nerve structures thus hypothetized to be exposed to a HSROR+Gz stress become functionally inefficient, not because of the lack of oxygen, but because of a sudden increase in intracerebral mechanical stresses, creating early intracranial hypertension. In this biomechanical approach, the concept of energy becomes paramount. A simple model was proposed (1). The first results of this modeling study indicate that ROR + Gz acceleration could augment mechanical stresses inside nerve tissues, therefore result in sudden intracranial hypertension hypertension. The threshold intracranial pressure at which LOC could occur can be discussed with respect to the instantaneous arterial pressure and heart parameters. Four effects were analyzed : chronological effects of the cardiac cycle vs.load application time, volume effects of systolic stroke, heterometric adjustment of myocardium, neurohormonal inotropic adjustment of cardiac function (homeometric adjustment).

1)Quandieu P.,Gaffié D., Liébaert Ph: Interprétation biomécanique des pertes de connaissance en vol des pilotes de chasse sous l'effet de l'application d'une accélération +Gz d'installation rapide(fort jolt). C.R. Acad. Sci.Paris 312,(II) 185-190, 1991.

10.6

ENTAINMENT OF CIRCADIAN RHYTHMS IN THE RAT BY DAILY ONE HOUR G PULSES. C.A. Fuller, D.M. Murakami and V. Demaria Pesce. Dept. of Animal Physiology, University of California, Davis, CA 95616-8519.

This study examined the effect of daily one-hr hyper-G pulses on the circadian rhythms of body temperature and activity in rats. Ambient temperature [~26°C] and lighting [LL 30 lux] were constant. During a three week baseline period free-running rhythmic patterns were established. The animals were then exposed to a 2G field for one hr at the same time every day. Body temperature and activity were monitored via an implanted telemetry transmitter [MiniMitter] and data collected via a microcomputer data system [DataQuest] in ten minute bins. At 1 G the animals demonstrated a free-running temperature rhythm with a period greater than 24 hrs. Two distinct changes were noted in the circadian rhythms with the daily centrifugation. First, body temperature and activity levels fell in response during the daily period of centrifugation; there was no habituation to this response over the four weeks of daily centrifugation. Second, the rhythms maintained a constant phase relationship with the 24 hour G cycle. Further, when the animals were released into constant 1 G, they free-ran from a phase point which would suggest that the rhythm was entrained by the G-pulse. This study was supported by NASA Grant NAGW-1950.

10.7

EFFECTS OF ACCELERATION STRESS ON THE SECRETION OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN RATS. K.H.Seul, J.K.Park*, B.O.Park*, S.H.Kim[1], K.W.Cho[1]. Aeromedical Training Center, ROKAF, Cheungwon 363-380, [1]Dept of Physiology, Jeonbug National University Med Sch, Jeonju 560-180, Republic of Korea

It is well known that an exposure to acceleration stress is accompanied by severe reduction of venous return to heart, reflexive increase of heart rate and increase of plasma renin concentration (PRC). ANP is released from the heart, and its secretion is controlled chiefly by changes in atrial volume. Effects of acceleration stress on the plasma ANP, atrial ANP, PRC and heart rate were examined in rats. Rats were exposed to centrifugation up to +9Gz, for 120 sec, one time per day for one run (Day 1), three runs (Day 3), seven runs (Day 7) consecutively. Rats were decapitated immediately after last exposure. Results as follows (*: P<0.05 vs Day 0);

Day	0	1	3	7
Plasma ANP (pg/ml)	367±37	259±19	254±17*	197±31*
Atrial ANP (ng/mg),Left	136±11	176±33	214±33*	240±55*
Right	158±21	240±70	292±29*	272±58*
P R C (ngAI/ml/h)	42±3	38±9	41±7	34±9

Heart rate decreased significantly during centrifugation from 496±6 to 458±12 beats/min. We conclude that the decrease in plasma ANP by acceleration stress in rats is due to decrease of both heart rate and cardiac volume. But mechanisms of decrease of heart rate and no alteration of PRC by acceleration in rats require futher study.

10.8

PERIPHERAL VASCULAR RESISTANCE IN NON HUMAN PRIMATES EXPOSED TO REPEATED HIGH SUSTAINED +Gz ACCELERATION. P.Liscia*, C.Drogou* and P.Quandieu*. CERMA, Base d'Essais en vol 91228 Brétigny sur Orge. France.

The object of this study was to show whether repeated high sustained +Gz acceleration (+8Gz for more than 30 seconds over several months) affects peripheral vascular resistance in non human primates (baboons). A trinitrine test was run. This test was realised on 4 baboons exposed to +8Gz for five months[1] and on two control exposed to 1Gz. The test was realised one week after the end of expositions. Trinitrine (3mg/60 kg)was injected in vein. Intrafemoral pressure was measured using a Millar probe. The drop in arterial pressure and the concomitant increase in heart rate were displayed on a Hewlett-Packard system. Results: heart rate increased by 43±12bpm under trinitrine in the four trained baboons vs 28±6 bpm in the two control animals. The decrease in arterial systolic pressure was 63±7mmHg in trained baboons vs 22.5±10 mmHg in control animals. The decrease in arterial diastolic was 39±7 mmHg in trained baboons vs 21±1 mmHg in control animals. Conclusion: this test seems to indicate that permanent vasocons-triction develops in baboons regurlaly exposed to +Gz acceleration, resulting in increased peripheral resistance.

1)Investigations was conformable to APS"Guiding principles in the care and use of animals".

10.9

LACTACIDEMIA IN NON HUMAN PRIMATES EXPOSED TO REPEATED HIGH SUSTAINED +Gz ACCELERATION. A.Guillaume*, P.Liscia*, C.Drogou* and P.Quandieu*. CERMA, Base d'Essais en Vol 91228 Bretigny-Sur-Orge. France.

Lactacidemia, which reflects the muscular work induced by hypergravity has been measured in baboons [1] regularly exposed to +8Gz accelerations sustained for more than 30 seconds. It has also been measured during an adaptation period of 1Gz exposure. Lactacidemia was determined on venous blood prior to centrifugation and immediately after centrifugation was stopped. Seven animals were trained. Five were exposed to 40 +8Gz runs. For two other baboons, training was interrupted after 29 exposures as they showed signs of intolerance. Results: During training, lactacidemia induced by acceleration exposures was 3.28±0.23 $mmol.l^{-1}$ for the five baboons. This increase, higher than 1.5 $mmol.l^{-1}$ is physiologically significant. The statistical analysis shows that levels are different with a risk $\alpha<0,5\%$. For the two others animals this increase was only 0.89±0.17 $mmol.l^{-1}$. During adaptation, the difference in lactacidemia between samples was 0.29±0.09 $mmol.l^{-1}$ for the group of five animals, and 0.13±0.09 $mmol.l^{-1}$ for the group of 2 animals. Conclusion: The five animals whose lactacidemia had significantly increased under the effect of centrifugation, showed good tolerance to acceleration. The two animals whose lactacidemia had not increased, did not tolerate training. 1) Investigations was conformable to APS "Guiding principles in the care and use of animals".

10.10

CHANGES IN THE RENIN-ANGIOTENSINE RESPONSE OF NON-HUMAN PRIMATES EXPOSED TO REPEATED HIGH SUSTAINED +GZ ACCELERATION. P.Liscia*, C.Drogou* and P.Quandieu*. CERMA, Base d'Essais en vol 91228 Bretigny sur Orge. France

The renin-angiotensin response of five baboons[1] (Papio Papio) exposed to +8Gz acceleration for 30 seconds, twice a week for 5 months, was investigated. High Gz exposure was preceded by an adaptation period of 1Gz exposure. The hormonal response was evaluated as a 5 point kinetic distributed during pre-test rest, immediately after centrifugation and during recovery. Six kinetics were run during the experiment. Results: the mean plasma-renin activity value at rest increased from 7.95±1.9 ng/ml/h during adaptation period to 12.27±0,8 ng/ml/h at 16 expositions and to 14.40±3.4 ng/ml/h at 40 expositions. Half an hour after the centrifugation, the mean plasma renin activity value was 11.12±2.1 ng/ml/h for +1Gz period adap-tation, it increased to 14.40±1.8 ng/ml/h at 16 expositions +8Gz and to 18.5±0.7 ng/ml/h at 40 expositions +8Gz. The statistical analysis shows that the kinetics were significantly different with a risk $\alpha<2\%$. Conclusion: the repeated exposure to high sustained +Gz acceleration induces a general increase plasma renin activity.

1) Investigations was conformable to APS" Guiding principles in the care and use of animals".

10.11

THE EFFECT OF THE HYPERGRAVITATION ON THE PROTEIN METABOLISM IN JAPANESE QUAIL. JANKELA J.,BARANOVSKÁ M.,ANTALÍKOVÁ J. Institute of Animal Biochemistry and Genetics SAS, 900 28 Ivanka pri Dunaji, Czecho - Slovakia.

Japanese quail-cockerels continuosly for 5 days subjected to 2 G hypergravitation /by means of centrifugation/, or hypodynamy in jackets without contact with floor were compared to control animals reared in normal conditions. Only one interruption lasting for half an hour on the 4th day was performed in order to applicate the solution of C^{14}-lysine /0,5 ml/100 g body mass/ to the animals. The quails were fed "ad libitum" a commercial mash during the whole experimental period. 24 hours after isotope application the animals were killed and m. supracoracoides or m. fibularis superficialis were taken. Protein and nucleic acids contents as well as specific radioactivity of free and protein bound lysine in above mentioned muscles were determined. The differences especially in protein synthetic rate were observed between control animals and animals subjected to hypergravity.

10.12

CARDIOVASCULAR EFFECTS OF DOBUTAMINE AND THEIR INFLUENCE ON G-TOLERANCE IN PIGS. J.R. Cooper, B. Girten, R.D. Latham and A.A. Karl (SPON: R.W. Gotshall). Armstrong Laboratory, Wright-Patterson AFB, OH.

The objective of this study was to determine if chronic administration of dobutamine (DOB) would increase G-tolerance. Charles River Minipigs were divided into two groups of 8 animals each and administered either DOB (24 mcg/kg/min) or sterile saline (SAL) intravenously 5 days a week for 6 weeks. Base line data collected forty-eight hours following the last treatment period demonstrated that cardiac output was significantly greater ($P=0.03$) in DOB treated animals as compared to SAL treated animals. Base line aortic blood pressures between these groups was not significantly different. Immediately following the collection of base line data, the pigs were restrained on a centrifuge and accelerated to 9 $+G_z$. Time to loss of consciousness (GLOC) was determined by closed circuit television. Following the initial centrifuge run all pigs received Brevibloc administered intravenously. Hemodynamic measurements taken at this time revealed that the cardiac output of DOB treated animals remained significantly elevated ($P=0.03$) over SAL treated controls. Aortic blood pressure recordings in SAL treated animals following Brevibloc administration were however, lower than precentrifuge base line values ($P=0.06$). Pigs were once again accelerated to 9 $+G_z$ and time to GLOC recorded a second time. SAL treated animals maintained consciousness for significantly shorter periods of time ($P=0.02$) at 9 $+G_z$ following administration of Brevibloc. Time to GLOC remained relatively constant following Brevibloc administration for animals who were treated with DOB. Results demonstrate that the chronic administration of DOB induces adaptive changes in the heart. These adaptations may contribute in part to changes observed in G-tolerance following beta-1 adrenergic blockade.

10.13

VENOUS RETURN, GRAVITATIONAL STRESS AND PHYSICAL TRAINING. Francis Louisy* and Charles Yannick Guezennec* (SPON : A. GÜELL). C.E.R.M.A. - C.E.V., 91228 Brétigny sur Orge Cedex, France.

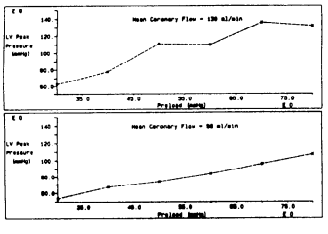
Leg plethysmographic measurements (mercury strain-gauge plethysmography) were made to study the effects of different types of physical training on lower limb venous distensibility and emptying. Seven endurance trained subjects (ET, VO_2 max = 65 ± 6 ml.mn^{-1}.kg^{-1}), seven weight trained subjects (WT, VO_2 max = 48 ± 4 ml.mn^{-1}.kg^{-1}) and seven untrained subjects (UT, VO_2 max = 44 ± 4 ml.mn^{-1}.kg^{-1}) were submitted to a tilt-table test. Leg filling volume FV (ml.100 ml^{-1}) during 30° vertical tilt, half-emptying time $T_{1/2}$ (seconds) and venous output at the 6th second (VO_6) (ml.100 ml^{-1}.mn^{-1}) during return to horizontal position were measured. Comparative tests were made using a factor analysis of variance. Results show a significant increase in FV and VO_6 in ET compared with UT and WT (FV : 4.2 ± 0.7 ml.100 ml^{-1} in ET vs 2.4 ± 0.8 and 2.1 ± 0.5 ml.100 ml^{-1} respectively in WT and UT, $p < 0.001$; VO6 : 9.7 ± 4.2, 7.7 ± 2.9 and 5.4 ± 1.8 ml.100ml^{-1}.mn^{-1} repectively in ET, WT and UT, $p < 0.05$ ET vs UT and ET vs WT) whereas no significant difference was observed between groups for $T_{1/2}$. Therefore, UT and WT have the same lower limb physiologic venous responses to orthostatic stress. Endurance training seems to increase lower limb venous distensibility and these results suggest that the greater blood pooling in lower limbs recruits elasticity of venous walls to insure better emptying and therefore maintain adequate venous return. Data available in this experiment also suggest that the greater venous distensibility in ET is a physiological response to hypervolemia resulting from the aerobic type training.

CARDIAC PHYSIOLOGY

11.1

SEGMENTAL LEFT HEART TRANSPLANTATION. RR Lazzara*, CM McCarty*, and TL Demmy* (sponsor: BE Pennock). Medical College of PA at Allegheny General Hospital, Pittsburgh, PA 15212

The canine left ventricle can be isolated and transplanted for purposes of physiologic study. Three pairs of mongrel dogs were used. The left ventricle was surgically isolated by: 1) incising the ascending aorta down through the non-coronary cusp, 2) opening the right atrium and exposing the coronary sinus ostia, 3) incising the anterolateral wall of the right ventricle. PTFE grafts were then sutured to the coronary ostia and coronary sinus ostia. The graft conduits were then anastomosed to the recipient carotid artery and jugular vein. The following pressure volume curves and biochemical data were obtained.



Myocardial Biochemical Data

	Trial #1	Trial #2	Trial #3
ATP (μM/gm protein)	34.6±8.1	28.1±13.0	50.7±9.6
ADP (μM/gm protein)	11.7±2.0	9.8±1.4	17.4±1.9
Glycogen (μM/gm protein)	11.9±1.2	13.6±4.1	10.8±1.5
Lactate (μM/gm protein)	0.04±0.02	0.41±0.16	.11±.06

We conclude that the canine left ventricle can be surgically isolated and transplanted with maintenance of hemodynamic and physiologic functions.

11.2

THE EFFECTS OF ACUTE PHARMACOLOGICAL HYPOTENSION ON INDICES OF LEFT VENTRICULAR DIASTOLIC FUNCTION IN MAN. Joe M. Moody*, Steven R. Bailey*, and Bernard J. Rubal. Brooke Army Medical Center, Fort Sam Houston, TX 78234

This study examines the effects of amyl nitrite inhalation on indices of left ventricular diastolic function in seven patients (ages 44±7 yrs). High-fidelity left ventricular and aortic pressures were obtained using a multisensor micromanometric catheter. Following a control period patients inhaled (3 deep breaths, 10±5 seconds). Amyl nitrite caused a significant decrease in aortic systolic blood pressure (27 percent, $p<0.05$) and left ventricular ejection time (8 percent, $p<0.05$). Although left ventricular end-diastolic pressure did not change peak negative left ventricular dP/dt decreased 30 percent ($p<.05$). It was observed that left ventricular end-systolic pressures were influenced by late systolic aortic wave reflections. These data suggest that arterial wave reflections may influence left ventricular pressure-derived indices of diastolic function especially at low arterial pressures.

11.3

COMPARISON OF INTRACARDIAC AND INTRAVASCULAR SOUNDS IN MAN. Randolph E. Modlin*, Joe M. Moody*, James K. Gilman*, and Bernard J. Rubal. Brooke Army Medical Center, Fort Sam Houston, TX 78234

Intracardiac micromanometric pressures and piezoelectric phonocardiograms were simultaneously recorded from the left ventricle and aorta in six patients (ages = 41±11 yrs) undergoing elective cardiac catheterization using a custom-designed, 8F multisensor catheter. Data were recorded at rest, deep breathing, physiologic maneuvers and supine exercise. Phonocardiograms were bandpass filtered and digitized at 5kHz. Power spectral analysis revealed significant ($p<0.05$) differences in the frequency content of the left ventricular and aortic phonograms during the conditions studied. When the phonograms were compared with simultaneously recorded high-fidelity pressures the onset of heart sounds could be correlated with physiologic events. Intracardiac phonocardiography may prove useful as a research tool for investigating the etiology of normal and pathologic heart sounds in man.

11.4

COUPLING OF LEFT VENTRICULAR CONTRACTILITY AND COMPLIANCE IN MATHEMATICAL AND ANIMAL MODELS. John A. Ward, Charles P. Kingsley and Noel M. Diaz (SPON: R.D. Latham). Brooke Army Medical Center, Fort Sam Houston, TX 78234

A three compartment numerical model was developed to determine the effect of changes in compartment volumes, compliances and resistances and cardiac contractility on pressure-volume loop geometry. Swine hearts were instrumented with a micromanometer for LV pressure (P) and sonomicrometer crystals for LV dimension (D) according to an approved protocol. PD loops obtained during i.v. administration of anesthetic and inotropic agents were compared with loops from the mathematical model. Thiopental, ketamine and calcium caused changes in both preload and contractility, suggesting that ventricular compliance and contractility are coupled. PD loops recorded from the animal model during pulsus alternans also suggest coupling. Coupling maintains stroke volume by switching between pressure and volume pumping modes with changes in contractility.

11.5

VASOPRESSIN RELEASE DURING EXERCISE DOES NOT ATTENUATE COLLATERAL-DEPENDENT FLOW OR FUNCTION IN AMEROID-OCCLUDED MINISWINE. J.D. Symons,* J.C. Longhurst, and C.L. Stebbins. University of California, Davis, CA 95616.

Canine coronary collateral vessels are believed to be more sensitive to pathophysiological levels of vasopressin (VP) than vessels in adjacent myocardium. Since lysine VP significantly increases during treadmill exercise in the miniswine, we hypothesized that VP release would attenuate collateral-dependent blood flow and regional function during exercise. An ameroid occluder was placed around the left circumflex coronary artery (LCX) in 13 miniswine. 10 weeks later, V_1 receptor blockade using $d(CH_2)_5Tyr(Me)$ arginine-vasopressin (10-12 μg/kg, iv; n=2) increased resting LCX flow in all regions, indicating that V_1 receptors were present. During treadmill exercise, at 80% heart rate reserve, regional myocardial blood flow (radioactive microspheres) and systolic wall thickening (sonomicrometer dimension gauges) were measured in the LCX region. During a control run (C) HR, mean arterial pressure (BP), rate-pressure product (systolic blood pressure X HR;RPP), endocardial flow ratio (LCX/left anterior descending flow; Q ratio) and systolic wall thickening (% Wth) were measured at 20 min of exercise. The workload was repeated in the presence of V_1 receptor blockade (V_{1x}). Blockade was verified by an attenuated pressor response to intravenous injection of vasopressin after V_{1x}.

	HR(b/min)	BP(mmHg)	RPP(x10⁴)	Q RATIO	% WTH
C	223±4	105±2	3.6±.10	.80±.1	37±4
V_{1x}	222±5	97±1†	3.5±.10	.79±.1	42±4

Means ± SEM; †p<0.05 C vs V_{1x}; Q ratio n=9

These results suggest that physiological concentrations of VP are not sufficient to cause coronary collateral vasoconstriction or constriction-induced myocardial dysfunction during prolonged exercise in the miniswine.

11.6

FUNCTION OF CORONARY ARTERIES DURING ACUTE REJECTION OF A TRANSPLANTED HEART. A.J. McLarty, C.G.A. McGregor, V.M. Miller, Mayo Clinic, Rochester, MN 55905

Experiments were designed to determine the effects of acute rejection on the function of coronary arteries in transplanted hearts. Pigs underwent heterotopic heart transplantation (i.e. transplanted donor heart in series with the recipient heart). Immunosuppression was maintained for five days and then discontinued. Rejection typically occurred after three days. Rings of right coronary arteries with and without endothelium were suspended in organ chambers for measurement of isometric force. Responses were compared with rings from transplanted hearts, recipient native hearts and unoperated hearts (controls). All rings contracted comparably to potassium chloride. Endothelin and angiotensin-1 caused concentration-dependent contractions which were less in arteries from rejecting and native hearts compared to control. Endothelium-dependent relaxations to UK14304 were reduced in arteries from rejecting hearts compared to those from control and native hearts. These results demonstrate decreases in receptor operated mechanisms of the vascular smooth muscle and endothelium from an acutely rejecting transplanted heart. These changes may affect perfusion of the organ and also may contribute indirectly to the onset of accelerated atherosclerosis.

11.7

THE LOCALIZATION AND EFFECTS OF NEUROKININS AND VIP IN THE RABBIT HEART. Eric A. Accili*, Alison M.J. Buchan*, Yin Nam Kwok*, John C. Brown* and John R. Ledsome. M.R.C. Regulatory Peptide Group, Faculty of Medicine, Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

The objectives of these experiments were to localize substance P and vasoactive intestinal peptide-like immunoreactivity (SP-LI and VIP-LI) in the rabbit heart and to examine the effects of SP, neurokinin A (NKA), neurokinin B (NKB) and VIP on the isolated perfused rabbit heart. Hearts were removed from chloralose-urethane anaesthetized rabbits and either fixed in Bouin's solution for immunocytochemistry (ICC) or perfused using the Langendorff method. Both SP-LI and VIP-LI were found in the cell bodies of neurons located in the atrial septa of rabbit hearts using ICC. Bolus injections of SP, neurokinin A and VIP caused dose-dependent reductions in the perfusion pressure (PP) of hearts perfused at constant flow with ED50 values of 75 fmol, 15 pmol and 75 pmol respectively while neurokinin B did not have an effect. The rank order of potency for the neurokinins was SP>NKA>NKB which implicates the NK-1 receptor subtype in the vasodilatation observed. There were no significant changes in heart rate, left ventricular pressure or contractility when these peptides were given at these doses. These results argue in favor of a physiological role for intrinsic VIP and SP in coronary vasodilatation in the rabbit.

Financial support from the Medical Research Council of Canada, the Heart and Stroke Foundation of British Columbia and the Yukon, and the British Columbia Health Care Research Foundation is gratefully acknowledged.

11.8

STRUCTURE AND PHARMACOLOGY OF CANINE CORONARY COLLATERAL ARTERIES. Jane E. Ward*, Joe J. Smolich*, Grant A. McPherson* and James A. Angus* (SPON: J.W. Funder). Baker Medical Research Institute, Melbourne, 3181, Australia.

We studied the relationship between structure and vascular reactivity in mature coronary surface collateral arteries removed from 17 dogs 24 weeks after implantation of a casein occluder on the circumflex coronary artery. Normal (N) and collateral (C) artery segments (≃ 500 μm internal diameter) were mounted in a myograph for isometric force recordings. C arteries contracted to half the maximum force generated by N vessels in response to K^+ (124 mM), endothelin-1 (1 – 100 nM) and a thromboxane A_2 mimetic U46619 (1 – 300 nM). Partially contracted C and N vessels (K^+ 25–30 mM) had similar sensitivity to the relaxants acetylcholine, sodium nitroprusside and cromakalim. C arteries had relatively thicker adventitia, thinner media, ruptured internal elastic laminae and a thick neointima lined by endothelium. Theoretical calculations predict that, under isotonic conditions, despite the poor contractility of C arteries, the neointimal lumen encroachment would reduce the lumen to zero in response to constrictor stimuli, an exaggerated response compared with N arteries. Coronary collateral arteries are thus compromised flow conduits that may play a role in vasospastic angina.

Supported by Glaxo Australia and the National Heart Foundation.

11.9

Renin-Angiotensin System Dependency of Myocardial Fibrosis in Hypertrophied Ventricles of Aorta-constricted Hypertensive Rats *K. C. Chang, §S. W. Norby, *J. E. Zehr & §H. M. Swartz

*Dept. of Physiology, §College of Medicine, Univ. of Ill., Urbana, IL 61801

Myocardial hypertrophy in systemic hypertension may become pathological with ventricular dysfunction. We used the inter-renal aortic constriction to induce arterial hypertension and cardiac hypertrophy within 7 days post-surgery. These rats developed focal myocardial cell necrosis accompanying progressive perivascular and interstitial fibrosis which might account for such deterioration of cardiac function. Simultaneous measurement of oxygen concentration, using *in vivo* electron paramagnetic resonance (EPR) spectroscopy, showed significant difference between fibrotic and hypertrophied myocardium of the same animal (0.20±0.05 versus 2.00±0.05%, $p<0.005$). Induction of hypertension was the result of the activation of renin-angiotensin system (RAS) due to renal ischemia/hypoxia. EPR oximetry showed significantly decreased oxygen concentration in the kidney below constriction after surgery (0.50±0.02 versus 1.15±0.05%, $p<0.01$). Plasma renin activity was also shown to be increased in these hypertensive rats. Myocardial fibrosis occurred not only in the left ventricle but also in the right ventricle. Due to the in-series alignment, with respect to the systemic circulation of the ventricles, the occurrence of myocardial fibrosis in the right ventricle can not be explained by pressure-overload because right ventricle has a normal afterload in this hypertensive model. Therefore, myocardial fibrosis occurred in hypertrophied ventricles of these aorta-constricted hypertensive rats might be the consequence of RAS activation. The cause of these phenomena is under investigation. (AHA Illinois Affiliate)

11.10

CONGESTIVE HEART FAILURE IN MALIGNANT HYPERTHERMIA SUSCEPTIBLE SWINE. Charles H. Williams, Dorothy D. Ekery*, and Marcos Martinez*. Texas Tech Univ. HSC-RAHC, El Paso, TX 79905-1298

Stroke volume data from our malignant hyperthermia susceptible (MHS) pigs indicated that the MHS pig heart was pumping only 63% of the blood volume to peripheral tissues as compared to normal pigs. We have evaluated hemodynamic data collected from (n=23) MHS outcross, (n=14) MHS inbred, and (n=22) normal pigs. Increased (CVP), increased (MPAP), increased (PCWP), decreased stroke volume index (SVI) and decreased left ventricular contractility in inbred MHS pigs are all consistent with Congestive Heart Failure (CHF). CHF in inbred MHS pigs is one of the key pathophysiologic factors which makes the MHS or Porcine Stress Syndrome (PSS) pig acutely susceptible to various environmental stressors. Left ventricular hypertrophy decreases SV and/or SVI. This left ventricular hypertrophy leads to diastolic dysfunction of the left ventricle, causing problems with left ventricular filling, left-sided pressures, and left ventricular relaxation. The left ventricular hypertrophy and dysfunction cause a marked decrease in left ventricular contractility (dP/dT) in MHS inbred pigs. This decrease in dP/dT further reduces the effectiveness of the left ventricle to pump blood resulting in a decreased cardiac reserve thereby reducing tolerance to physical exercise. In addition, the MHS inbred pig must dissipate a higher metabolic heat load as reflected in increased core and rectal temperatures which would cause them to be particularly susceptible to heat stress. Inbred MHS pigs have CHF as a basic cardiovascular problem induced by the MH gene defect. Heart function is affected and may lead to sudden death and arrhythmias.

11.11

SPATIAL AND TEMPORAL PATTERN OF ATRIAL NATRIURETIC PEPTIDE (ANP) SYNTHESIS IN RIGHT VENTRICULAR HYPERTROPHY (RVH). Kirk B. Kelley*, James C. McKenzie*, Elaine M. Merisko*, Jasiri Kennedy* and Robert M. Klein* (SPON: Ronal R. MacGregor) Department of Anatomy and Cell Biology, University of Kansas Med. Ctr., Kansas City, KS 66103, Department of Anatomy, Howard University College of Medicine, Washington, D.C. 20059, and Malvern Research Group, Malvern PA 19355

ANP synthesis occurs in the adult mammalian ventricle in congestive heart failure and hypertension. The present study analyzed ANP expression in the right ventricle of rat heart during establishment and recovery from hypoxia-induced pulmonary hypertension. Significant RVH and immunoreactive ANP (ANP-ir) were detected in the right ventricle after only 3 days of hypoxia. Initial focal concentrations of ANP-ir were observed in cardiomyocytes near the junctions of the right ventricular free wall and the septum, as well as surrounding isolated blood vessels in the right ventricular free wall. Initial sites of ANP-ir may represent zones of maximum wall tension following increased workload. At 14 days, ANP-ir enlarged to encompass the entire right ventricular wall and right half of the septum and ANP-ir became apparent in the left ventricle as well. Twenty-one days of normoxia following 21 days of hypoxia reduced RVH and ANP-ir to the levels seen at 3 days. EM immunocytochemistry revealed few ANP-positive secretory granules similar to those found abundantly in the atria. In order to determine if ventricular cardiomyocytes have the secretory machinery to package ANP in a regulated pathway, anti-clathrin immunocytochemistry was performed. In ventricular cardiomyocytes from hypoxic rats, perinuclear anti-clathrin staining typical of atrial cardiomyocytes was rarely observed. These data support the view that ANP release from the hypertrophied right ventricle occurs via a constitutive rather than a regulated pathway. Supported by the American Heart Association/Kansas Affiliate Inc. and NIH grant HL 45241

11.12

CARDIAC MYOSIN PHENOTYPE CHANGES WITH ATROPHY AND HYPERTROPHY. *C. D. Ianuzzo, B. Li*, S. E. Ianuzzo*, C.A.M. Barrozo* and T.A. Salerno**, York University and University of Toronto, Toronto, Canada M3J 1P3

Physiological demands imposed on the myocardium lead to changes in expression of the myosin isoforms. In this study the changes in the cardiac myosin isoforms were compared in hearts pumping against an increased afterload with hearts that were beating but not pumping. Female Lewis rats(190-210 g) were exposed to an arterial pressure overload by constriction of the abdominal aorta using neurosurgical hemoclips. Hearts were unloaded by a heterotopic transplant, in which the donor heart was anastomosed to the abdominal aorta and inferior vena cava of the recipient rat. Sacrifice occurred 30 days post-operative. Hypertrophy ranged from 24-110 % with a mean of 60%. The unloaded hearts atrophied an average of 52%. The hypertrophied left ventricular free wall(VFW) had up to a 50 % decline in the V1 isoform with commensurate increases in the V2 and V3. In hearts with 100% increase in mass myosin changes were observed in the septum and right VFW. The atrophied transplanted(T) hearts showed similar relative changes in myosin expression(C=100% V1 vs. T=55% V1) as did the hypertrophied hearts with changes also occurring in both ventricles. These comparative findings show that both hemodynamic hypertension and hypotension lead to similar changes in myosin expression.

Supported by Ontario Heart and Stroke Foundation and Canada NSERC grants

11.13

CARBON MONOXIDE-INDUCED CARDIAC HYPERTROPHY IS NOT REDUCED BY ALPHA OR BETA- BLOCKADE IN THE RAT

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The stimulus for CO-induced cardiac hypertrophy was investigated. Two exper's were carried in which adult male Sprague-Dawley rats were exposed continuously to 700 ppm CO for 30 days (CO) or inhaled room air (AIR). In each exper., 2/3s of the rats received either the alpha blocker, prazosin (PR), or the beta-1 blocker, atenolol (AT), in the food daily, at a low and a high dose. Systolic blood pressure (SBP) was signif. lowered (20-25 mmHg) by CO alone. PR lowered SBP only at the high dose, but failed to further lower SBP in the CO rats. AT alone lowered SBP marginally, even at the high dose. Low dose, and particularly high dose AT, lowered SBP even more in the CO rats. Heart rate (HR) was signif. lowered by PR and AT alone at both doses in the AIR rats. HR was signif. elevated (30-50 beats/min.) by CO. This HR rise was blunted slightly by PR, but not by AT. Body wt. (BW) was unaffected by CO, PR or AT treatment. Hematocrit increased from 50% in the AIR to 77% in the CO rats; it was unaltered by PR or AT. CO alone resulted in 30-43% and 18-25% wt. increases in right ventricle free-wall (RV) and left ventricle + septum (LV+S), respectively. Neither low nor high dose PR signif. decreased RV and LV+S wts in the CO rats. Low dose AT failed to alter RV and LV+S wts., in the CO rats; however, high dose AT, significantly ($P<0.01$) increased RV wt. in the CO rats. CO exposure increased lung/BW ratio; AT, but not PR, attenuated this effect. Thus, CO-induced cardiac hypertrophy develops in spite of lowered SBP (i.e. lowered LV afterload), and the blockade of either alpha or beta-1 receptors.

11.14

FREE RADICAL SCAVENGERS PREVENT INTESTINAL ISCHEMIA-REPERFUSION-MEDIATED CARDIAC DYSFUNCTION J.W. Horton and D.J. White,*UT Southwestern Medical Center, Dallas, TX 75235

Our previous studies show that intestinal ischemia impairs cardiac function. This present study examined the contribution of oxygen-derived free radicals to cardiac dysfunction after intestinal ischemia-reperfusion in a rat model of superior mesenteric artery (SMA) occlusion (atraumatic clip for 20 minutes) and collateral arcade ligation. Controls were sham operated (Group 1, n=10). In Group 2, 10 rats with SMA occlusion were sacrificed 2-3 hours after reperfusion without treatment. SOD and catalase (17,140 units/kg each) were given one minute after SMA occlusion (Group 3, n=11) or 2 minutes after reperfusion (Group 4, n=10). To examine the contribution of neutrophils as a source of free radicals, additional groups of animals were treated with pentoxifylline (PTX) either 1 min after SMA occlusion (Group 5, n=10) or 2 minutes after reperfusion (Group 6, n=10). Cardiac contractile depression occurred in the untreated ischemic group as indicated by a fall in LVP (from 77±3 to 63±4 mmHg, $p<0.01$) and +dP/dt max (from 1827±60 to 1558±98 mmHg/sec, $p<0.03$) and -dP/dt max (from 1267±57 to 953±68 mmHg/sec, $p<0.02$). Contractile depression after untreated ischemia-reperfusion was confirmed by a rightward shift in LV function curves and decreased responsiveness to perfusate calcium. Both free radical scavengers (SOD and catalase) and neutrophil stabilization (PTX) prevented ischemia-reperfusion-mediated cardiac dysfunction, and left ventricular function curves for these treated groups were identical to those generated by control hearts. We conclude that oxygen-derived free radicals from activated neutrophils contribute, in part, to intestinal ischemia-reperfusion-mediated cardiac dysfunction.

11.15

INTRACORONARY DYNORPHIN 1-9, NALOXONE, NOREPINEPHRINE (NE) OVERFLOW AND MYOCARDIAL CONTRACTILE RESPONSES IN DOGS. J.L. Caffrey, B.A. Barron*, H. Gu and J.F. Gaugl. Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Repeated left cardiac nerve stimulation at 20 minute intervals gradually reduced the stimulated coronary venous NE overflow. Intracoronary dynorphin 1-9 infusions lowered stimulated NE overflow further and reduced the increase in left ventricular contractile function (LV dP/dt). Both responses were prevented by naloxone. After naloxone, the previous decline in NE overflow was reversed and approached initial control responses. In controls pretreated with naloxone, the decline in NE overflow was prevented or substantially delayed. Dynorphin did not alter resting LV dP/dt. The ability of dynorphin to modify stimulated post-junctional responses was evaluated by comparing contractile responses to infused NE, before, during and after intracoronary dynorphin. Contractile responses to each of these three infusions were identical. These data suggest endogenous opioids were responsible for the declining overflow observed in original controls and that suppression of myocardial contractile function observed during dynorphin results primarily from dynorphin's ability to suppress NE overflow and presumably NE release. Supported by NIH Grants RO1HI33380 & KO4HL01658

11.16

OPIOID PEPTIDES IN THE RAT HEART: AGE AND SEX STUDIES. Meryl J. Fullerton* and John W. Funder. Baker Medical Research Institute, Prahran, Victoria, Australia 3181.

Opioid peptides arise from the processing of three precursors (proopiomelanocortin, proenkephalin and prodynorphin). Previous studies have reported the presence of βendorphin (βEP) in rat heart, and modulation of its levels by castration and testosterone administration. In the present study we have determined tissue concentrations of processing products of each of the opioid peptide precursors in male and female rat hearts at a variety of ages, and in preliminary studies examined the effect of castration. Hearts were excised from rats aged 3, 10, 50 and 80 days, and extracted in 2M acetic acid; 250mg tissue equivalents were further extracted on Sep Pak cartridges for assay of βEP, Metenkephalin–Arg–Phe (MERF) and dynorphin A(1–17) (DYN). In male rats tissue concentrations of all three peptides changed progressively with age. Total levels per heart remained relatively constant at 3–10 days; at 50 days female levels were 3–fold (MERF, DYN) to 100–fold (βEP) lower than male levels. In male rats, MERF and βEP levels changed 7 days post–castration, but not those of DYN. We interpret these studies as evidence for a possible neuromodulator role for endogenous opioid peptides in the heart, and for the possibility of substantial sex differences in their physiological activity. Supported by National Heart Foundation of Australia.

11.17

SEX STEROID MEDIATED CHANGES IN MYOCARDIAL ANOXIC RESISTANCE.
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Eight treatment groups of ten CD strain castrate male or female rats per group (Charles River) were injected daily with cottonseed oil (sham), or with testosterone proprionate (50 µg/100 g BW), estradiol benzoate (7 µg/100 g BW), or a combination of both sex steroids dissolved in cottonseed oil. These physiological replacement dosages of sex steroids, determined by bioassay procedures, were injected in a 0.1 ml bolus of cottonseed oil daily (I.P.) for sixteen weeks. Myocardial anoxic resistance was quantitated by means of an in vitro right ventricular strip preparation which evaluated the ability of the isolated ventricle to maintain contractions in response to electrical pacing at 1 Hz following ten minutes of anoxia. While this parameter was elevated by 162% in the estrogen-treated groups of male and female castrates compared to the sham (oil) injected groups, neither testosterone treatment alone nor combination steroid treatment produced anoxic resistance values which differed significantly from those of the sham injected animals. Thus, while estrogen alone may afford anoxic protection to the myocardium, testosterone is able to abolish this hormone-induced protection.

11.18

THE EFFECT OF GLUCOCORTICOIDS ON RAT MYOCARDIAL CONTRACTILE PROTEIN ATPASE ACTIVITIES.
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Myocardial ATPase activity after adrenalectomy (Adx) was compared with that after glucocorticoid replacement. Sprague-Dawley rats were sham-operated (SO), adrenalectomized (Adx), or Adx treated with corticosterone (AdxB), hydrocortisone (AdxHC), or dexamethasone (AdxD) administered in oil s.c. (2 mg/kg, corticosterone and hydrocortisone; 12 ug/kg, dexamethasone) daily for 6 weeks. The data (means±SEM) are summarized in the table:

	SO	ADX	AdxB	AdxHC	AdxD
Myofibrillar	0.234	0.176	0.183	0.223	0.237
ATPase	±.013	±.013	±.007	±.016	±.014
Ca^{2+}-activated	2.12	1.41	1.64	2.01	2.237
Myosin ATPase	±.055	±.057	±.114	±.044	±.162
Actin-activated	0.142	0.107	0.085	0.178	0.071
Myosin ATPase	±.010	±.007	±.009	±.084	±.011

Ca^{2+}-dependent myofibrillar ATPase activity and Ca^{2+}-activated myosin ATPase activity were spared the effects of Adx by hormone treatment reflecting glucocorticoid potency. Actin-activated myosin ATPase rats remained sub-normal in AdxB and AdxD, but AdxHC rats exhibited values equivalent to SO rats. The latter, unexplained, phenomenon has been observed previously. The data support our hypothesis that glucocorticoid hormones are important to the function of the heart as a pump.

11.19

ADRENERGIC STIMULATION INDUCES A CONSISTENT MAGNESIUM EFFLUX FROM CARDIAC CELL VIA ELEVATION OF INTRACELLULAR cAMP LEVEL.
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The adrenergic stimulation of perfused rat hearts or collagenase dispersed rat ventricular myocytes induced a considerable Mg^{2+} efflux across the plasma membrane. The Mg^{2+} movement, measured by atomic absorbance spectrophotometry or $^{28}Mg^{2+}$ distribution, was regulated via a modulation of intracellular cAMP level. The addition of permeable cAMP analogues (DBu-cAMP, 8-Cl-cAMP, 8-Br-cAMP) or forskolin mimicked the adrenergic stimulation in both experimental models. By contrast the addition of carbachol (inhibiting the adenyl cyclase activity via muscarinic receptor) stimulated a consistent magnesium uptake. A consistent Mg^{2+} efflux was also observed by inhibiting the protein kinase C with 50 uM H7, whereas its stimulation with TPA or PDBu induced a limited Mg^{2+} uptake. The presence of physiological concentrations of Na^{+} and Ca^{2+} in the extracellular medium was necessary for the adrenergic induced Mg^{2+} efflux. As the concentration of each ion was decreased a progressive reduction in Mg^{2+} efflux was observed. Interestingly, changes in extracellular Mg^{2+} (from 0 mM up to 1.2 mM) did not affect the amplitude of Mg^{2+} release. These data indicate that: i) a mechanism(s) of Mg^{2+} transport is located in the plasma membrane of rat cardiac cells; ii) this transport is regulated via a modulation of cellular cAMP level through *beta*-adrenergic receptors and muscarinic receptors; iii) it is possible that the protein kinase C can counter-regulate this mechanism or can stimulate a different one, mimicking a carbachol-dependent Mg^{2+} uptake; iv) this mechanism(s) requires physiological concentrations of extracellular Ca^{2+} and Na^{+}; v) it is operating over a broad concentration of extracellular Mg^{2+}. (Supported by NIH 18708 and by AHA F227).

11.20

EFFECTS OF THYROXINE ON MYOCARDIAL CONTRACTILITY.
George Kaldor and David R. Hoak. VAMC, Allen Park, MI, 48101 and Dept.Path. Wayne State Univ.School of Med. Detroit, MI, 48102.

The effect of thyroxine on the myocardium is complex in that it increases the repolarization time of the transmembrane potential, the Ca++ pumping of the sarcoplasmic reticulum and the concentration of the V1 iaomyosin. We found that the recombination of actin with myosin and the shortening of the myofibrills was also much faster with the V1 as compared to the V3 containing proteins. The V1 - ATP interaction generated 70% more heat than the V3 -ATP interaction. Although the free energy change of the nucleotide - isomyosin interaction was similar with both proteins the heat capacity and entropy change was significantly higher in the V1-ATP than in the V3-ATP interaction. The heat capacity change during the myosin-ATP interaction was connected to the ATP induced conformational change of the myosin which may be important in the mechanochemical energy transduction in the contration cycle. Our data indicate a difference in this process between the thyroxine treated and normal myocardium.
Supported by a VACO grant.

11.21

THYROID HORMONES AFFECT GROWTH OF UNLOADED FETAL MYOCARDIUM *IN OCULO*.
Aurora Torres* and Diane C. Tucker. University of Alabama at Birmingham, Birmingham, AL 35294

Atria, ventricles and whole hearts from fetal rats (E-12) were grafted *in oculo* to study thyroid hormone effects on the growth of developing myocardium. Grafts develop a mature morphology *in oculo* in the absence of hemodynamic load. To examine the interactions of sympathetic innervation with thyroid hormone stimulation, sympathetic innervation of the grafts was prevented by ipsilateral superior cervical ganglionectomy (SCGx) of the host. Experiment I compared whole heart grafts in hosts receiving daily s.c. injections of l-thyroxine (T4, 0.1 mg/kg); propyl thiouracil (PTU, 20 mg/kg) or saline (SAL, 0.9%). PTU treatment of hosts greatly reduced graft growth (PTU vs SAL: 1.01 ± 0.26 vs 3.18 ± 0.69 mg). T4 treatment produced cardiac hypertrophy in adult host rats but did not increase graft growth *in oculo*. Experiment II compared grafts of ventricles or atria cultured in hosts treated with tri-iodothyronine pellets (T3, 1.5 mg/3 wk, s.c.) to euthyroid controls. Initial data indicated that after only 2 weeks *in oculo* excess T3 facilitates growth of atrial grafts in SCGx eye chambers (Graft surface area of T3 vs CON: 2.4 ± 0.5 vs 1.2 ± 0.1 mm^2). T3 treatment had no effect on growth of ventricular grafts. Thyroid hormones were shown to be important for normal myocardial growth *in oculo*. The data also suggests that in the absence of hemodynamic load, excess T3 potentiates growth of atrial but not ventricular grafts.

11.22

SYNERGISM OF ATRIAL NATRIURETIC PEPTIDE, VERAPAMIL, ADENINE AND RIBOSE FOR RAPID RECOVERY OF REPERFUSED RAT HEART.
Z Tan* and J Gu*. Deborah Research Institute, Browns Mills, NJ 08015 (Spon: A.Malik)

Postischemic cardiac failure is characterized by a low level of myocardial high energy compound, increased intracellular calcium and myocardial stiffness. Isolated working rat hearts were perfused by Krebs-Henseleit buffer, subjected to 25min global ischemia and 60min reperfusion, resulting in cardiac dysfunction. Adenine (1mM), ribose (1mM), verapamil (10^{-7}M) or atrial natriuretic peptide (ANP) ($3.26x10^{-8}$M) were added separately or in combinations during reperfusion (n=5). Cardiac output following reperfusion was 50±7ml/min in Group A (Aden +Ribo+Verap+ANP), 39±5ml/min in group B (Aden+Ribo+Verap), 21+4ml/min in group C (ANP alone) and 20±4ml/min in group D (no treat-ment). Stroke volume was 0.18±0.02ml/beat(A), 0.14±0.02ml(B), 0.08±0.01ml(C) and 0.08+0.01ml(D). Aortic systolic pressure was 145±18mmHg (A), 140±16mmHg(B), 94±11 mmHg(C) and 90±10mmHg(D), ($P<0.05$ for A vs B, B vs C or D for all the 3 parameters). Although, ANP alone did not show any effect when combined with adenine, ribose and verapamil there were distinct inotrophic effects. The mechanism may be that adenine and ribose supplied precursors for ATP resynthesis, verapamil reduced Ca^{++} influx and protected mitochondria, and ANP improved postischemic myocardial compliance and increased stroke volume.
Supported by: Deborah Research Foundation DRG-1990-32

11.23

GLUCAGON PROCESSING INTO MINI-GLUCAGON (GLUCAGON (19-29)) IS ESSENTIAL FOR ITS POSITIVE INOTROPIC EFFECT.

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Glucagon is well known for its cardiotonic effect but its mechanism of action remains undetermined. In the present study, we show that glucagon, under minimal degradation conditions, has no effect on the amplitude of contractility of beating chick embryo ventricular cells. This raised the question as to the contribution of the active metabolite glucagon (19-29), (mini-glucagon), to the positive inotropic effect of glucagon. Incubation of glucagon with heart cells led to its rapid conversion into mini-glucagon, as measured by radioimmunoassay. Accumulation of the metabolite was maximal after 8 min and remained stable until 15 min, reaching 6% of the initial glucagon concentration. Bacitracin inhibited this processing of glucagon into mini-glucagon. Mini-glucagon, from 0.1 pM to 1 nM, exerted a potent negative inotropic action. The most striking observation was a 45% increase in the amplitude of cell contractility elicited by the combination of 30 nM glucagon with 1 nM mini-glucagon. A similar effect was obtained when glucagon was replaced by a low concentration (75 μM) of 8-bromo-cyclic AMP. We conclude that glucagon prodessing into mini-glucagon may be essential for the positive inotropic effect of glucagon on heart contraction.

11.24

PEPTIDYLGLYCINE α-AMIDATION EXPRESSION IN PRIMARY HEART CELL CULTURE.

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In the heart, expression of peptidylglycine α-amidating monooxygenase (PAM; EC1.14.17.3) is developmentally regulated and levels in adult atrium are higher than in any other tissue. The function of PAM in the heart is unknown. To begin to understand the function of PAM in this tissue, we have established primary cultures of atrial and ventricular myocytes from newborn rats by trypsin-collagenase dissociation of the tissue. We have assayed cell extracts and spent medium for the 2 catalytic activities found in the bifunctional PAM precursor: peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidyl-α-hydroxyglycine α-amidating lyase (PAL). In cultured atrial and ventricular cells, PHM and PAL activity is stably expressed throughout a culture period of 15 days. In both atrial and ventricular cultures more than 50% of the total cellular content of PHM and PAL activity is secreted in 24 hours throughout the 5- to 15-day culture period. Western blot analysis of extracts of atrial cultures with antibodies to PHM and PAL identified proteins corresponding to rPAM-1 and rPAM-2; rPAM-2 was more prevalent than rPAM-1. Lesser amounts of 55 and 40 kDa PHM proteins were also detected in the atrial cells while the medium contained a 55 kDa PHM protein. Although a single gene encodes rat PAM, alternative splicing generates at least seven forms of PAM mRNA. By reverse transcription-PCR, the seven different known forms of PAM mRNA were detected in atrial and ventricular cells; rPAM-2 mRNA was the predominant form of PAM mRNA present. Some changes in PAM expression were observed as a function of time in culture; levels of rPAM-1 mRNA in both atrial and ventricular cultures increased after 6 days, while levels of rPAM-5 mRNA increased after 11 days. Thus primary cultures of rat cardiocytes exhibit some changes in PAM expression and are well suited to investigate the role of PAM in the heart.

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11.25

ENERGY REACTIONS IN ATHEROSCLEROSIS; THE ROLE OF TRICARBOXYLATES IN THE PATHOGENESIS OF THE DISEASE.

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Acetyl CoA is the genesis of steroid synthesis and the carbon atoms of the acetate provide the molecular basis for all endogenous cholesterol. In decreased metabolic respiration, decarboxylation and phosphorylation are inhibited and an increase of the tricarboxylate will occur. The underutilized high energy citric acid substrate, by virtue of its feedback to acetyl CoA, may enhance steroid synthesis as effectively as it does lipogenesis. It is conceivable that endogenous and possibly exogenous citrates are important in mediating cholesterol synthesis and its pathological analogue, vascular occlusion.

11.26

CHRONIC ß-GUANIDINOPROPRIONIC ACID (BGPA) FEEDING AND MYOCARDIAL STUNNING

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Myocardial stunning is associated with normal or supranormal creatine phosphate (CrP) levels. To determine whether decreased availability of CP would further exacerbate stunning, male F344 rats (B) were fed a diet containing 3% BGPA for 15 weeks to decrease myocardial CrP. Control rats (C) were fed standard rat chow. Cardiac function was determined via the working heart model (in mM; 1.25 Ca^{+2}, 10 glucose and 0.5 acetate). Preload and afterload were set at 10 and 80 cm H_2O, resp. Normothermic, global ischemia was induced for 25 min. by clamping the atrial and aortic lines. A subgroup of C (O2) was perfused identically, substituting a normoxic Langendorff perfusion for ischemia. Pre-ischemic cardiac output (Q) was not significantly different between groups (47.4±0.9 and 43.7±2.4 ml/min/g, C and B, resp.), though cardiac work (Q*aortic systolic pressure (ASP)) was significantly lower in B (4260±89 vs. 3660±283, C and B, resp.;p=0.01). Post-ischemic Q and Q*ASP were significantly higher in B compared to C, and not significantly different from O2 (Q=16.0±4.8, 37.5±3.0, 45.8±3.1; Q*ASP=1834±450, 3106±257, 3780±274'; C,B and O2, resp.). Relative to pre-ischemic values, Q recovered 34.0±9.0% in C compared to 88.5±1.8 in B and 96.7±2.5 in O2 ($p<0.01$ C vs. B and O2). Total coronary flow (CF) during 0-10 min. reperfusion was signifcantly higher in B (132.6±9.4 vs. 95.9±7.8 ml/g, B and C, resp.). LDH release during this time was 4-fold less in B (747±204 vs. 3515±491, B and C, resp.). Thus, chronic feeding of BGPA increased functional recovery and decreased lethal injury subsequent to ischemia/reperfusion.

11.27

FIBER AND CAPILLARY MORPHOMETRIC CHANGES ACCOMPANY FUNCTIONAL DEFICITS IN POST-ISCHEMIC LEFT VENTRICLE.

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We reported previously that perfusion deficits are associated with loss of left ventricular function (LVF) in isolated rabbit hearts following extended periods of normothermic global ischemia. To determine whether these changes are accompanied by intracellular edema, fiber cross-sectional area [ā(f)] of sub-epicardium (EPI) and sub-endocardium (ENDO) were measured in isolated rabbit hearts following 5 (n=3) and 30 (n=3) minutes of normothermic global ischemia. Subsequently, hearts were perfused with India ink solution then glutaraldehyde fixative. Morphometric determination was made of ā(f) normalized to 1.82 μm sarcomere length. After 5 min ischemia, ā(f) was 121.2±15.6 (EPI) and 117.6±20.1 (ENDO) μm^2. After 30 min ischemia, intracellular edema resulted in ā(f) of 189.1±39.4 (EPI) and 156.1±22.6 (ENDO) μm^2. This fiber enlargement may contribute to the gross impairment of LVF reported after 30 min ischemia. Candidate mechanisms include disruption of fiber geometry and interference with alignment of actin and myosin filaments. Furthermore, it is likely that increased tissue pressures consequent to fiber enlargement act to compress the microvasculature, suggesting a potential basis for the 58% (EPI) and 60% (ENDO) reduction in perfused capillary length per fiber volume after 30 min ischemia (as determined by the presence of India ink in capillaries). These data lend further support to the concept that tissue alterations occur during normothermic global ischemia which act to compromize LVF and capillary perfusion. Importantly, these alterations do not depend upon blood-endothelial interactions which occur on myocardial reperfusion.

11.28

PHOTOAFFINITY LABELLING AND FUNCTIONAL EXPRESSION OF THE ATP RECEPTOR FROM VENTRICULAR MYOCYTES.

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Micromolar extracellular ATP generates a temporary calcium increase in ventricular myocytes trough the activation of plasma membrane receptors. Neurosecretory granules within the heart, where ATP is copackaged with norepinephrine, are likely the physiological source of extracellular ATP. Additional local ATP release could occur during ischemia or myocyte lysis.

The response to ATP was inhibited by the photoaffinity analogue 8-azido-ATP only when the compound was UV photolysed. Other photoaffinity analogues like 2-azido-ATP or Bz-ATP did not have the same effect. The 8-azido-ATP effect was not prevented by GTP or UTP as predicted by ATP receptor specificity. We used P32 8-azido-ATP to label the receptor in intact cells in presence of high concentration of UTP in order to reduce aspecific binding. Preliminary results show labelling of two bands on a SDS gel of molecular size of about 36 and 90 Kd.

ATP receptor was expressed in Xenopus Laevis oocytes by injection of mRNA. The functional assays were done by detecting transients of calcium as the luminescence generated by external ATP after the oocytes had been injected with Aequorin. The expressed receptor had similar specificity to the one on myocardial cells. We size fractionated the message on glucose gradient finding peak expression corresponding to mRNA size of 2.0-2.5 Kb. Size selection increased the response at the assay.

12.1

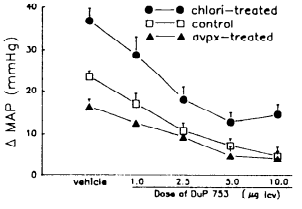
H-1 HISTAMINE RECEPTORS STIMULATE NOREPINEPHRINE RELEASE FROM THE PARAVENTRICULAR NUCLEUS/ANTERIOR HYPOTHALAMUS (PV/AH) IN THE RAT. Steven L. Bealer, Univ. of Tennessee, Memphis, TN 38163

Histamine (HA) releases norepinephrine (NE) from the PV/AH in conscious rats. These experiments investigated the HA receptor subtype mediating this response. Microdialysis probes were placed adjacent to the PV/AH 24 hr prior to the experiment. During testing, probes were perfused with artificial cerebrospinal fluid (ACSF) followed by ACSF containing HA (3mg/ml) alone or HA and either the H1 antagonist, chlorpheniramine, or the H2 antagonist, cimetidine. Dialysate NE was subsequently measured by radioenzymatic assay. Separate experiments measured dialysate NE following perfusion of ACSF containing the selective H1 agonist, 2-thiazolylethylamine (3 mg/ml) or the H2 agonist, dimaprit (3 mg/ml). HA alone increased dialysate NE concentration. H1 receptor blockade abolished this response, while H2 receptor blockade had no effect. In addition, perfusion with the H1 agonist significantly increased dialysate NE, while perfusion with the H2 agonist had no effect. These data demonstrate that stimulation of H1 histamine receptors, but not H2 receptors releases NE from the PV/AH region of the hypothalamus. (Supported by USPHS Grant HL-25877 and American Heart Association Grant 88-1103)

12.2

AT$_1$ RECEPTOR BLOCKADE BY CENTRAL DUP 753 COMPLETELY PREVENTS THE SYMPATHETIC BUT NOT THE VASOPRESSIN COMPONENT OF THE CENTRAL ANGIOTENSIN II PRESSOR RESPONSE. G.M. Toney and J.P. Porter. Department of Physiology, Univ. of Louisville Louisville, KY 40292.

Central (icv) injection of angiotensin II (AII) causes a pressor response characterized by two pressor components (1)increased sympathetic nervous outflow and (2) increased vasopressin (AVP) secretion, and is prevented by blockade of AII type 1 (AT$_1$) receptors. Experiments were performed in conscious, unrestrained male Sprague Dawley rats to determine the role of AT$_1$ receptors in each component of the central AII pressor response. AII (100ng) was injected icv alone, or following icv DuP 753 (1.0, 2.5, 5.0, 10.0 μg) in control animals and in animals pretreated with either the ganglionic blocking agent chlorisondamine (3mg/kg iv) or a peripheral AVP (V$_1$) receptor antagonist (AVPX). In both control and AVPX groups the central AII pressor response was almost completely blocked by DuP 753 (5 and 10 μg). However in chlorisondamine treated rats, which had an exaggerated central AII pressor response, 5 and 10 μg doses of DuP 753 only reduced the pressor response by about 65%.



The response that remained was due to AVP release since subsequent administration of AVPX completely prevented the pressor response. These data indicate that a portion of the AVP released in response to central AII is not mediated by AT$_1$ receptor activation. Supported by HL38993.

12.3

EFFECT OF HYPOTHALAMIC INJECTIONS OF INSULIN ON BLOOD PRESSURE AND RENAL NERVE ACTIVITY IN URETHANE-ANESTHETIZED RATS. James P. Porter. Department of Physiology University of Louisville, Louisville, KY 40292

Hyperinsulinemia has been associated with hypertension and increased sympathetic nerve activity. In the present study, the effect of microinjections of insulin directly into the hypothalamus on mean arterial pressure (MAP), heart rate (HR) and renal nerve activity (RNA) was investigated in normal rats and rats drinking a 10% sucrose solution for at least 6 days. Rats were anesthetized with urethane (1.5 g/kg, ip) and prepared with a femoral arterial catheter and a bipolar stainless steel hook electrode attached to a branch of the left renal nerve with Wacker Sil-Gel. MAP, HR and RNA were monitored before and for 15 min after injections of insulin (300 μU in 300 nl) into the ventromedial (VMH) or lateral (LH) hypothalamic areas. The change from baseline (15 min after injection) for each of the variables is shown below.

Drink	Site	n	ΔMAP (mmHg)	ΔHR (bpm)	%ΔRNA
water	VMH	4	-16 ± 7	8 ± 13	-8 ± 9
water	LH	4	0 ± 5	4 ± 4	3 ± 5
sucrose	VMH	3	12 ± 3	18 ± 3	18 ± 9
sucrose	LH	5	-3 ± 2	-6 ± 8	-10 ± 6

Hence, the VMH injections tended to decrease MAP and RNA in normal rats, but increase both variables in the rats drinking sucrose. Blood glucose was markedly elevated by urethane anesthesia (356 ± 48 and 441 ± 23 mg/dl, respectfully, in rats drinking water or sucrose), and was not consistently affected by the insulin injections. These data raise the possiblity that the elevated MAP and sympathetic activity in carbohydrate-fed rats may be due to an action of insulin in the VMH. Supported by HL38993.

12.4

Cardiovascular effects of interleukin-1 (IL-1) microinjected into the dorsal vagal complex (DVC) of anesthetized rats.

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The cytokine, IL-1, has been implicated as a mediator of cardiovascular depression during sepsis. IL-1 has also been found in the CNS. We evaluated the cardiovascular effects of human recombinant IL-1α and IL-1β in the medullary DVC, which contains the first synapse of the baroreflex arc. IL-1β and IL-1α were stereotaxically microinjected (25-100nl) into the DVC of urethane-anesthetized rats. IL-1β (0.25-1.0 pmoles) caused dose-dependent reductions in heart rate without consistent changes in blood pressure. The maximal changes were -46±10 or -115±8 beats/min after unilateral and bilateral microinjections, respectively. IL-1α (13-51 pmoles) had a similar effect, but it was 10-100 times less potent. The bradycardic responses to IL-1α and IL-1β were completely prevented by cholinergic muscarinic blockade with methyl atropine (2mg/kg, i.v.). The bradycardic effects of IL-1β were also attenuated by the opioid antagonist, naltrexone (2mg/kg, i.v.) as well as by a recombinant IL-1β receptor antagonist protein (Synergen), 1 pmole bilaterally injected into the DVC. These data suggest that IL-1 receptors are functional at important baroreceptor neurons in the DVC and that their activation increases vagal outflow to the heart through a mechanism that involves endogenous opioids.

12.5

FOS EXPRESSION REVEALS THE CENTRAL COMPONENTS OF THE BARORECEPTOR REFLEX. A.F. Sved, M.G. Backes*, W.L. Mays*, B.R. Olson*, G.E. Hoffman. *Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

The present study examined the localization of Fos protein in brain following stimulation of the baroreceptor reflex, with the goal of determining whether this approach is useful for identifying neurons involved in central mediation of the baroreceptor reflex. To activate the baroreceptor reflex, rats underwent bilateral sino-aortic denervation (SAD) while anesthetized with halothane. Following the completion of the surgery rats were allowed to regain consciousness. Forty-five minutes to 6 hours later rats were deeply anesthetized and perfused with sodium nitrite followed by phosphate-buffered 4% paraformaldehyde and 2% acrolein. Brains were sectioned and stained immunohistochemically for Fos-like immunoreactivity using 2 antisera raised against the N-terminal region of c-Fos (T. Curran Alu-Fos and Cambridge Res. OA-11-823/03429). In rats sacrificed 2-3 hours after SAD numerous Fos-positive neurons were present in 4 regions of the brainstem: nucleus tractus solitarius, caudal ventrolateral medulla in the region of the A1 cell group, rostral ventrolateral medulla in the region of the C1 cell group, and the paratrigeminal region. Fos staining was very light in animals sacrificed 45 minutes after SAD and was not detected in rats sacrificed 6 hours following SAD. Control and sham-SAD rats had few or no Fos-positive neurons in these regions. Activation of a gastrointestinal reflex by i.v. administration of CCK (10 ug/kg) elicited a different pattern of staining. These results indicate that neurons of the baroreceptor reflex pathway transiently express Fos in response to SAD, and that expression of Fos following manipulation of baroreceptor afferent activity may be useful in the characterization of the central pathways mediating the baroreceptor reflex.

12.6

'CENTRAL' CARDIORESPIRATORY EFFECTS OF EQUIPRESSOR I.V. INFUSIONS OF AII AND AVP IN AWAKE DOGS. D.B. Jennings and P.J. Ohtake. Dept. Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6

To examine 'central' effects of AII on respiration, 7 awake dogs were given I.V. infusions of angiotensin II (AII; ~ 5 ng.kg^{-1}.min^{-1}) to raise mean arterial blood pressure (MAP) 19 mm Hg ± 1.5 S.E. relative to control measurements. Data were obtained over 20 min and then, while continuing AII infusion, MAP was titrated back to the control level by simultaneous I.V. infusion of sodium nitroprusside (SNP). To control for potential effects of SNP, arterial pressure changes were induced by arginine vasopressin (AVP) in a comparable study. The effects of AII or AVP infusion at 'normal' arterial pressure were assumed to represent 'central' effects of these peptides. At 'normal' MAP, AII caused tachycardia, whereas AVP caused bradycardia. The effect of AII was to stimulate ventilation ($\dot{V}_E$) and decrease $PaCO_2$ and $[H^+]a$. In contrast, AVP did not affect $\dot{V}_E$ or $PaCO_2$. Although plasma levels of AVP were unchanged during pressor infusions of AII, when MAP was normalized during AII infusion there was a 400% increase in plasma AVP. These results indicate that a central nervous system action of systemic AII increases AVP release, heart rate and respiration which opposes arterial baroreceptor inhibitory reflexes. However, systemic AVP results in a centrally mediated bradycardia with no respiratory response. Supported by MRC and OTS.

12.7

THYROIDAL VASCULAR RESPONSIVENESS TO PARASYMPATHETIC STIMULATION IS INCREASED IN HYPERTHYROIDISM. **M. Dey, M. Michalkiewicz, L. Huffman and G.A. Hedge, Dept. of Physiology, West Virginia University Health Sciences Center North, Morgantown, WV 26506.**

It has been suggested that thyroid blood flow (TBF) is regulated by both parasympathetic and sympathetic nerves. Since T_4-pretreatment increases the sensitivity of the thyroid to the effects of thyrotropin (TSH), the present study was conducted to determine whether T_4-pretreatment can also sensitize the thyroid to the effect of parasympathetic stimulation on TBF. Untreated or T_4-pretreated (5μg/100g BW/day; ip; 3days) male rats (225±6g) were anesthetized and both superior laryngeal nerves (SLN) were transected close to the nodose ganglia. The distal cut ends of the nerves were placed on bipolar electrodes and stimulated (30 Hz; 10V; duration 0.5 ms) in one half of the rats for 2 minutes. TBF was continuously monitored by laser Doppler flowmetry (LDF) and also determined using ^{141}Ce-labelled microspheres [15 μm diam]. In stimulated groups, microspheres were injected 1 1/2 minutes after the beginning of the stimulation. Stimulation of the SLN had no effect on TBF in untreated rats when measured by LDF or microspheres (9.18±1.04ml/min x g, unstimulated *vs.* 11.83±0.87ml/min x g, stimulated). In contrast, stimulation of the SLN after T_4-pretreatment increased TBF by 65±21% over prestimulus levels when measured by LDF. By the microsphere technique, stimulation of the SLN increased TBF (25.72±6.08 ml/min x g) significantly ($p < 0.05$) in these rats compared to TBF in a non-stimulated T_4-pretreated group (6.80±1.96 ml/min x g). This increased sensitivity of the thyroid vasculature to T_4- pretreatment could be due to altered thyroidal metabolism, or to altered afferent or efferent neural input to the thyroid, or to some change at the intrathyroidal neuro-vascular smooth muscle junction. (Supported by NSF DCB-8904470).

12.8

ELEVATIONS IN FREE FATTY ACIDS STIMULATE THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL AXIS IN RATS. Eric P. Widmaier, Kimberly Rosen* and Brian Abbott*. Boston University, Boston, MA 02215

Increases in plasma free fatty acids (FFAs) inhibit growth hormone secretion in mammals. Physiological stimuli (stressors) that activate the hypothalamic-pituitary-adrenocortical (HPA) axis in rats rapidly increase plasma FFA levels, which serve as an important fuel source during stress. We tested the hypothesis that FFAs would also inhibit secretion of adrenocorticotropic hormone (ACTH) as part of a negative feedback loop. Conscious, cannulated male rats were injected i.v. with saline or a mixture of FFAs and heparin (Intralipid 10%, 2.85ml/kg); repeated blood samples were obtained via the carotid artery. At this dose, FFA levels increased transiently to 6.5mEq/l by 15 minutes and returned to baseline by 60 minutes. There was no effect of the injection on plasma osmolarity or pH, but plasma glucose was significantly increased by 60%. By sixty minutes after Intralipid injection, ACTH and corticosterone (B) were significantly increased above resting levels (pre-injection) and saline-injected time-controls, even at Intralipid doses as low as 0.285ml/kg. There was no B response after Intralipid injection in adrenalectomized rats (i.e., no interference with steroid assay), and no increase in ACTH or B immunoreactivity in hypophysectomized rats (no direct adrenal action or interference with ACTH assay). There was no significant ACTH response to 2.85ml/kg of Intralipid 4 hours after treatment with dexamethasone (DEX). There was also no B response to Intralipid after DEX suppression (no direct adrenal action). We conclude that moderate to large increases in plasma FFA levels activate, rather than inhibit, the HPA axis of rats (positive feedback), and this occurs at a level above the adrenal and is dex-suppressible. We are currently determining whether the effects of FFAs are direct or indirect (e.g., secondary to systemic hypotension). Support: (NIH) RO1 DK-41263.

BLOOD PRESSURE REGULATION

13.1

MODEL ANALYSIS: CONTROLLED RESPONSE TO AORTOTOMY HEMORRHAGE IN SWINE

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Hemodynamic changes following 5 mm aortotomy hemorrhage in conscious swine were analyzed using a mathematical model of the cardiovascular system plant. Potential controllers for this plant model incorporated effector mechanisms such as heart rate (f), arteriolar resistance (R_a), venous capacitance, and heart stroke volume (V_S), and sensed variables such as mean arterial pressure (P_{MA}), rate of arterial pressure change, oxygen debt (DO_2), end-diastolic heart volume (V_{ED}), and right heart pressure (P_{RH}). Only three relationships between effector and sensed variables significantly reduced prediction error (i.e., percentage of unexplained variance in mean arterial pressure, cardiac output, heart rate, and hematocrit): 1) $f = -G_1\Delta(P_{MA}/P_{RH})$; 2) $\Delta R_a = -G_2 \cdot DO_2$; and 3) $\Delta V_S = G_3 \cdot \Delta V_{ED}$. These represent combined vagal/sympathetic activity on the heart, the local effects of hypoxia on arteriolar resistance, and Starling's Law of the heart. An additional, nonlinear forcing function was required to predict venous capacitance. Using this control-system model, the percentage of unexplained variance was less than 5.5%.

13.2

VASOPRESSIN AND RENIN RESPONSIVENESS TO CHANGES IN BLOOD VOLUME IN CONSCIOUS MONKEYS. T.V. Peterson, L.C. Keil, C.D. Morley*, A.B. Carter*, R.A. Miller* and J. Evans*. Dept. of Medical Physiology, Texas A&M Univ. Hlth. Sci. Ctr., College Station, TX 77843 and NASA-Ames Res. Ctr., Moffett Field, CA 94035.

In nonhuman primates, changes in plasma arginine vasopressin (AVP) and plasma renin activity (PRA) during alterations in blood volume have not been well-studied. We assessed these hormonal responses in conscious Macaca fascicularis monkeys subjected to hemorrhage or autologous volume expansion, both equal to 20% of blood volume and performed over 90 min. The results (mean ± SE) for control (C) and experimental (E) samples taken every 30 min are shown below.

Hemorrhage

	C	E30	E60	E90
MABP (mmHg)	101±3	102±4	100±4	88±5*
AVP (pg/ml)	3.4±1.1	3.0±0.7	7.3±1.7*	34.2±10.4*
PRA (ng Al/ml/hr)	5.5±0.6	6.9±0.9*	7.7±0.9*	8.7±1.0*

Volume Expansion

	C	E30	E60	E90
MABP (mmHg)	102±3	105±3	108±3*	110±3*
AVP (pg/ml)	4.9±0.8	3.8±0.9*	3.6±1.0*	3.5±1.0*
PRA (ng Al/ml/hr)	6.3±0.7	5.7±0.8	5.3±1.1*	4.8±0.7*

MABP=mean arterial blood pressure
* Significantly different from C at $p<0.05$.

These results show that the AVP and PRA responsiveness to both decreases and increases in blood volume are similar in that they occur with close to the same amount of volume change (E30-E60=7-13% of blood volume). (Supported by NIH Grant HL31987.)

13.3

INTERACTION OF GENES AND ENVIRONMENT WITH THE BIOLOGICAL FACTORS CORRELATED TO THE BLOOD PRESSURE

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High blood pressure (BP) and heart disease are becoming major problems in Asian Indians (AI) throughout the world. To estimate of the role of genetic and environmental factors in BP variation in AI, we measured BP, anthropometrics, and blood and 24 hr urine samples in 14 sets of monozygous (MZ) (6 male, 8 female) and 6 dizygous (DZ) (4 male, 2 female) twins in Madras. The mean age =22 ± 6 (SD) (range 15-38)yrs, Systolic BP=115±8 (102-136), diastolic BP=68 ±9 (51-96) mm Hg. The factors **significantly** correlated with the systolic blood pressure were:

Variable	r coef	rMZ	rDZ	h^2
UCa	0.11	0.24	0.32	-.16
UNa	-3.1	0.42	0.13	0.58
SH Girth	3.2	0.97	0.81	0.32
Height	0.48	0.99	0.95	0.08
Weight	-1.13	0.94	0.83	0.22
Systolic	1.00	.85	.30	1.10

The relative genetic and environmental influences on these above parameters and Heritability (h2) was calculated as = 2(rMZ-rDZ) where r=intraclass correlation coefficient. The high rMZ value for height, weight and shoulder girth signifies that genes play an important role on these factors, which in turn control BP; while UCa and UNa are mostly regulated by non-gene factors. Twin studies in Asian Indian populations throughout the world may yield new clues to the epidemic of heart disease.

13.4

SEXUAL DIMORPHISM IN THE COMPENSATORY RESPONSE TO BLOOD LOSS. Lisa M. Slimmer and M.L. Blair, Dept. of Physiology, Univ. of Rochester, Rochester, N.Y. 14642.

We have examined the compensatory responses of mean arterial pressure (MAP), heart rate (HR) and plasma renin activity (PRA) to slow graded hemorrhage (0.8 ml/kg bw/min) in conscious age-matched Sprague-Dawley male rats and female rats in each phase of the estrus cycle. Measurements were taken via femoral artery catheter at 8%, 12%, 16%, 20% and 24% loss of initial blood volume (iBV). In male rats MAP was 106 ±2, 106±3, 101±3, 82±9, 58±9 and 42±3 mmHg at 0, 8, 12, 16, 20 and 24% iBV loss, respectively. MAP and HR significantly decreased from control beginning at 16% iBV loss for males ($n=6$, $p<.01$) and at 20% iBV loss for each female estrus cycle phase ($n=6$ except estrus $n=4$, $p<.05$). However, there were no significant differences between males and females in any phase for MAP at 0-24% iBV loss, or for HR at 0% or 20-24% iBV loss. In proestrus rats, HR significantly increased above control and was greater than male HR at 8-16% iBV loss ($p<.05$). PRA was 6.0±1.6, 7.6±2.2, 12.1±.5, 16.2±1.2, 24.6±2.2 and 39.8±5.0 ng/ml/hr at 0, 8, 12, 16, 20 and 24% iBV loss, respectively, in males. PRA was significantly increased from control at 12-24% iBV loss for all groups ($p<.05$; $n=4-6$) and was not significantly different in males and females at any time. In conclusion, we found few sex-related differences in the compensatory responses to slow graded hemorrhage. (Supported by AHA NYSA #89-032G and PHS S7RR05403-29).

13.5

MANIPULATION OF FOOTSHOCK PREDICTABILITY DIFFERENTIALLY AFFECTS THE BLOOD PRESSURE (BP) RESPONSE OF THE BORDERLINE HYPERTENSIVE RAT (BHR). J.E. Lawler, S.K. Naylor, M.M. Abel and D.R. Baldwin. Department of Psychology and Physiology Program, University of Tennessee, Knoxville, TN 37996.

Most studies which have attempted to study the effects of stress on BP responses have confounded the physical stressor with the psychological manipulation. Yet it is possible to separate them. In the present study, all animals received the same number of shocks, but differed in how predictable the shocks were. In the 100% group, shock always occurred during a 30 sec tone-on period. In the 75% group, most of the shocks occurred during the tone. Finally, in the 50% group, only half of the shocks occurred during the tone. Animals were studied for 5 days on this schedule. After this, arterial lines were implanted for direct BP determinations. Following recovery, the stress session was repeated. Within each predictability group, both normal (WKY) and BHR were studied. For WKY, systolic BP in the seconds immediately preceding footshock was slightly higher for the 50% group compared to the other two conditions (158±5 vs 153 ±4 mm Hg, respectively). However, for BHR, the 50% condition significantly elevated BP compared with the other two conditions (186±3 vs 172±4 mm Hg, respectively). We conclude that predictability manipulations can affect BP during an acute stress session. Furthermore, this manipulation interacts with genetic factors to determine the magnitude of the pressor response. (Am Heart Assoc 88-791)

13.6

REGULATION OF BLOOD PRESSURE AND ACTH DURING HEMORRHAGE IN CONSCIOUS, PREGNANT DOGS. V.L. Brooks. Dept. of Physiol., Oregon Hlth. Sci. Univ., Portland, OR 97201-3098.

To test the hypothesis that reflex regulation of ACTH is unaltered by pregnancy, the ACTH response to a graded hemorrhage (H) of up to 25% of blood volume was examined in conscious, pregnant (P) dogs (n=5) and in the same dogs at least 1 month post-partum (nonP dogs). Surgery was performed to implant femoral arterial and venous, and left and right atrial catheters. In nonP dogs, H decreased ($p<0.05$) arterial pressure (BP; 109±6 to 90±5 mmHg), right atrial pressure (RAP; 3.1±1.6 to -2.8±0.5 cmH_2O), and left atrial pressure (LAP; 7.9±1.9 to 0.6±0.7 cmH_2O), and increased ($p<0.05$) heart rate (HR; 70±6 to 98±4 b/min), ACTH (40±4 to 138±30 pg/ml), and plasma cortisol concentration (CS; 9±2 to 54±13 ng/ml). In P dogs, H caused larger ($p<0.05$) decreases in BP (107±6 to 73±10 mmHg) but smaller ($p<0.05$) decreases in RAP (1.1±1.5 to -1.0±0.7 cmH_2O) and LAP (5.9±0.9 to 2.2±0.9 cmH_2O). HR first increased, but by the end of H was unchanged (110±8 to 96±10 b/min). ACTH increased from 40±10 to 185±28 pg/ml ($p<0.05$), CS increased from 22±5 to 57±11 ng/ml ($p<0.05$), and the relationship between BP and ACTH, or BP and CS, was not different between P and nonP dogs. These data suggest that the hemodynamic response to H is altered by pregnancy, but that reflex regulation of ACTH remains unchanged. Supported by NIH Grant HL39923.

13.7

Effects of Sympathetic Nervous System Blockade on Cardiovascular Responses to Ethanol in Conscious Sheep. R.M. Thornton, B.A. Burak*, E. Krasney* and J.A. Krasney. Department of Physiology, SUNY at Buffalo, Buffalo, NY 14214.

The purpose of this study was to determine if ethanol (ETOH) has a vasodepressor effect during sympathetic nervous system (SNS) blockade in chronically instrumented conscious sheep. Sheep, with an intact SNS or following blockade of the SNS with bretylium tosylate (10 mg/kg IV), were given 1.2 g/kg of ETOH intravenously over 10 min. Mean arterial pressure (MAP) and heart rate (HR) were measured prior to and for 180 min following infusion of ETOH. Blood alcohol levels reached 195±11 mg/dl and 226±8 mg/dl in intact and SNS blocked animals, respectively, 20 min after beginning the ETOH infusion and thereafter declined progressively to 64±8 mg/dl and 79±12 mg/dl at the end of the study. In the intact sheep MAP did not change over the course of the study from the pre-ETOH infusion level of 93±5 mmHg while HR increased slightly from 102±6 beats/min to 113 beats/min at 180 min after beginning the ETOH infusion. In the SNS blocked animals MAP fell significantly ($p<0.05$) from the pre-ETOH infusion value of 96±4 mmHg to 79±3 mmHg by 40 min after beginning the ETOH infusion and remained at this level for the remainder of the study. HR in the SNS blocked animals increased significantly ($p<0.05$) from 100±3 beats/min to 144±8 beats/min at 100 min after beginning the ETOH infusion and stayed at this level until the end of the study. These results indicate that ETOH has a direct vasodepressor effect that is revealed only after removal of SNS. In intact animals alcohol results in an activation of SNS that offsets the depressor effects of alcohol. (Supported by NIH P01 HL-28542 and HL-36126).

13.8

DEPRESSOR RESPONSE TO PROPRANOLOL IS RELATED TO THE LEVEL OF PLASMA RENIN ACTIVITY (PRA) IN CONSCIOUS MONKEYS ON A LOW SODIUM DIET. Peter Frei*, Steven Kerr*, Steven Weldon*, Mary McFarland*, Paul Harrison*, Sudha Desai*, Jeffrey Madwed, Raymond Winquist and Maret Panzenbeck. Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT 06877

We investigated the hypotensive effects of propranolol in 8 conscious cynomolgus monkeys with elevated PRA. Monkeys were maintained on a low Na^+ diet for 1 week with supplemental furosemide which increased PRA from 5.7±2 to > 41±14 ng AI/ml/hr (range 8 to >105). Baseline mean arterial blood pressure (BP) was 104±3 mmHg. Propranolol (0.03 to 1.0 mg/kg, i.v.) caused significant, dose-related decreases in heart rate (-20±3% to -39±2% from a baseline of 184±10 bpm) in all monkeys. However, propranolol had variable effects on BP. In 6 of 8 monkeys, PRA values were 21±5 ng AI/ml/hr, and propranolol at the highest dose caused a modest fall in BP (-15±7%, $P < 0.05$). Subsequent administration of the angiotensin converting enzyme inhibitor enalaprilat (0.3 mg/kg i.v.) caused no additional decrease (-4±5%) in BP. Without propranolol, this dose of enalaprilat caused substantial decreases (22±1%) in BP in these monkeys. In the other 2 monkeys, propranolol caused significant dose-related decreases in BP (15±2 to 41±3%). In these 2 animals PRA was 5-fold higher than those from monkeys who showed a weak depressor response. In all 8 monkeys, PRA decreased by approximately 67% after propranolol. However, plasma angiotensin II levels did not significantly change in the 6 monkeys who had a weak depressor response to propranolol. Therefore, in conscious primates on a low Na^+ diet with a marked elevation of PRA, propranolol effectively lowers heart rate and BP in a dose-related manner. However, in animals with only a modest elevation of PRA, administration of propranolol is less effective in lowering BP and unexpectedly prevents any subsequent depressor response to enalaprilat.

13.9

ALTERED REFLEX CONTROL OF PERIPHERAL CIRCULATION SECONDARY TO CARDIOPULMONARY BYPASS Robert M. Lust*, Edward R. Norris, and W. Randolph Chitwood*. East Carolina University School of Medicine, Greenville, NC 27858-4354

The loss of peripheral vascular tone (PVT) during cardiopulmonary bypass (CPB) and the disturbed regulation of blood pressure during and after CPB are well described, but poorly understood. Baroreceptor reflex control of PVT before and during CPB was examined to test the integrity of the neural reflex control mechanisms. Six anesthetized dogs were instrumented to measure cardiac output, arterial blood pressure and heart rate. Peripheral vascular resistance (neosynephrine, nitroprusside) or output was altered (volume expansion or depletion) and changes in the other variables were measured before and again after 30 min of normothermic CPB. Spontaneous sinus rhythm existed throughout the experiment. Femoral arterial cannulation was used and each vena cava was cannulated separately, outside the pericardium, so as not to disrupt pathways to or near the sinoatrial node. Before CPB, cardiac output and blood pressure were inversely and exponentially related, ($Y=157.9X - 0.54$, $r = 0.965$). After CPB, this relationship had reversed and become a direct, linear function ($Y = 23.7X + 24.6$, $r = 0.977$). Also, before CPB blood pressure and heart rate interval were directly related in a sigmoid manner ($Y = 86.4 + 0.535X - 0.001X^2 + 2.21*10^{-7}X^3$, $r = 0.943$). However, after CPB, heart rate no longer changed appreciably with changes in blood pressure, and the function became linear and essentially vertical ($Y = 1.6X - 532.5$, $r = 0.982$). These data suggest that CPB produces a dramatic loss of baroreceptor-mediated reflex peripheral vascular control. The loss of reflex reactivity occurred early after CPB and was apparently unrelated to either hypothermia or cardiac arrest.

13.10

PSYCHOLOGICAL STRESS RESPONSE IN PREPUBERTAL CHILDREN: BLOOD PRESSURE (BP) AND BLOOD LEVELS OF ACTH,CORTISOL(C),ADH,RENIN (R) AND ALDOSTERONE (A). C.Dacou-Voutetakis,N.Alikatora,N.Georgopoulos,A.Gaviotaki. First Department of Pediatrics,Athens University,Athens Greece.

"Idiopathic" hypertension is the most frequent diagnosis in hypertensive adults. We elected to test the hypothesis that a different "alarm" response in these subjects,from early life, represents one of the pathogenetic mechanisms. We examined the cardiovascular and hormonal response to the stress of surgery anticipation in 13 healthy children undergoing elective minor surgical procedure. ACTH,(C),ADH,(R) and (A) were determined by radioimmunoassay prior to any manipulation. The study protocol also included recording of BP,pulse rate, personality characteristics,and family history of cardiovascular disorder. Below we compaire the hormonal response of 6 children (group A) with high BP (systolic > 120mm Hg and/or diastolic ≥ 90mm Hg) to 7 children (group B) whose BP was normal (BP < 120 systolic and ≤ 80 diastolic).

	ACTH pg/ml	C mcg/ml	ADH pg/ml	R ng/ml/hr	A ng/dl
Group A	76.6±61	22.8±3	10.95±18	6.8±3	33.4±8
Group B	34.7±16	18.78±5	2.8±3	2.65±1	6.88±6
p	>0.1	>0.1	>0.1	p<0.05	p<0.05

The data showed that, even in prepubertal period, psychological stress increases the BP and is associated with elevated R and A. These stress responses may play a role in the pathogenesis of hypertension.

14.1

THE INFLUENCES OF SIMULATED MICROGRAVITY CONDITIONS ON BLOOD PH, PCO_2, and PO_2 LEVELS IN RATS: PRELIMINARY RESULTS. Charles M. Tipton*, Lisa A. Sebastian, Kim A. Monnin, Elik Essif, and Ralph F. Fregosi*. Department of Exercise and Sport Sciences, University of Arizona, Tucson, AZ 85721

To better understand why rats experience a decrease in $\dot{V}O_2$ max after 7 to 35 days of head-down suspension (HDS), we obtained, and replaced, blood from rats before and during 1 and 7 days of HDS or cage control (CC) conditions. In addition, select cardiovascular measurements were made before the blood samples were taken. The group results, corrected for colonic temperatures ($\bar{X}$, SE, * = .05 level of significance) were from 8 rats in the CC and 11 in the HDS groups, respectively.

Parameter	Group	Before	After 1 Day	After 7 Days
pH	CC	7.47 ± .015	7.48 ± .016	7.44 ± .008
(mmHg)	HDS	7.46 ± .015	7.50 ± .004	7.48 ± .014*
PCO2	CC	34.1 ± 1.40	34.1 ± 1.16	36.0 ± 1.90
(mmHg)	HDS	36.1 ± 1.33	28.9 ± 0.81*	32.2 ± 1.21
PO2	CC	89.5 ± 2.08	87.2 ± 1.93	93.6 ± 2.17
(mmHg)	HDS	84.4 ± 2.56	74.4 ± 3.20*	81.2 ± 2.17*
Colonic Temp.	CC	38.0 ± 0.21	37.9 ± 0.25	38.7 ± 0.16
(°C)	HDS	37.8 ± 0.14	36.9 ± 0.39*	36.6 ± 0.30*
HR	CC	397 ± 15	396 ± 12	383 ± 18
(b/m)	HDS	394 ± 12	440 ± 10*	409 ± 11
HCT	CC	41.4 ± .50	39.6 ± .75	39.4 ± 0.97
(%)	HDS	42.3 ± .50	43.8 ± .78*	44.0 ± 1.38*

Calculation of the plasma bicarbonate changes revealed no significant changes during the experimental period. Collectively, the results suggest that hyperventilation is occuring to produce hypocapneia and a tendency for alkalosis. Although the hypoxemia trends follow those reported by Stazhadze et al. Life Science Digest 18:24-25, 1988, for HDS humans, it is unlikely that they are contributing to the lower $\dot{V}O_2$ max observed after 7 days of HDS. (Supported in part by NASA grant NAG-2-392).

14.2

LOCATION AND EXPRESSION OF DNA TAKEN UP BY MYOCYTES *IN SITU*. T. Toliver*, T. Raabe*, R. Walter*, and J. Koke, Dept. Biology, Southwest Texas State Univ., San Marcos, 78666.

We investigated uptake and expression of plasmid DNA (pCH110, 7224 bp, containing the SV40 early promoter and the ß-galactosidase reporter gene) by myocytes *in situ*. In separate experiments, covalently-closed circular and linearized DNA, and denatured, biotinylated DNA (bDNA), were injected into rat cardiac and skeletal muscle (100 µl, at 1 µg/µl, in buffered saline). The affected tissue was removed at intervals and fixed in 4% formaldehyde, then Vibratome sectioned at 70 µm. Sections of tissue exposed to bDNA were treated with avidin-conjugated peroxidase, reacted with diaminobenzidine and H_2O_2 followed by silver intensification, then processed for electron microscopy to visualize the location of the bDNA. 60 minutes after injection into skeletal and cardiac tissue, bDNA was found among the myofilaments in the cytoplasm. The bDNA was not membrane-bound or observed inside mitochondria, t-tubules, sarcoplasmic reticulum, or nuclei. In sections of bDNA-perfused hearts (100µg bDNA/liter Krebs-Hanseleit buffer), bDNA was also found in the capillary endothelium, the interstitial space, and associated with the sarcolemma, suggesting transport from the capillary lumen into the myocyte cytoplasm. Sections of tissue exposed to native circular and linearized DNA were examined histochemically for ß-Gal activity. Early results suggest expression occurs within 5 hours after injection of DNA into skeletal and cardiac muscle. Immunocytochemical experiments are in progress to determine the location of expression of exogenous DNA in myocytes.

14.3

EFFECTS OF EXERCISE TRAINING AND IMMOBILIZATION ON STRUCTURE-FUNCTION RELATIONSHIPS IN CANINE GASTROCNEMIUS MUSCLE. D. E. Bebout, M. C. Hogan, O. Mathieu-Costello and P. D. Wagner. Dept. of Medicine, University of California, San Diego, La Jolla, CA 92093-0623

To investigate the effects of exercise training and immobilization on structure-function relationships in skeletal muscle, 6 sets of purpose-bred hounds (3 litter mates in each set) were studied with each litter mate being allocated to one of 3 groups: control (C), trained (T) and immobilized (I). T trained on a treadmill 5 d/wk for 8 wk. C and I were cage-confined for 8 wk with I undergoing left hind limb immobilization for the last 3 wk. Each dog's left gastrocnemius was then surgically isolated, pump perfused and electrically stimulated to work maximally in situ under 3 different conditions of arterial oxygenation [normoxia (N), moderate hypoxia (MH) and severe hypoxia (SH)] in random order. O_2 delivery for 100 g tissue was kept constant among the 3 groups at each PaO_2. Simultaneous arterial and muscle venous blood samples were taken, muscle blood flow measured and $\dot{V}O_2$max calculated. Muscle diffusive conductance (DO_2) was estimated according to Fick's first law of diffusion by a numerical Bohr integration analysis. After experimental study, 3 sets of these animals were randomly chosen for morphological analysis after glutaraldehyde perfusion-fixation. Samples were taken from the mid belly of the medial gastrocnemius muscle and capillary-fiber geometry was subsequently quantified by standard morphological techniques. There were no differences in muscle weights between T and C but there was a decrease of 31% in I ($P<0.01$). Compared to C, T revealed an increase in $\dot{V}O_2$max and DO_2 for 100 g tissue of 38, 33 and 19 % and 71, 75 and 68 % during N, MH and SH, respectively ($P<0.02$). Capillary-to-fiber number ratio and capillary density were increased in T by 45 and 19 %, respectively, while capillary diffusion distance was reduced by only 8 % ($P<0.05$). In I, $\dot{V}O_2$max and DO_2 for 100 g tissue did not change, capillary-to-fiber number ratio decreased by 9 %, capillary density increased by 47 % and capillary diffusion distance decreased by 17 % ($P<0.05$ for each). We conclude that muscle $\dot{V}O_2$max and DO_2 are more closely related to the absolute amount of capillaries than to capillary density and diffusion distance, indicating that the major resistance to O_2 flux in skeletal muscle resides at the capillary level and not within the myocyte itself. (supported by NIH HL 17731 and TRD 1RT 227).

14.4

STIMULATION VOLTAGE AND POWER OUTPUT OF LATISSIMUS DORSI MUSCLE. R.L. Kao, S.S. Choi*, and G.J. Magovern*. Allegheny-Singer Research Institute, Pittsburgh, PA 15212.

Successful dynamic cardiomyoplasty and biomechanical assist depend on an adequate level of power output from skeletal muscle under chronic conditions. Six sheep under general anesthesia had their left and right latissimus dorsi muscles mobilized with careful preservation of the neurovascular bundle. Perineural electrodes were implanted for all 12 muscles, and Itrel pulse generators were connected only to one side in each animal. After recovery, the muscles with implantable pulse generators were conditioned with a gradually increased duration and rate over a 3 month period. At 4 months after the initial operation, each muscle was freed from its humeral insertion and attached to a lever-actuated piston pump which was connected to a mock circulatory system. The stimulation voltage (minimum voltage required to produce full muscle contraction) increased from 0.40 ± 0.06 to 1.49 ± 0.16 volts during the 4 month period with no difference between the conditioned and unconditioned muscles. The conditioned latissimus dorsi muscles had a lower power output initially (0.81 ± 0.05 vs 1.20 ± 0.10 watts/kg); however, this was reversed after 4 minutes of isotonic contraction. Maintaining the same stimulation for 2 hours, the conditioned muscles retained a significantly higher percentage (38 ± 5%) of initial power output as compared to the control muscles (12 ± 6%). By histological evaluation the perineural electrode did not cause any nerve injury and required a significantly lower stimulation voltage as compared to other electrodes (intramuscular). Skeletal muscle fatigue remains a limiting factor for dynamic cardiomyoplasty and biomechanical assist. Supported by NIH grant HL 38078.

14.5

CALCIUM ACTIVATED FORCE AND CALCIUM SENSITIVITY OF SKINNED SKELETAL MUSCLE FIBERS FOLLOWING FATIGUE J.H. Williams & C.W. Ward, Muscular Function Laboratory, H&PE Division, Virginia Tech, Blacksburg, VA 24061-0326

Force production of rested, skinned skeletal muscle fibers is reduced by conditions which mimic the fatigue state (e.g. elevated H^+, P_i, etc.). This study sought to examine the direct effects of fatigue on Ca^{2+} activated force and Ca^{2+} sensitivity of skinned fibers. Frog semitendinosus muscles were fatigued by tetanic trains of stimuli (80Hz, 100msec) delivered at $2sec^{-1}$ for 5min. This protocol reduced tetanic force to 1.8±0.2% of P_o with a recovery $t_{\frac{1}{2}}$ of 66.0±4.7min (n=4). Immediately after stimulation, single fibers were chemically skinned (saponin, 250µg/ml) for 15 min. Rested fibers were obtained from contralateral muscles. All fibers were sequentially transferred to increasing concentrations of Ca^{2+} (pCa 9-4.5). Fatigue experiments were complete within 35min of the stimulation protocol. Maximal Ca^{2+} activated force (pCa 4.5) was not significantly different between rested and fatigued fibers (198.5±12.3 and 179.7±16.9mN·mm^{-2}, $p>.05$, n=8). Likewise, the maximal rate of force development (dP/dt) was not different between conditions (81.8±4.2 and 94.9±6.6mN·mm^{-2}·s^{-1}, $p>.05$). At submaximal activating concentrations of Ca^{2+}, force and dP/dt of rested and fatigued fibers were not different although pCa_{50} of rested fibers (6.8±0.2) was slightly less than that of fatigued fibers (7.0±0.1, $p<.05$). These results suggest that maximal Ca^{2+} activated force of skinned fibers is not altered by fatigue, per se, while Ca^{2+} sensitivity is only minimally increased.

14.6

EFFECTS OF COCAINE ON RAT TRACHEAL SMOOTH MUSCLE CONTRACTION. G.E.Santacana and C.A. Jimenez-Rivera*. UCC-Sch. Med., Dept., Physiology, Bayamon, P.R. 00621.

Cocaine (COC) is a natural ocurring substance with local anesthetic and psychomotor stimulant properties. COC abuse, specially in the smoked form (crack), has become a serious health problem in our society. Notwithstanding, the direct effects of this drug on the respiratory system has not been elucidated. The actions of COC (10-5 M) on the acetylcholine (Ach) and potassium chloride (KCl) induced contraction of the isolated intact rat trachea (RT) were studied. Preincubation of the RT in COC (60 min.) produced a significant (t=8.48, n=5) increase (15%) in the maximal response to Ach. When COC was applied during the platcau of the Ach induced contraction, a significant (t=6.53, n=5) potentiation of the contractile response was also observed (72%). Addition of propanolol (1 uM), prazosin (10 uM) or nifedipine (1 uM) to the incubation medium produced no significant effect on the potentiating action of COC. However, incubation of the RT in a calcium (Ca^{+2}) free medium totally inhibited the potentiating effect of COC. In a medium with KCl (40 mM), COC augmented (20%) the Ach-induced maximal response (t=6.15, n=5). COC also had potentiating effects on the contraction induced by KCl after atropine (1 uM) pretreatment. These results suggest that COC augments the Ach induced contraction of rat trachea by an extracellular Ca^{+2} dependent effect and not via COC actions on cathecholaminergic receptors. In addition, the observation that COC potentiated the KCl induced contraction even in the presence of atropine indicates that this effect is independent of endogenous cholinergic neurotransmission. (Supported by RO3-DA07175 and 1-G12-RR03035-01A).

14.7

MASTOPARAN-INDUCED RELAXATION IN RABBIT AORTIC STRIPS IS NOT POTENTIATED BY A PHOSPHODIESTERASE INHIBITOR. Patricia B. Lieberman and R. Clinton Webb. University of Michigan, Ann Arbor, MI. 48109

Mastoparan, a tetradecapeptide purified from wasp venom, has recently been tested in many systems. It is thought that mastoparan works by bypassing cell membrane receptors and directly interacting with G-proteins. Since mastoparan's effects have not been characterized in smooth muscle, we examined the effects of mastoparan on isolated strips of rabbit aorta. Due to the observation that mastoparan caused relaxation in vascular smooth muscle, we hypothesized that it acts through an adenylate cyclase linked G-protein. Helical strips of rabbit aorta (endothelium removed) were suspended in tissue baths for isometric force measurement. Vessels were contracted with prostaglandin F_{2a} (1-5 uM) in the presence of phentolamine (1 uM). Isoproterenol and mastoparan both caused relaxation. Isoproterenol (.1-1 uM) caused a rapid 50% relaxation in 1 minute whereas mastoparan (1 uM) caused a 50% relaxation over a much longer interval (30 min). The role of cAMP in the relaxation response was investigated using aminophyllin, a phosphodiesterase inhibitor. In the presence of aminophyllin (.1 mM), relaxation to isoproterenol was augmented to 75% whereas relaxation to mastoparan was not enhanced. In fact at higher doses of mastoparan (10 uM), in the presence of aminophyllin, contraction was observed. These data do not support the hypothesis that the mechanism for mastoparan-induced relaxation is due to activation of a stimulatory G-protein coupled to adenylate cyclase. (Supported by NIH grant HL 18575)

AGING

15.1

EFFECTS OF AGE AND STRESS ON SERUM 3,5,3'-TRIIODOTHYRONINE (T3) CONCENTRATIONS IN FISCHER 344 RATS. H.A. Bertrand and J.T. Herlihy. Univ. of Texas Health Science Center, San Antonio, Texas 78284.

Previous longitudinal studies from our laboratory showed that serum total T3 levels rise with age, a finding different from reports in the literature which show either no change or a decrease in T3 levels with age. The experiments reported here were performed to uncover possible reasons for the discrepancy. Cross-sectional studies were performed in which the serum T3 levels were measured in blood obtained both from the tail and, after decapitation, from the neck. In both methods of sampling, the serum T3 concentrations increased from 3 to 19 months, confirming the observations of our longitudinal studies and eliminating the design and method of blood sampling as possible reasons for the discrepancy. The effects of trauma were also examined in 12 month old rats. Anesthesia (methoxyflurane for 40 min) lowered serum T3 concentrations by 43% (148±16 vs 84±9 pg/dl) in blood sampled 3 days after anesthesia. Even simple relocation from our barrier facilities in which rats are raised into conventional housing produced a 26% decrease (190±28 vs 139±21 pg/dl) in T3, measured 3 days after the move. These results show that trauma arising simply from shipment or anesthesia can significantly lower serum T3 levels and the failure to detect an age-related increase in serum T3 may be due, in part, to a stress-induced decrease in hormone levels. (Supported by NIA grant #AG01188)

15.2

PROTECTION OF DNA DAMAGE BY DIETARY RESTRICTION. M.H. Chung[1] and B.P. Yu[2]. [1]Dept. Pharmacol., Sch. Med., Seoul Natl Univ., Korea; [2]Dept. Physiol., Univ. TX Hlth Sci. Ctr, San Antonio, TX 78284-7756.

DNA damage produced by endogenous free radicals has been proposed to be a major contributing factor to many degenerative diseases and aging. In this report, the extent of damage in rat liver nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) was examined by quantitating the amount of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) which is a major product of oxidative damage. The liver from 3 and 24 mo old male SPF Fischer 344 rats, either ad libitum fed (AL) or 40% dietarily restricted (DR) rats, was used. The amount of 8-OH-dG was determined by the HPLC method of Kasai et al (Carcinogenesis 8:1959, 1987). The results showed that mtDNA damage was 15-fold higher than nuDNA damage in both AL and DR rats at 24 mos of age. Dietary restriction significantly reduced the amount of 8-OH-dG in both nuDNA ($p<0.05$) and mtDNA ($p<0.01$), indicating protection of the genomic integrity against oxidative damage by DR. A similar significant reduction ($p<0.005$) of 8-OH-dG by DR was observed in nuDNA at 3 mos. The conclusion drawn from this study is that the reduction of DNA damage in both nuclei and mitochondria by DR can be the underlying mechanism in support of the anti-radical hypothesis of DR and the interventive action of dietary restriction on aging and age-related neoplastic diseases. (Supported by Korea Sci. & Eng. Found. and AG01188).

15.3

ALTERATION OF MEMBRANE FLUIDITY BY LIPID PEROXIDATION IN AGED RATS. B.P. Yu, E.A. Suescun and S.Y. Yang, Dept. Physiol., Univ. TX Hlth Sci. Ctr., San Antonio, TX 78284-7756.

Age-related changes in membrane fluidity are generally accepted. Accumulation of membrane cholesterol has been the explanation for such changes with age. In this report, data are presented to show that the age-dependent lipid peroxidation is the major factor responsible for the increased membrane rigidity with age. Male SPF Fischer 344 rats were sacrificed at 3, 6, 12, 18, and 24 months of age, and mitochondrial and microsomal membranes were prepared. Membrane fluidity measurements were made by quantifying changes in polarization and anisotropy of a cationic fluorescence probe. The results showed that the fluidity of membrane preparations from ad libitum fed rats (AL) undergoes a substantial alteration with age, while little changes in the fluidity were detected in dietarily restricted rats (DR). Simultaneous measurements of the changes in fluidity and in vitro lipid peroxidation showed a strong correlation, suggesting the possible causal relationship. This notion was further strengthened by the data that no age-related changes in membrane fluidity were observed in DR rats, in spite of substantially increased membrane cholesterol with age. It is concluded that the age-related decrease in membrane fluidity is likely caused by increased lipid peroxidation of the membrane due to high hydroperoxide content and peroxidizability of the membranes. (Supported by AG01188).

15.4

EFFECTS OF AGE AND DIET ON PERIPHERAL CATECHOLAMINE (CAT) METABOLISM. J.T. Herlihy and H.A. Bertrand. Univ. of Texas Health Science Center, San Antonio, Texas, 78284.

The CAT contents of several organs were measured in male Fischer 344 rats fed throughout life either ad libitum (Group A) or 60% of that consumed by Group A (Group B). The norepinephrine (NE) content decreased from 6 to 24 months of age in heart, kidney, spleen and brown adipose tissue (BAT) of Group A rats. No age-related changes were observed in skeletal muscle, while total catecholamine content in adrenals increased. The NE contents of all tissues from Group B rats were higher than those of Group A rats but declined with age only in kidney, spleen and BAT. Heart and skeletal muscle content of Group B rats did not change with age and increased in adrenals. No consistent age or diet related pattern was observed in the TH activity of heart, kidney and adrenals. In Group A rats, the TH activity decreased with age in heart, did not change in kidney and increased in adrenals. In Group B rats, TH activity decreased with age in heart but increased in kidney and adrenals. Only sporadic differences in TH activity between the two dietary groups were observed. These results demonstrate that age and diet alter in a complex manner the peripheral catecholamine metabolism.
(Supported by grant AG01188 from the NIA)

15.5

PLASMA NOREPINEPHRINE INCREASES BY AGE: CORRELATION TO TOTAL BLOOD VOLUME AND LONG-TERM SMOKING HABITS. Ebbe Winther Jensen*, Ebbe Eldrup*, Henning Kelbæk*, Steen Levin Nielsen* and Niels Juel Christensen. Department of Internal Medicine and Endocrinology and Department of Clinical Physiology and Nuclear Medicine, Herlev Hospital, University of Copenhagen, 2730 Herlev, Denmark.

Plasma norepinephrine (NE) was measured together with cardiac output, total blood volume (BV) and other hemodynamic parameters in 12 young (28±2 years) and 10 elderly (69±3 years) healthy, non-obese male subjects in the resting supine and sitting position. Sitting plasma NE was found to be significant higher in the elderly (0.418±0.047 vs. 0.305±0.026 ng/ml, p=0.038). Cardiac index was not different in the two groups and did not correlate to plasma NE. Four of the 12 young subjects and half of the elderly subjects were smokers. Elevated plasma NE levels were confined to elderly smokers. There was a highly significant correlation between sitting plasma NE and BV corrected for body weight (r=-0.720, p=0.0002), but BV was not different in smokers and non-smokers. It is concluded that the increase in plasma NE in the elderly is confined to long-term smokers. It is suggested that this abnormality is a compensatory mechanism to vascular dilatation induced by long-term smoking.

15.6

HINDLIMB BLOOD FLOW RESPONSES TO SCIATIC NERVE STIMULATION AND ADENOSINE IN ADULT AND AGED F-344 RATS. R.C. Tyler, N.M. Abbey and A. Tucker. Department of Physiology, Colorado State University, Fort Collins, CO 80523.

Aging is characterized by a decline in maximal exercise O_2 consumption; this may be due to a reduced hyperemic response in exercising skeletal muscle. The effect of aging on blood flow during simulated exercise or injection of 5'-(N-ethylcarboxamido)-adenosine (NECA) was studied in anesthetized (ketamine 16 mg, and acepromazine 1 mg) aged (23 mo, n=8) and adult (10 mo, n=9) female F-344 rats. Blood flow was measured with a Transonic flow probe on the caudal abdominal aorta. The contralateral iliac artery, inferior mesenteric artery and the tail were ligated. Blood pressure and heart rate were measured from a cannulated carotid artery. An achilles tendon and sciatic nerve were isolated for tension measurement and electrical stimulation (4-V, 4-hz, 7 min). Skeletal muscle tension during rest and stimulation were similar between groups. Absolute resting (8.2 vs 4.9 ml/min) and peak exercise (13.2 vs 8.3 ml/min) blood flows were higher in the aged vs adult rats. Vascular resistance was less in the aged vs adult animals at rest (10.6 vs 21.4 mm Hg/ml/min) and during 'exercise' (7.3 vs 12.8 mm Hg/ml/min). Heart rate, mean blood pressure and gastrocnemius and soleus weights were similar between the groups. NECA (1.5 ug I.V.) had little effect on resting blood flow, but depressed heart rate to a greater extent in adult rats (adult 436 vs 280 bpm; aged 453 vs 375 bpm). We conclude that aged skeletal muscle does not exhibit a decreased hyperemic response to simulated exercise and that the cardiac sensitivity to NECA is reduced in aging.
(supported by AFAR, AHA of Colorado and NIA animal colony)

25.1

THE EFFECT OF SIMULATED WEIGHTLESSNESS ON FEMORAL ARTERY BLOOD FLOW IN THE RAT. Robert D. Roer and Richard M. Dillaman*. Univ. of N.C. at Wilmington, Wilmington, NC 28403

In order to determine if the blood supply of the bones of the hindlimbs of rats is altered by the tail-suspension model of weightlessness, rats were chronically instrumented for the measurement of femoral artery flow. Ultrasonic, transit-time flow probes (Transonic Systems, Inc.) were implanted into 8 wk. old Wistar Furth rats under ketamine/xylazine anaesthesia. Following 24 hr. recovery, flow was measured in the normal, ambulatory posture with the rat tethered to the meter via an electronic swivel. The animals were then suspended and flow was measured immediately and daily over the next 4-7 d. Animals were then returned to normal posture and flow was monitored daily for 1-3 d. Flow data were recorded directly to a computer and demonstrated a marked reduction in flow upon suspension. Flow decreased until a new steady state was attained at 5 d. Upon return to normal posture, flow increased, but did not return to presuspension levels until 1 d. Quantile-quantile plots of data illustrated a decrease in flow both during systole and diastole.
This work was supported by NASA Grant NAG 2-391.

25.2

ALTERATIONS IN GLOMERULAR HEMODYNAMICS AND TUBULAR REABSORPTION AFTER 24 HOURS HEAD-DOWN TILT (HDT) AND FOLLOWING ACUTE RETURN TO ORTHOSTASIS. Bryan J. Tucker and Margarida M. Mendonça*. Univ. of Calif., San Diego, La Jolla, CA 92093.

Previous studies have demonstrated that glomerular filtration rate (GFR) and urine flow are elevated after 24 hr HDT in the rat. This increase is associated with increased renal plasma flow (PF). However, neither the specific changes in glomerular hemodynamics and tubular fluid reabsorption due to 24 hr HDT nor the renal response to acute return to orthostasis has been examined. Munich-Wistar rats (n=7) were tail suspended for 24 hrs (25° HDT), anesthetized with inactin (100 mg/kg BW i.p.), and surgically prepared for micropuncture measurements in 25° HDT. After the initial period, the rats were returned to a horizontal position (Post 24 hr), and after 1 hr, the measurements were repeated. Single nephron GFR (SNGFR), single nephron PF (SNPF), glomerular hydrostatic pressure gradient (ΔP), glomerular ultrafiltration coefficient (LpA) proximal tubule fluid reabsorption (APR), and loop of Henle fluid reabsorption (LHR) measurements were compared to controls. § P<0.05 to control, * P<0.05 to 24 hr HDT.

	SNGFR	SNPF	ΔP	LpA	APR	LHR
	[nl/min	][mm Hg][nl/sec/mm Hg][			nl/min	]
Control	33±2	93±8	34±1	.05±.01	13±1	12±1
24 hr HDT	45±2§	166±11§	34±1	.08±.02	19±1§	16±1§
Post 24 hr	37±2*	122±10*	35±1	.05±.01	13±1*	13±1*

In 24 hr HDT, SNGFR increased due to increases in SNPF. The rise in APR and LHR in 24 hr HDT were insufficient to prevent increased flow rate in the distal tubule (from 8 to 11 nl/min) potentially contributing to increased urine flow. One hour orthostasis after 24 hr HDT restored most renal function parameters to values not different from control.

25.3

EFFECT OF 14 DAY HEAD-DOWN TILT (14d HDT) ON RENAL FUNCTION AND VASCULAR AND EXTRACELLULAR FLUID VOLUMES IN THE CONSCIOUS RAT. Stacey B. Provost* and Bryan J. Tucker. Univ. of Calif., San Diego, La Jolla, CA 92093.

Previous studies from our laboratory have examined the effects of 25° HDT for 7 days on renal function and extracellular fluid volume (ECF) in the rat but have not examined the changes in blood volume (BV), longer duration HDT, or post-tilt recovery (pHDT). Wistar rats (n=8) were chronically cannulated in the femoral artery and vein and the bladder. Measurements of mean arterial pressure (MAP, mm Hg), glomerular filtration rate (GFR, ml/min/100 g BW), renal plasma flow (RPF, ml/min/100 g BW), and ECF (% BW) were obtained twice prior to HDT and at days 1,3,7,10, and 14 HDT followed by measurements at 1 hr, and 1,3 and 7 days pHDT. BV was measured in separate groups of rats at 1,7 and 14d HDT and compared to non-tilt controls (n=4 in each group). Body weight remained constant during 14d HDT and increased during the pHDT period. * P<0.05 compared to pre-tilt values.

	Pre-tilt	1d HDT	3d HDT	14d HDT	7d pHDT
MAP	104±2	107±2	111±3*	113±2*	114±2*
GFR	0.82±.03	0.80±.07	0.96±.06*	0.85±.05	0.78±.05
RPF	3.1±0.1	3.1±0.2	3.6±0.2*	3.3±0.2	2.9±0.2
ECF	28±2	32±2*	30±2	28±2	30±2

BV increased slightly, but not significantly at 1d HDT and was significantly decreased at 7d and 14d HDT indicating a dissociation between ECF and BV by 7d HDT. The increase in ECF early in HDT most likely contributed to the elevation in GFR and RPF observed at 3d HDT. None of the fluid shifts correlated well with MAP, implying potential neuro-hormonal changes and altered volume homeostasis due to HDT.

25.4

INFLUENCE OF HEMATOLOGICAL PARAMETERS IN TEN-WEEKS TAIL-SUSPENSION RATS. M. NAKAYA, K. KOSUGI,* AND S. TAKEUCHI,* Space Med. Lab. and First Dept. of Anatomy*, The Jikei Univ. Sch. of Med., Tokyo 105, Japan

The influence of simulated micro-gravity on hematological parameters in rats were investigated. Thirty male rats (Wistar strain, average body weight 250g) were used, and divided into a control group and tail-suspension group. After ten weeks exposure, the rats were laparotomized under ether anesthesia and blood samples were obtained from the abdominal aorta. Hematological tests were performed thereafter immediately using Automatic Blood Cell Counter F-800(Toa Co.) and TDX analyzer(Dynabot Co.). No significant difference in red blood cell(RBC), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), and mean corpuscular hemoglobin concentration(MCHC) were found between the two groups. But significant lower mean values of hematocrit, hemoglobin, and transferrin were found in the tail-suspension group.

25.5

PARS DISTALIS VASCULATURE: DISCOVERY SHUTTLE STS-29 RATS COMPARED TO GROUND-BASED ANTIORTHOSTATIC RATS. A. Pattison*, T. Pattison* and J. Schechter* (SPON: A. Mircheff). Univ. of So. Cal., Los Angeles, California 90033

The anterior pituitaries (AP) of male, adult Long Evans rats carried 5 days in the Space Shuttle Discovery (STS-29) have been compared with two groups of ground-based controls. All animals were part of a study (SE82-08) into the effects of gravity versus a microgravity environment on fracture healing. All had sustained a right mid-shaft fibular osteomy. The duration of the study was 10 days, and animals in all groups were weight bearing for 5 days prior to shuttle lift off. The 3 experimental groups consisted of 4 rats each: Flight (F), and two ground-based control groups, weight bearing (WB) and suspended (S). The suspension group was in a Holton/Sweeney head-down suspension apparatus (antiorthostatic) for the final 5 days of the study. The AP of F and WB rats were essentially identical. The AP vasculature and parenchymal cells appeared unaffected in both instances. However, the AP of S rats were dramatically altered. The AP vasculature was widely expanded with proteinaceous deposition covering the lumenal endothelial surfaces, and entrapping numerous platelets and aggregates of red blood cells. Parenchymal cells were highly vacuolated, occasionally with membranous vacuoles, but most often revealing large clear cytoplasmic zones unlined by any membranes. Whereas profiles of exocytosis were numerous in F rats, and present in WB rats, they were essentially absent in S rats. These results indicate that weightlessness over a 5-day flight period does not influence the structural integrity of the AP and may, in fact, promote secretory granule release. However, the head-down tilt model, frequently used to study fracture repair under weightless conditions, has a profound impact on the AP vasculature which then affects the structural and functional characteristics of the AP parenchymal cells. (NIH-DK35904)

25.6

SINGLE HINDLIMB WEIGHT-BEARING BY THE RAT DURING SIMULATED MICROGRAVITY. Craig S. Stump and Charles M. Tipton. University of Arizona, Tucson, AZ 85721.

This study was designed to examine the relationship between non-weight-bearing per se and the systemic influences of simulated microgravity on rat hindlimb muscle. For this purpose, a single hindlimb suspension (SHS) model was developed in which rats (n=4-10) were suspended in a head down position (45°) with the left hindlimb non-weight-bearing (L) and the right bearing 20% of pre-suspension body mass (R). Weight-bearing by SHS-R was accomplished using a platform connected to a rod in sleeve, cable, and pulley apparatus to which weight could be added. Rats (250-325g) were assigned to SHS or CC conditions for 14 days. Knee-ankle-foot angles were determined on days 2, 6, and 12. On day 14, soleus (SOL), plantaris (PL), extensor digitorum longus (EDL) and gastrocnemius (G) masses and glycogen concentrations were measured. Joint angles for SHS-R and CC remained similar (20-30°) throughout the experiment while the SHS-L limbs extended to angles of ~150° by day 12. Significant atrophy of the SOL, PL and G muscles were observed in the SHS-L when compared to pre-suspension values. SHS-R prevented the atrophy of these muscles, but they did not reach the final muscle mass values of the CC. Separate groups of SHS and CC rats were surgically prepared and their hindlimbs perfused for 15 min with Krebs-Henseleit buffer containing aged-rejuvenated erythrocytes, 4% albumin, 1 mM 2-deoxyglucose, 0.5 mM mannitol, 0.15 mM pyruvate, 90 μU/ml insulin and either ^{3}H 2-deoxyglucose or ^{14}C mannitol tracer. Extracellular space (ml/g) was greater (16-68%) in the SOL, PL, and white G in both SHS-L and SHS-R limbs when compared to CC, reaching statistical significance ($p < 0.05$) for the SOL. 2-deoxyglucose uptake was also significantly higher in both SHS limbs compared to CC for the SOL (85-145%) and EDL (90%) muscles. However, only SOL 2-deoxyglucose uptake was greater (33%) for SHS-L compared to SHS-R. These results suggest that the increase in hindlimb muscle glucose uptake and extracellular space associated with simulated microgravity persists with hindlimb weight-bearing despite the prevention of muscle atrophy.
(Supported by NASA NAG 2-392 and NGT-50493).

25.7

NEUROHUMORAL RESPONSES TO ISOHEMIC HYPERVOLEMIA: A MODEL FOR WEIGHTLESSNESS. V.M. Chenault*, M. Morris, C.D. Lynch*, S.J. Maultsby*, P.M. Hutchins. Bowman Gray School of Medicine of Wake Forest University, Winston Salem, NC. 27157

Space flight induces a cephalad shift of fluid that prompts compensatory neurohumoral changes. This study was undertaken to explore these adaptation processes in response to acute isohemic blood volume expansion (30%) in conscious rats. Whole blood, drawn from a femoral artery catheter of conscious donor rats, was infused into the jugular vein of recipient rats. Blood samples were drawn from a carotid artery of recipient rats at 6 time points beginning immediately post volume expansion (IPVE) up through 24 hours post volume expansion (PVE). To characterize the attendant compensatory mechanisms, the plasma concentrations of electrolytes and fluid regulatory hormones were determined. Hematocrit began to rise IPVE and was significantly elevated above control IPVE through 24 hours PVE. Atrial natriuretic factor (ANF) was elevated 2.5 fold above the pre-volume expansion (Ctrl) values 0 to 30 minutes PVE. Volume expansion produced an unexpected increase in plasma aldosterone (ALDO) (294.3 ± 16.5 vs. 90.7 ± 14.3 pg/ml) at 30 minutes PVE, while plasma renin activity (PRA) was significantly decreased. Plasma sodium, potassium, osmolality and arginine vasopressin levels were not altered by the volume expansion except plasma sodium was significantly increased at 6 hours PVE. Thus, isohemic volume expansion produced compensatory endocrine changes in ANF, ALDO (both increased) and PRA (decreased) suggesting that in this paradigm there is dissociation of the conventional neurohumoral associations. The results of these studies are also consistent with the findings from simulated weightlessness (bedrest and head-down tilt) and space flight.

25.8

PREADAPTATION OF THE CIRCULATION BY REMOVAL OF DIFFERENT BLOOD VOLUMES TO COUNTERACT CENTRAL FLUID SHIFTS IN WEIGHTLESSNESS. K.E. Simanonok*, R. Srinivasan*, and J.B. Charles* (SPON: S.F. Fortney). NASA Johnson Space Center, Houston, TX 77058

Fluid shifts in weightlessness cause a central volume expansion, activating reflexes to reduce the blood volume (BV); the final adapted state of the circulation is a reduced BV at normal composition. Preadaptation of the BV prior to exposure to weightlessness may counteract the central volume expansion due to fluid shifts and thereby attenuate the circulatory and renal responses that result in large losses of fluid from body water compartments. This hypothesis was tested using computer simulation with a modified version of the Guyton Model of Fluid, Electrolyte, and Circulatory Regulation. After ten weeks of simulated five degree head down tilt the new equilibrium BV was 11% less than the starting volume. Simulated BV reductions of 5%, 11%, and 15% immediately before head down bedrest show that preadaptation of the BV is beneficial in damping the physiologic responses to fluid shifts and reducing body fluid losses. After several hours of head down tilt, preadaptation results in better fluid retention which persists for twenty to thirty days of bedrest, producing higher BV, extracellular volume, and total body water. Simulations suggest that the optimum preadaptation BV reduction to counteract fluid shifts is the amount lost in adapting to head down bedrest without countermeasures (11% in this study).

25.9

DEPENDENCE OF BLOOD VOLUME CHANGES ON INCREASED ORAL SODIUM UPTAKE

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We studied blood volume changes in six healthy men (mean age 23,8 ± 1.4 SEM; mean body weight (BW) 76.74 ± 3.76 kg SEM) who followed a diet consisting of an increasing sodium uptake and constant water intake covering a period of 24 days. The whole study was subdivided in three 8 day periods. Sodium intake was held constant at 2.8 mmol sodium/kg BW in the first phase, 5.6 mmol sodium/kg BW in the second phase and 8.4 mmol sodium/kg BW in the third phase. Urinary excretion of water, sodium and potassium were determined. Metabolic water and sodium balances were calculated as 24 hour balances. Blood volume, serum osmolality, serum sodium concentration, the biological half life of the erythrocytes and plasma levels of vasopressin, renin, aldosteron, and cortisol were measured on the fifth and seventh day during each phase after the dietary adaptation. Correspondingly to the higher sodium ingestion the urinary sodium excretion increased significantly whereas urine flow was almost unaffected. A linear increase of blood volume observed during the experiment resulted in a raise of 0.60 l ± 0.13 l (mean ± SEM) at the end of the third phase. Blood volume increase was accompanied by a decrease in plasma renin, aldosteron concentration, a tendency to an increase in plasma vasopressin and by decreased plasma renin-aldosteron levels. The metabolic sodium balances revealed a sodium storage which was not accompanied by positive metabolic water balances. Serum sodium concentration and serum osmolality did not change significantly. Determination of the biological half life of the erythrocytes showed a significant decrease of 7.5 days compared to the average value of 28 days.

25.10

INTRAMUSCULAR PRESSURE MEASUREMENT AS AN INDEX OF TORQUE DURING DYNAMIC EXERCISE. Richard E. Ballard, Michael Aratow, Albert Crenshaw, Jorma Styf, Norman Kahan, Donald E. Watenpaugh, and Alan R. Hargens. Life Science Division (239-11), NASA-Ames Research Center, Moffett Field, CA 94035-1000

Muscle atrophy is commonly experienced by astronauts in microgravity. Studies of muscle function in space require measurement of joint torque, yet volume and weight limitations prevent the use of existing devices to measure this variable. Electromyography (EMG) correlates with muscular contraction force in some instances, but this relationship can be highly variable with dynamic exercise. We hypothesized that intramuscular pressures (IMP) during isometric and dynamic exercise correlate better with joint torque than EMG. IMP and surface EMG activity were measured in the tibialis anterior and soleus muscles of twelve male volunteers during lower leg isometric and isokinetic exercise. Ankle torque was measured using the Lido Active System dynamometer and IMP was measured directly by the Myopress catheter. Tibialis anterior and soleus IMPs correlated directly with dorsiflexion and plantarflexion ankle torques, respectively, during both isometric (R^2 = 0.99, 0.99) and dynamic (R^2 = 0.85, 0.94) exercise. EMG signals in both muscles correlated well with joint torque during isometric contractions (R^2 = 0.92, 0.99), but during dynamic exercise, tibialis anterior EMG correlated less closely with torque (R^2 = 0.71), and soleus EMG did not correlate. Although more invasive than EMG, IMP provides a more reliable and direct index of torque for specific muscles during dynamic exercise. Thus, the IMP technique will aid development of exercise protocols and flight hardware for astronauts (Supported by NASA).

25.11

PARABOLIC FLIGHT PROFILE DETERMINES THE EFFECTS OF MICRO-GRAVITY ON THE CARDIOVASCULAR SYSTEM. John M.Karemaker and Rick D.Latham. Univ.of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands and Armstrong Lab., Brooks AFB, Tx 78235-500.

Parabolic flight is a frequently used means of obtaining short-lasting microgravity for experiments in human physiology. While studying its effect on the cardiovascular system we compared two different types of parabolic flight. The single parabola profile (SPP) entails steady horizontal flight (1G-condition), pull-up for extra thrust (up till 2G), suddenly changing into micro-gravity (typically 25 seconds of 0G), followed by pull-out with extra motor power to brake the high speed of the parabola's down-slope (up till 2G again), returning to 1G level flight (all G-levels refer to the head-foot direction in the upright position). This protocol is repeated after a period of steady level flight. The alternative method is the so-called roller-coaster profile (RCP) where pull-out and pull-up are flown in continuity, ideally resulting in 2G-0G-2G-0G etc. sequences in which the micro-gravity takes up one third of one entire period of 75 seconds. This profile generates responses of the human cardiovascular system different from those by the SPP where the period of horizontal flight will allow the autonomic system to return to baseline levels. In the upright position the 2G-periods constitute a strong sympathetic stimulus, the 0G-periods induce a vagal reflex to the heart and suppress sympathetic outflow. This pattern has been shown in earlier experiments. As a striking difference to these earlier observations we noticed in recent RCP experiments an unpredictable 'onset'-response to the first few parabolas, only then to be followed by a pattern that can be compared to the one observed in SPP, however, lacking the intermediate return to baseline levels. We conclude that the interpretation of physiological responses to micro-gravity in RCP can only be made with utmost caution and should, preferably, be compared to SPP responses.

25.12

ECHOCARDIOGRAPHIC EVALUATION OF CARDIAC FUNCTION DURING PARABOLIC FLIGHT. J.P. Johns,* M.N. Vernalis,* C.D. White,* J.M. Karemaker,* and R.D. Latham. Brooke Army Med. Ctr., Ft. Sam Houston, TX, Walter Reed Army Med. Ctr., Washington, D.C., Univ. of Amsterdam, The Netherlands, and The U.S. Army Aeromedical Research Lab, Ft. Rucker, AL 30362

We have undertaken a noninvasive analysis of cardiac function in normal man during transient microgravity induced by parabolic flight. The subjects were not medicated prior to flight and had normal cardiac anatomy on screening echocardiograms prior to enrollment. NASA JSC's KC-135A was used to perform parabolic flight profiles in 4 sets of 10 parabolas with 15-30 sec of 1G baseline between each parabola (4 flights, n=8) or sets of 10 parabolas without 1G baselines (1 flight, n=4). Echocardiograms and Doppler velocity recordings were obtained with an Acuson model 128/XP10 echocardiograph. Pulsed Doppler recordings of right and left ventricular (LV) inflow and outflow velocities and carotid artery diameter and velocity were performed. Finapres™ was used for continuous digital blood pressure recording. Respiration, ECG, and head-to-foot gravity (Gz) were also recorded simultaneously. Imaging quality was good, and recordings of LV volumes suggested an increase in LV dimensions during rapid ascent followed by a decrease in dimensions with entry into microgravity. Carotid artery velocities tended to decrease or remain unchanged in microgravity. We conclude that sophisticated noninvasive evaluations of cardiac and vascular function are feasible during parabolic flight profiles using 1G baselines between parabolas to restore control levels between periods of microgravity.

25.13

DATA ACQUISITION SYSTEM FOR THE ARTIFICIAL GRAVITY SIMULATOR (AGS). David Cardus, WG McTaggart
Baylor College of Medicine, Houston, TX 77030

Tha data acquisition system for the Artificial Gravity Simulator (AGS) is designed to meet requirements for safety and physiological monitoring of subjects submitted to variable acceleration stresses of the apparatus. In its present state of development, the data acquisition system allows the continuous recordings of the electrocardiogram, blood pressure, heart rate, stroke volume, cardiac output, thoracic body fluid index and sleep parameters (EEG, EOG and EMG). Data are collected sequentially from each of the four beds of the AGS through computer controlled multiplexing switches, using one set of instruments only. Blood pressure and cardiac data are read with instruments equipped with RS232 ports. The digital information generated by these instruments is processed on line with a PC computer. Custom software developed with ASYST allows for collection, tabular and graphical display, and storage of the desired physiological data. Analog signals for the ECG, EEG, EOG and EMG are transmitted by telemetry, converted to digital and processed by the computer for analysis and display.

RENIN-ANGIOTENSIN SYSTEM

26.1

Chronic Effects of Angiotensin on Atrial Natriuretic Peptide Secretion During Controlled Increments in Right Atrial Pressure. T.E. Lohmeier, Y. Shin, R.L. Hester, and G.A. Reinhart. Univ. Miss. Med. Ctr., Jackson, MS. 39216

It has been reported that angiotensin (ANG II) has a direct stimulatory effect on the secretion of atrial natriuretic peptide (ANP). To determine whether high physiological levels of ANG II increase ANP secretion independent of effects on atrial pressure, right atrial pressure (RAP) was continuously servocontrolled at 5.4±0.1 mmHg above control (1.5±0.2 mmHg) for 8 days in 5 dogs with an externally adjustable occluder implanted around the pulmonary artery; additionally, the dogs were infused with Captopril and plasma ANG II concentration was fixed at normal or high levels (↑ ANG II) by infusion of ANG II at 0.6 (days 1-3 and 7-8) or 5.0 ng/kg/min (days 4-6), respectively. During ↑ ANG II, partial deflation of the occluder prevented increments in RAP. Results for RAP, ANP, and mean arterial pressure (MAP) were:

	RAP (mmHg)	ANP (pg/ml)	MAP (mmHg)
Control	1.5±0.2	88±15	100±4
DAY 3	6.9±0.1*	453±86*	79±3*
DAY 6 (↑ ANG II)	6.8±0.1*	465±86*	131±4*+
DAY 8	6.9±0.1*	478±84*	86±2*+

*p<0.05 vs. control +P<0.05 vs. Day 3.

These findings indicate that the increments in ANG II achieved in heart failure do not stimulate ANP secretion in the absence of elevations in atrial pressure. (HL 11678).

26.2

DIFFERENTIAL ANG II RESPONSE BY THE PITUITARY GLAND DURING LOW AND HIGH SODIUM INTAKE IN RAT. K. Qian*, S.M. Galli*, G. Aguilera* and M.I. Phillips, Dept. of Physiology, College of Medicine, Univ. of Florida, Gainesville, FL 32610 and NIH, Bethesda, MD.

We have recently shown in rat that sodium diet modulates the amount of Ang II content in the hypothalamus (HTS) and whole pituitary but not in brainstem (BRS) (S.M. Galli et al., FASEB Vol. 5, 7949, 1991). The aim of the present study was to investigate which of the pituitary lobes is involved in the Ang II response to 7 days of low and high sodium intake and also its correlation with the HTS Ang II response. A control group of 6 rats was set up with subcutaneous Ang II osmotic pumps for 7 days to artificially simulate the increments of plasma Ang II. Ang II content of the anterior lobe (AL), intermedia lobe (IL) and neural lobe (NL) were determined by RIA. The Ang II values, expressed in pg/g tissue, are the average of n=9 ± SE.

	Control normal sodium	↓ Na^+ diet	↑ Na^+ diet	+ Ang II osmotic pump
AL	444.5 ± 89.4	633.6 ± 25.8	45.7 ± 28.5	330.8
NL	374.7 ± 105.9	997.5 ± 148.4	142.6 ± 1.7	634.5

Compared to the normal sodium group, low Na^+ intake increases Ang II content in the NL by 166.2% while the AL Ang II increase is only 42.3%. Sodium load decreases Ang II content to the same extent in both pituitary lobes (AL=67.2% and in NL=62%). The artificial increase in plasma Ang II by the Ang II osmotic pump does not alter AL Ang II content. Although Ang II in the NL increases in this group, the levels are still lower than those found in the low Na^+ group. The activation of the hypothalamic Ang system during sodium depletion may account for the pronounced increase of Ang II content by the NL of the pituitary gland.

26.3

DIFFERENCES IN ANGIOTENSIN-II-INDUCED DRINKING IN SALT-SENSITIVE (SHR-S) AND SALT-RESISTANT (SHR-R) SHR. M.I. Meldrum* and P. Moran* (Spon: M.J. Katovich) Dept of Pharmacodynamics, Univ of Florida, Gainesville, FL 32610

Increased dietary salt intake exacerbates blood pressure in SHR-S (Taconic Farms) while similar increases in salt intake have no effects on blood pressure in SHR-R (Charles Rivers). Angiotensin II induced responses are also known to be modulated by the addition or removal of dietary salt. We therefore examined angiotensin II induced drinking as a measure of the angiotensin system in SHR-S and SHR-R on basal and high salt diets. Animals were placed in individual cages and allowed two hours to acclimatize. Each cage had a 100 ml graduated spill-proof water bottle (Bio-Serve Inc) with the water temperature at 25±1°C. Intake was measured by the volume differences at both 30 and 60 min after I.P. injections. Isotonic saline (1 ml/kg), angiotensin II (Ang II) (200 μg/kg), isoproterenol (20 μg/kg) and 24 hour water deprivation were all used to induce drinking. SHR-S and SHR-R animals showed no differences in water intake after either saline or isoproterenol injections or 24 hour water deprivation; however, SHR-S drank significantly more after Ang II injections than did SHR-R. Placement of SHR-S and SHR-R rats on high salt (3.17% NaCl) diets for 7 days or chronic (5 day) injections of Ang II attenuated the difference in Ang II induced drinking by increasing the drinking in SHR-R to the levels seen in SHR-S. The drinking response to isoproterenol was not altered by high salt treatment in either SHR-S or SHR-R. These differences in angiotensin II responses may reflect differences in the angiotensin system between the SHR-R and SHR-S which may affect blood-pressure regulation. (Supported by American Heart Association, Florida Affiliate)

26.4

THE EFFECT OF DIRECT NEURAL STIMULATION ON RENIN SECRETION IN YOUNG SHR. Jennie Mangun and James P. Porter. Dept. of Physiology, Univ. of Louisville, Louisville, KY 40292.

Previous studies have shown that the canine kidney responds to direct renal nerve stimulation in a frequency dependent manner: renin secretion rate (RSR) reaches threshold first, while sodium excretion (UNaV) and renal blood flow (RBF) changes require greater increases in the frequency of stimulation. Neurally mediated RSR has been implicated in the hypertension of the SHR, yet the effect of direct neural stimulation has not been characterized. In the present investigation, the splanchnic nerve of the SHR was stimulated at frequencies of .125, .250, .50, and 1.0 Hz. to determine the threshold for changes in RSR, UNaV, and RBF. In initial experiments, the threshold for changes in RSR, only, were determined in rats instrumented with catheters in a femoral artery and the left renal vein, and a Doppler flow probe on the left renal artery. In subsequent experiments, the threshold for changes in UNaV and RBF were determined. In addition to the Doppler flow probe, these animals were instrumented with a ureter catheter. For RSR and UNaV, the threshold was defined as the first frequency where a statistically significant change could be detected. For RBF, the threshold was determined as the first frequency where a decrease could be visually detected.

	Δ CONTROL			
	.125	.250	.500	1.00 Hz
RSR	27.4±28.1	102.9±26.7*	71.5±27.4*	34.6±12.9
UNaV	-24.7±25.1	-24.7±87.6	-110±66.7	-110.4±48.5*
RBF				-.46±.07

These results indicate that in the SHR, the threshold for neurally mediated RSR occurs at a lower frequency than for UNaV or RBF. Supported by AHA, Kentucky Affiliate.

26.5

VASOPRESSIN AND RENIN RESPONSES TO GRADED REDUCTIONS IN ATRIAL PRESSURE IN CONSCIOUS DOGS. C.P. O'Donnell, C.J. Thompson, L.C. Keil, and T.N. Thrasher. Dept. of Physiology, University of California, San Francisco, CA 94143 and NASA, Ames Research Center, Moffatt Field, CA 94035.

It is generally assumed that unloading atrial receptors is a sufficient stimulus to cause a reflex increase in the secretion of arginine vasopressin (AVP) and renin. To test this hypothesis, venous return was decreased gradually by graded constriction of the thoracic inferior vena cava in conscious dogs (n=5). Left atrial pressure (LAP) was reduced in four steps without reducing mean arterial pressure (MAP) and a fifth step in which both LAP and MAP were reduced. Decreasing venous return sufficient to reduce LAP by 4.0 ± 0.7 mmHg did not change MAP (from 87 ± 6 to 84 ± 5 mmHg, NS) but stimulated a threefold rise in plasma renin activity (from 0.34 ± 0.10 to 0.97 ± 0.23 ng ANG I/ml/3h, P<0.05). This decrease in LAP was not associated with a change in either heart rate (from 68 ± 7 to 69 ± 11 beats/min, NS) or plasma AVP (from 0.9 ± 0.1 to 1.1 ± 0.1 pg/ml, NS). Plasma renin activity continued to increase as LAP fell but plasma AVP did not increase significantly until there was a decline in MAP, at which time AVP increased to 64 ± 50 pg/ml (P<0.05). We conclude that under the conditions of this study, a decrease in venous return sufficient to lower LAP by 4 mmHg but not change MAP, is a sufficient stimulus to increase renin secretion but not secretion of AVP. (Supported by HL 41313)

26.6

ANGIOTENSIN-LIKE IMMUNOREACTIVITY AND CATECHOLAMINE RESPONSE TO CAPTOPRIL IN THE NURSE SHARK. Sara M. Galli* and K. Qian* (SPON: M.I. Phillips). Department of Physiology, College of Medicine, University of Florida, Gainesville, FL 32610.

We have previously discovered the presence of Ang II-like immunoreactivity (i.r.) in plasma and tissues of the Nurse shark. This elasmobranch species was found to respond to hemorrhage and sodium depletion by increasing plasma Ang II i.r. The next step was to study the effect of the converting enzyme blocking agent Captopril (CAP) on plasma i.r. for Ang I and Ang II. Epinephrine (E) and norepinephrine (NE) were also measured. The sharks (male, b.w. 6-8 lb) were anesthetized by immersion in MS222 (0.1-0.01%) for 2 hrs during which CAP was administered (80 mg/kg i.m.) and blood samples taken by caudal aorta puncture after 20 min, 1 hr and 2 hr. The sharks were active and fully recovered after 15 min. The results show that in control samples from six sharks the levels of Ang I i.r. (103.35±15.4 pg/ml) are higher than Ang II i.r. levels (21.73±4.3 pg/ml) and that NE levels (31.0 ng/ml) are 10 times higher than E levels (3.0 ng/ml). After CAP administration the levels of Ang II i.r. compared to controls did not change significantly. However, Ang I i.r. levels increased after 20 min (31.0%), 1 hr (36.4%) and at 2 hrs the levels still remained high (41.8%). During CAP the levels of E and NE did not change.

The increased plasma Ang I i.r. under CAP suggest that in the shark's angiotensin system a converting enzyme is present, and that plasma catecholamines do not influence this response.

NEURAL CONTROL OF CIRCULATION

27.1

INFLUENCE OF POSTURE ON CAROTID BARORECEPTOR CARDIAC-VAGAL REFLEX RESPONSES IN HUMANS. Janice M. Fritsch*, Michael L. Smith, Dwain L. Eckberg. VA Medical Center Richmond, Virginia 23249.

The influence of body position on arterial baroreflexes has been the subject of many studies, but with disparate results. This may be due in part to differences in technique. We defined carotid baroreceptor cardiac-vagal response relations in 10 healthy subjects while supine, sitting and standing. R-R intervals and blood pressures were measured during sequential, R-wave triggered, 15 mmHg steps of neck pressure and suction (encompassing threshold and saturation regions). R-R intervals were plotted against carotid distending pressure (systolic - neck pressure) at each step. The slope of the response did not change [5.1±0.8 ms/mmHg supine, 5.1±0.8 sitting, and 3.9±1.4 standing (p=NS)], nor did range of response [219±23 ms, 228±30, and 177±48 ms, respectively, (p=NS)]. However, position of the operational point (a measure of hypotensive vs hypertensive buffering capacity) decreased toward threshold from 33±7% supine to 19±5% sitting (p<0.05) and 13±3% standing (p<0.05). **Conclusions:** Upright posture 1) does not affect carotid baroreceptor response slope or range but 2) shifts the point of operation closer to threshold. Thus, knowledge of threshold and saturation regions and operational position are important for correct assessment and interpretation of baroreflex function.

27.2

NALOXONE DOES NOT PREVENT VASOVAGAL SYNCOPE DURING SIMULATED ORTHOSTASIS IN HUMANS. Michael L. Smith, Mark D. Carlson*, Helen M. Sheehan*, Marc D. Thames. Dept. of Medicine, University Hospitals of Cleveland, Case Western Reserve University, and VA Med Ctr, Cleveland, Ohio 44106.

Hemorrhagic hypotension leads to paradoxic bradycardia and sympathoinhibition in conscious rabbits which is prevented by administration of naloxone. In humans, orthostasis produces a similar paradoxic effect for which the mechanism is unknown. We hypothesized that endogenous opiates may be important in the mediation of vasovagal syncope. Heart rate (HR), blood pressure (BP) and forearm blood flow (BF) were recorded in 6 subjects during graded lower body negative pressure (LBNP) to pre-syncope (PS; rapid onset bradycardia and hypotension) before and after a 30 minute rest period to determine the reproducibility of LBNP. In 4 additional subjects, i.v. naloxone (0.1 mg/kg) was given at the end of the rest period to determine the role of endogenous opiates in vasovagal syncope. **Results:** The responses to LBNP were reproducible: HR, BP and BF changes at each level of LBNP were similar (p>0.05), and the cumulative stress achieved at PS (LBNP level x time [min]) was not different between the two tests (-749±35 vs. -721±41 mmHg x min; p=0.47). Naloxone did not alter the cumulative stress achieved at PS (-672±47 vs. -604±70 for saline and naloxone, respectively; p=0.33). Moreover, vasovagal-like responses (>30 mmHg fall in BP and >20 bpm fall in HR during final 30 sec of stress) occurred despite naloxone pre-treatment. **Conclusion:** Endogenous opiates do not appear to contribute importantly to the vasovagal-like responses elicited by simulated orthostasis in humans.

27.3

DIURNAL VARIATION OF CALCITONIN GENE-RELATED PEPTIDE AND BLOOD PRESSURE LEVELS IN NORMAL MAN. Ettore degli Uberti,* Giorgio Trasforini,* Francesco Portaluppi,* Angelo Margutti,* Maria Rosaria Ambrosio,* Roberta Rossi* and Luciana Vergnani* (SPON: E. Roti). University of Ferrara, Ferrara, Italy I-44100

Calcitonin gene-related peptide (CGRP) is the most effective physiological vasodilator, yet virtually nothing is known of its normal circadian variation and its relation with the diurnal blood pressure (BP) pattern. We have assessed the daily pattern of CGRP in eight recumbent healthy volunteers after careful standardization of daily diet and routine schedules, using a radioimmunoassay after extraction by immunoaffinity chromatography. Noninvasive monitoring of BP was also performed with an oscillometric device. A diurnal pattern was apparent for CGRP and also for BP levels in each subject. The peak value of CGRP plasma concentration (36±3 pmol/L) occurred at midnight simultaneously with the lowest BP levels, while the lowest CGRP concentration (23±1 pmol/L) occurred at noon during the high portion of the daily BP curve. Our findings demonstrate that CGRP circulates in plasma of normal man with a circadian rhythm antiphasic to the BP rhythm. These observations suggest a physiological role for CGRP in BP regulation.

27.4

CAROTID BAROREFLEX FUNCTION DURING LOW INTENSITY DYNAMIC EXERCISE IN MAN. J.T. Potts, X.R. Shi *, J.W. Williamson, C.G. Crandall and P.B. Raven. Dept. of Physiology, Texas College of Osteopathic Medicine, Fort Worth, TX. 76107

We utilized rapid changes in neck-pressure / neck suction (+40 to -65 torr) to alter carotid sinus transmural pressure (CSTP) in six volunteer men during upright rest, 0, 50, and 75 watts steady state exercise. Beat-to-beat changes of interbeat interval (RRI) and blood pressure (MAP) were recorded using a cardiotachograph and a finger plethysmograph (Finapres), respectively. RRI progressively decreased from rest to 75 watts and was significantly decreased from rest at 50 and 75 watts. The calculated RRI threshold for the carotid-cardiac reflex progressively increased to higher estimated CSTP during 50 and 75 watts ($p<0.01$). Simlarily, the calculated MAP threshold of the carotid-vascular reflex was negatively correlated with the mean steady-state exercise RRI ($r=-0.97$, $p<0.01$). These data indicate a resetting of the carotid sinus baroreflex which was directly related to the intensity of the exercise. However, as the MAP at 0, 50, and 75 watts was below the carotid-cardiac and carotid-vascular threshold CSTP, we conclude that the carotid baroreflex was not regulating exercise blood pressure.

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27.5

DISSOCIATION OF THE CUTANEOUS ACTIVE VASODILATOR AND SUDOMOTOR SYSTEMS DURING EXERCISE. D.L. Kellogg, Jr.*, J.M. Johnson, W.A. Kosiba. Department of Physiology and *Geriatric Research, Education, and Clinical Ctr., Univ. Texas Health Science Center, San Antonio, TX 78284.

A causal link between the sudomotor system and cutaneous active vasodilation has been postulated. However, exercise induces shifts in the internal temperature threshold at which cutaneous vasodilation begins, but does not shift the threshold for sweating. These findings imply separate mechanisms for these systems. We hypothesized that the relationship between the active vasodilator and sudomotor systems can be changed by exercise. Seven male subjects each had 2 forearm sites iontophoretically treated with bretylium tosylate for local blockade of vasoconstrictor nerves in skin to expose unambiguously active vasodilator system behavior. Skin blood flow was monitored there and at untreated control sites. Sweat rate (SR) was measured from the opposite forearm by dew point hygrometry. Subjects underwent cold stress to verify vasoconstrictor blockade followed by hyperthermia (skin temperature = 38°C) at rest or with supine bicycle exercise. Exercise induced a shift of +0.35±0.08°C ($p<0.05$) in the threshold for active vasodilation, but did not alter the SR threshold ($p>0.05$). These findings suggest separate mechanisms for the active vasodilator and sudomotor systems rather than the traditional concept of a causal series relationship between them. (Supported by HL20663).

27.6

AUTONOMIC MECHANISMS OF MUSCLE METABOREFLEX CONTROL OF HEART RATE DURING POST-EXERCISE MUSCLE ISCHEMIA IN CONSCIOUS DOGS. D.S. O'Leary, Dept. of Physiology, Wayne State University School of Medicine, Detroit, MI. 48201.

When blood flow to active skeletal muscle is inadequate for metabolic demands, metabolites accumulate inducing a reflex increase in sympathetic activity (muscle metaboreflex). Several studies have shown that when ischemia of active muscle is maintained during the recovery from exercise, systemic arterial pressure (SAP) remains elevated however, heart rate (HR) decreases normally. Is muscle metaboreflex-induced sympatho-activation to the heart maintained during post-exercise muscle ischemia? Muscle metaboreflex was stimulated during mild treadmill exercise (3.2 KPM, 0% grade) by reducing hindlimb blood flow by ~ 50% which induced significant increases in SAP (Δ 53.1 ± 9.5 mmHg) and HR (Δ 24.6 ± 5.3 bpm) from the control values during exercise of 112.4 ± 6.4 mmHg and 118.0 ± 5.5 bpm. When the treadmill was abruptly stopped but the occlusion maintained for 1 minute, SAP remained elevated ($p > 0.2$ vs. pressor response during exercise) however, HR decreased markedly from 142.6 ± 4.4 to 119.3 ± 4.6 bpm ($p < 0.003$). After treatment with atropine similar increases in SAP (Δ 60.7 ± 10.4 mmHg) and HR (Δ 28.7 ± 6.0 bpm) occurred with muscle metaboreflex activation during exercise however, the reflex increases in SAP and HR were both sustained during post-exercise muscle ischemia ($p > 0.2$ vs. pressor responses during exercise). Conclusions are that parasympathetic activity rises at the cessation of exercise and obscures the tachycardia due to the sustained sympatho-activation during post-exercise muscle ischemia.
(Supported by AHA of MI 9012G10 and NIH HL45038)

27.7

PRESSURE-DISCHARGE CHARACTERISTICS AND RAPID RESETTING OF AUTOACTIVE BARORECEPTORS. Paul A. Munch. Univ. of Calif., Davis 95616

Previous studies identified baroreceptors (BRs) that discharge spontaneously below pressure threshold (P_{th}). These "autoactive" units (aBRs) contrast to more familiar BRs that are "quiescent" below P_{th} (qBRs). Seagard et al (Circ Res 66:1499-1509, 1990) found that carotid aBRs discharged slower and were less sensitive to pressure than qBRs, but operated over wider ranges of pressure. They proposed that aBRs may signal primarily the mean arterial pressure (MAP), whereas qBRs may signal mainly dynamic fluctuations. If so, aBRs should not rapidly reset to sustained changes in MAP, since their afferent signal would then be in error. Here I examined whether similar aBRs exist in the aortic arch and if they rapidly reset. Using an *in vitro* aortic arch/aortic nerve preparation, I constructed ramp-evoked pressure-discharge curves of 118 single-fiber BRs (64 qBRs, 54 aBRs). In 17 aBRs, I also compared individual curves 15 min after adjusting MAP to selected levels between 40 and 180 mmHg to test for resetting (defined as parallel curve shift along pressure axis). In general, compared to qBRs, aBRs in the aortic arch had significantly lower P_{ths} (mean±SE, 83±2 vs 91±2 mmHg) but similar threshold frequencies (13±1 vs 16±2 Hz), higher saturation pressures (138±4 vs 123±2 mmHg) but similar saturation frequencies (55±3 vs 55±3 Hz), and lower suprathreshold pressure sensitivities (slope of linear region: 1.1±0.1 vs 1.4±0.1 Hz/mmHg) but wider operating ranges (57±4 vs 35±3 mmHg). The aBRs also reset rapidly to changes in MAP (n=16), and the extent of their resetting ($\Delta P_{th}/\Delta MAP = 0.26$) was similar to that reported earlier for qBRs (0.23; Am J Physiol 244:H672-680, 1983). In conclusion, many aortic BRs are autoactive and exhibit discharge characteristics similar to carotid aBRs. However, unlike carotid aBRs, aortic aBRs fire at rates similar to aortic qBRs and both types reset to the same extent. Thus, neither group of aortic BRs seems better equipped to signal the absolute MAP. Supported by HL-44675.

27.8

NEURONAL RELEASE OF ATP MEDIATES ACTIVE THERMOREGULATORY VASODILATION IN THE CONSCIOUS RABBIT. W. Fred Taylor* and Vernon S. Bishop. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

We have shown previously that reflex increases in rabbit ear blood flow (EBF) in response to whole body heating (WBH) are mediated through an active neurogenic vasodilator system and requires endothelium derived relaxing factor (EDRF). It is also known that ATP induced endothelium dependent relaxations in the rabbit ear are antagonized by 8-phenyltheophylline (8-PT). This investigation tested the hypothesis that active thermoregulatory vasodilation of the rabbit ear during heat stress is an endothelium dependent process and occurs via the release of ATP from sympathetic perivascular nerves. Rabbits were chronically instrumented for the measurement of mean arterial pressure (MAP), heart rate (HR) and EBF. A catheter was placed in the lingual artery on the side of EBF measurement to allow local infusion of 8-PT into that ear. WBH was achieved by circulating warm water through a rubber pad placed around the rabbit. Internal temperature was monitored with a rectal thermocouple. When EBF was maximal during whole body heating, 8-PT infusion into the ear via the lingual artery catheter was begun (10^{-4} M, 0.5 ml/min) and continued during maintained heat stress. EBF was 0.12±0.03 kHz during control prior to WBH (C), 7.09±0.86 kHz during heat stress (HS) and 0.34±0.09 kHz after infusion of 8-PT (PTHS). On the average it took 52 minutes for 8-PT to reverse the thermoregulatory vasodilation. There were no changes in HR or MAP during any period. These results show that 8-PT abolished the endothelium-dependent thermoregulatory vasodilation and suggest that ATP is the neurotransmitter required for active neurogenic vasodilation. (Supported by NIH Grants HL12415 and HL07350)

27.9

ANGIOTENSIN II MODULATES THE ARTERIAL BAROREFLEX FUNCTION MEDIATED VIA THE CENTRAL α-1 ADRENOCEPTOR MECHANISMS. Yasuhiro Nishida* and Vernon S. Bishop. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764

To test the hypothesis that angiotensin II (ANG II) modulates the arterial baroreflex functions mediated via the central α-1 adrenoceptor mechanism, we examined the effects of vertebral arterial infusion of ANG II on the baroreflex function curves and the effects of vertebral arterial injection of α-1 adrenergic receptor antagonist, prazosin, on the ANG II-induced modulation of the baroreflex function. Rabbits were chronically instrumented with femoral and vertebral arterial catheters, a venous catheter and a caval occluder. Baroreflex curves were obtained by relating heart rate (HR) to mean arterial pressure (MAP) during ramp increases and decreases in MAP with phenylephrine and caval occlusion, respectively. Intra-vertebral infusions of ANG II (5, 10 and 20 ng/kg/min) produced dose-dependent increases in the maximum HR (272±2 to 277±4, 297±15, 321±27 bpm, respectively) at minimum MAP and in the minimum HR (163±13 to 169±8, 187±8, 205±13 bpm, respectively) at maximum MAP, shifted the mid range of the curve (53±2 to 57±2, 69±5, 76±5, respectively), and suppressed the gain (6.5±0.5 to 5.4±0.9, 4.8±1.4, 4.5±1.1, respectively). Pre-treatment with prazosin (10 μg/kg) via the vertebral artery abolished the ANG II (10 ng/kg/min)-induced shift in the baroreflex curve (maximum HR: 268 ± 7, minimum HR: 173±7, mid range: 54±2) but did not restore gain (4.4±0.7) to normal values. These data suggest that ANG II resets the operating point of the baroreflex curve to the right and that this effect is mediated via central α-1 mechanisms. (Supported by NIH Grants HL36080 and HL12415)

27.10

NALOXONE BLOCKS CARDIAC AFFERENT INHIBITION OF ACTIVE NEUROGENIC VASODILATION IN THE RABBIT EAR. **H.L. Collins* and S.E. DiCarlo. Department of Physiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.**

Endogenous opiates are reported to mediate cardiopulmonary baroreflex sympathoinhibition. The purpose of this study was to test the hypothesis that cardiac afferents inhibit active neurogenic vasodilation in the rabbit ear via an endogenous opiate mechanism. Rabbits (n=5) were chronically instrumented with a Doppler ultrasonic flow probe around the central ear artery. Catheters were positioned in the pericardial space, femoral artery and vein. Activation of cardiac afferents (intrapericardial nicotine 1 and 4 μg/kg) decreased central ear artery blood flow velocity (EBF), heart rate (HR) and blood pressure (MAP) and increased central ear artery resistance (ER). Naloxone (4 mg/kg bolus, followed with a continous infusion 0.12 mg/kg/min i.v.) prevented cardiac afferent inhibition of EBF. Table presents means ± SE

	HR (bpm)	MAP (mmHg)	EBF (kHz)	ER (mmHg/kHz)
1μg/kg	-13.3±1.8	-2.2±1.3	-2.9±0.2	+11.7±2.5
4μg/kg	-25.0±2.2	-4.6±0.9	-4.0±0.7	+17.6±4.7
Naloxone				
4μg/kg	-5.0±1.6	-2.0±0.9	-0.4±0.3	+0.4±0.5

Decreasing MAP with nitroglycerin did not significantly decrease EBF. These results demonstrate that cardiac afferents inhibit active neurogenic vasodilation through an endogenous opiate mechanism. (Supported by NIH-45245)

27.11

CARDIAC RECEPTORS DO NOT TONICALLY INHIBIT VASOPRESSIN SECRETION IN LATE-GESTATION FETAL SHEEP. Hong-Gen Chen* and Charles E. Wood. Department of Physiology, University of Florida College of Medicine, Gainesville, FL 32605.

In late-gestation fetal sheep, hemorrhage stimulates increases in arginine vasopressin (AVP). The AVP response to hemorrhage is not mediated by receptors with vagal afferent fibers, by arterial baroreceptors, or by arterial chemoreceptors. However, it is possible that AVP is controlled by cardiac receptors with non-vagal afferent fibers. This study was designed to test the hypothesis that AVP is tonically inhibited by cardiac receptors. We studied 8 fetal sheep between 122 and 134 days' gestation. Fetal sheep were chronically prepared with vascular, amniotic, and pericardial catheters. Fetuses were subjected to intrapericardial injection of saline (n=6) or of 4% procaine (n=6). The procaine injection blocked both afferent and efferent fibers from and to the heart. For two hours starting immediately after the injection, arterial, venous, and amniotic pressures were measured using a microcomputer on-line, blood gases using a blood gas analyzer, and vasopressin by radioimmunoassay. Neither saline nor procaine injection significantly altered arterial pressure (initial values: 42±2 and 41±1 mm Hg in the saline and procaine injected groups, respectively), plasma AVP concentration (2.2±0.3 and 3.0±0.9 pg/ml), arterial pH (7.39±.01 and 7.37±.02), PCO_2 (41.3±0.6 and 40.2±0.6 mm Hg), sodium concentration (145.4±1.2 and 145.1±0.9 mEq/L) or potassium concentration (3.62±0.20 and 3.68±0.15 mEq/L). Procaine did not alter arterial blood pressure, but did inhibit the normal variability in fetal heart rate. After both saline and procaine injection arterial PO_2 increased significantly but slightly (from 26.8±0.4 to 28.9±0.8 and from 24.7±1.4 to 25.8±2.5 mm Hg in the saline- and procaine-injected groups) and hematocrit decreased significantly (from 28±2 to 26±1 and from 28±1 to 26±1 %). We conclude that pharmacologic blockade of cardiac receptors does not stimulate AVP secretion. Therefore, cardiac receptors do not tonically inhibit AVP secretion in late-gestation fetal sheep. (Supported by a Grant-in-Aid and an Established Investigator Award from the American Heart Association to CEW.)

27.12

ARTERIAL BARORECEPTORS DO NOT MODULATE THE VASOPRESSIN RESPONSE TO HYPOXIA IN LATE-GESTATION FETAL SHEEP. Charles E. Wood and Hershel Raff. Department of Physiology, University of Florida College of Medicine, Gainesville, FL 32605.

Hypoxia stimulates reflex increases in fetal arterial blood pressure and in the secretion of various hormones which influence fetal cardiovascular function. It is possible that the increase in blood pressure during hypoxia partially inhibits the vasopressin response to hypoxia by stimulation of arterial baroreceptors. The present study was designed to test this hypothesis. Chronically catheterized fetal sheep between 115 and 127 and between 130 and 136 days' gestation (n=7 per group) were subjected to a 30-min period of isocapnic hypoxia (produced by allowing the pregnant ewe to breathe an hypoxic gas mixture). Each fetus was subjected to two experiments separated by at least 2 days. In one experiment, arterial blood pressure was controlled by intravenous infusion of sodium nitroprusside during the period of hypoxia, and in the other the arterial blood pressure was allowed to increase. Arterial PO_2 decreased from 24.8±1.2 to 13.9±0.8 mm Hg (pressure uncontrolled) and from 24.3±0.8 to 15.6±1.2 mm Hg (pressure controlled) in the younger fetuses and from 21.9±0.7 to 12.9±0.9 mm Hg (pressure uncontrolled) and from 21.2±2.8 to 12.6±0.8 mm Hg (pressure controlled) in the older fetuses. AVP increased in response to hypoxia from 1.9±0.8 to 74±60 (uncontrolled) and from 1.7±0.3 to 213±129 pg/ml (controlled) in the younger fetuses and from 6.1±1.1 to 296±90 (uncontrolled) and from 5.2±0.9 to 310±83.5 pg/ml (controlled) in the older fetuses. When analyzed by three-way ANOVA (after logarithmic transformation), there was a statistically significant effect of fetal gestational age on the AVP response to hypoxia but no statistically significant effect of the control of blood pressure during hypoxia on the magnitude of the AVP response. We conclude that the reflex AVP response to hypoxia is not modified by changes in arterial baroreceptor input in fetal sheep. (Supported by NIH grant HL36289 to CEW and HR and by an Established Investigator Award to CEW).

27.13

ROLE OF RENAL SYMPATHETIC NERVE ACTIVITY IN THE BLUNTED VOLUME REFLEX IN STREPTOZOTOCIN-INDUCED DIABETIC RATS. Ping L. Zhang and Kaushik P. Patel, Dept. of Physiology & Pharmacology, University of South Dakota, Vermillion, SD 57069 and Dept. of Physiology & Biophysics, University of Nebraska, Omaha, NE 68198-4575

To determine if a renal sympathetic nerve activity (RSNA) is responsible for the blunted volume reflex in diabetic rats, RSNA and excretory parameters in response to volume expansion were measured in control, diabetic and diabetic rats treated with insulin chronically. Diabetes was induced with streptozotocin (65mg/kg, ip) while control rats received only vehicle two weeks before the experiment. The rats were anesthetized with Inactin (0.1 g/kg). Graded volume expansion using isotonic saline (10% of body weight volume expansion in 40 min.) produced a smaller decrease in RSNA in diabetic rats compared to control rats. At the same time graded volume expansion produced smaller diuresis and natriuresis in the diabetic group compared to control group. Insulin treatment normalized the responses to graded volume expansion in the diabetic rats. However, when the relation between RSNA and natriuresis was evaluated, similar natriuresis per given change in RSNA during volume expansion was observed between diabetic and control groups. These results indicated that the relation between RSNA and renal excretory function is normal in the diabetic rats. Supported by NIH grant # 39046 and AHA Dakota affiliate grants.

27.14

IDENTIFICATION AND ISOLATION OF BARORECEPTOR NEURONS IN CULTURE. George Hajduczok and Francois M. Abboud. University of Iowa College of Medicine, Iowa City, IA 52242

The cellular mechanisms by which mechanical deformation of baroreceptor (BR) nerves is converted to electrical signals are not known. Technical limitations have prevented direct investigation of mechano-electrical signal transduction in BR nerve endings. We have developed a method to identify and isolate BR neurons in primary culture. The cell bodies of the aortic BR afferents are located in the nodose ganglia (NG). BR neurons were labelled with the unsaturated form (Δ^9-DiI) of the vital fluorescent carbocyanin dye, DiI, which is an oil at room temperature. Δ^9-DiI (20-50 μl) was microinjected into the wall of the aortic arch of anesthetized rats via a glass pipette (50-70 μm tip diameter). Fluorescent labelling of BR cell somata in the intact NG was evident within 48 hours post injection. NG were removed bilaterally 4 days to 2 months after Δ^9-DiI injection, minced, and incubated in 1% trypsin, 1% collagenase, and 0.1% DNAase. The NG were then triturated, resuspended in modified DMEM and cells were plated onto glass cover slips. Acutely dissociated NG neurons were spherical (20-60 μm diameter) and showed neurite outgrowth within 24-48 hrs after plating. Fluorescent BR neurons were identified acutely, and cell bodies and their neurites remained intensely labelled 14 days in culture. This approach can be used to study basic neurobiology of BR neurons and mechanisms of baroreceptor signal transduction.

27.15

SPECTRAL ANALYSES OF AUTONOMIC FUNCTION FOLLOWING WEIGHT LOSS IN MORBID OBESITY. R. De Meersman*, J. Albu*, J. Smolowitz* and I. Cohen* (SPON: K. Segal). Columbia University, NY 10027, St. Luke's Hospital, NY 10025, and Norwalk Hospital, CT 06856.

Aggregation of multiple cardiovascular risk factors with obesity have been reported. This study sought to explore changes in autonomic outflow and insulin sensitivity following weight loss. Spectral density analysis on 24 hour heart rate data was used to estimate autonomic components and decompose them into their characteristic frequencies. Comparisons of hourly spectral densities, using t-tests on data prior to- and following a 13.6% body weight loss in 5 morbidly obese patients revealed a significant shift ($p < 0.05$) of autonomic activity toward high frequency or parasympathetic outflow following weight reduction. Conversely, a lessening of sympathetic activity was noted as evidenced by diminished presence of low frequency spectra during diurnal and nocturnal episodes. Post weight loss areas under the insulin curves during oral glucose tolerance tests showed a 45.6% decrease, which was significantly related ($r=0.95$) to the weight loss ($p < 0.05$). These data suggest that weight reduction in morbidly obese patients is associated with favorable changes in autonomic outflow and insulin profile.

27.16

HEMODYNAMIC, RENAL & HORMONAL RESPONSES TO LOWER BODY POSITIVE PRESSURE. ET Mannix, MO Farber*, GR Aronoff*, P Palange*, and F Manfredi*. DVAMC & Indiana Univ Sch of Med, Indpls, IN 46202

Studies in normals subjected to lower body positive pressure (LBPP) using antishock trousers have failed to describe many of the effects of this maneuver. We studied the following responses to LBPP (35 mmHg, 1 hr, supine) in 7 healthy, 37±3 yr males, H2O loaded prior to LBPP (700 ml H2O/m2 BSA): systemic and renal hemodynamics; urine volume (UV), osmolality (Uosm), sodium (UNaV), potassium (UKV), free water (CH2O) and osmolar (Cosm) clearances; plasma atrial natriuretic peptide (ANP), vasopressin (AVP), osmolality (Posm), renin (PRA), aldosterone (PA), norepinephrine (NE), cortisol (CORT); serum sodium and potassium. Subjects were also studied on a control day under identical conditions, except that trouser pressure was 0 mmHg. The following changes ($\bar{X}$±SE, $p<0.05$) occurred only on the LBPP day: increases were noted in CORT (12.3±2.8 to 19.2±3.0 mg/dl), PRA (2.38±0.33 to 4.50±1.83 ng/ml/90 min), PA (7.2±1.1 to 9.3±1.5 ng/dl) and BP (89.4±4 to 112±4 mmHg), while FeNa decreased (1.15 ±0.19 to 0.73±0.07%). ANP and NE increased on the control day (47±7 to 97±21 pg/ml and 221±23 to 413±75 pg/ml, respectively) and on the LBPP day (49±10 to 104±30 pg/ml and 236±17 to 325±27 pg/ml). Conclusions: 1) LBPP decreased FeNa by increasing CORT, PRA and PA; 2) the significant ANP increases observed on both days, mediated by NE elevation, failed to affect renal sodium handling.

27.17

HIGH RESOLUTION ANALYSIS OF CONDITIONAL HR AND BP RESPONSE TO SHOCK IN RAT. D.C. Randall, D.R. Brown & R.M. Raisch*. Dept. Physiol. & Biophys. and Ctr. Biomed. Eng., Univ. Kentucky, Lexington, KY 40536.

Descriminative classical conditioning is useful to study the neural control of the circulation because the conditional response (CR) can be elicited multiple times under the precise control of the investigator. We developed a rat paradigm using behavioral procedures similar to those for dog (Physiol. & Behav. 48:333, 1990). A catheter was implanted in the caudal artery. The animals (n=5) were then habituated to a conical cloth restrainer for ≈2 hours daily for 2 days. Starting on day 3 they were given five 15 sec. tones (CS+) followed by a ½ sec. tail shock (≈0.5 ma). Another tone (CS-) was never followed by shock. The tones were interspersed pseudo-randomly with ≥ 5 min. between trials. The blood pressure (BP) and heart rate (HR) CRs were computed from days 3-5 as average changes over the 15 sec. tone relative to an 8 sec. pre-tone control. BP increased 5.2 ± 2.4 (mean ± SD) mm Hg during CS+; the change during CS- was -0.1 ± 1.3 mm Hg ($p<.01$). The average peak conditional pressor response was 13.9 ± 6.3 mm Hg at 5.0 ± 3.4 sec. after start of the tone. The HR CR varied across rats; the average change during CS+ was -4.2 ± 11.4 /min which was not different from the response to CS- (-2.2 ± 5.6 /min). Preliminary experiments indicate that the BP CR is due to multi-phasic changes in sympathetic nervous activity. (Supported by NIH grant HL 19343)

27.18

INTRAPERICARDIAL HEXAMETHONIUM DOES NOT SELECTIVELY BLOCK CARDIAC PARASYMPATHETIC GANGLIA. P.S. Clifford, S.W. Mittelstadt*, K.P. O'Hagan* and L.B. Bell. Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53295.

Previous anatomic studies have described the intrapericardial location of parasympathetic ganglia. Some recent studies have superperfused the epicardium with hexamethonium (HEX) to produce a cardioselective cholinergic efferent blockade (e.g. Circ Res 65:1212, 1989). The purpose of this study was to determine if intrapericardial infusion of HEX would block the heart rate (HR) response of parasympathetic stimulation. We evaluated the effect of intrapericardial infusion of HEX (250 mg) on HR response to vagal stimulation and on arterial blood pressure (BP). Stimulation of the left and right vagi before HEX infusion caused HR to decrease 43±12 and 59±46 b/min (mean±SD), respectively. After HEX infusion, vagal stimulation did not change HR. However HEX infusion decreased BP 34±25 mmHg. The change in BP was similar to that seen with IV infusion of HEX and suggested that the HEX may have been absorbed into the systemic circulation. A second set of experiments was conducted to determine if HEX in the pericardial space is absorbed into the circulation. The effect of intrapericardial HEX on BP and renal sympathetic nerve activity was evaluated in 4 additional dogs. Within 4 minutes of HEX infusion, blood pressure decreased 41±9 mmHg and renal sympathetic nerve activity decreased to 10.6±4.7% of resting activity. The results of these studies show that although HEX in the pericardial space blocks HR response to parasympathetic stimulation, it is also absorbed into the systemic circulation and blocks peripheral sympathetic ganglia. (Supported by VA and NIH).

27.19

INFLUENCE OF LUNG AFFERENT ACTIVITY ON BLOOD PRESSURE (BP) AND RENAL BLOOD FLOW (RBF) IN DOGS. K.P. O'Hagan*, L.B. Bell, S.W. Mittelstadt* and P.S. Clifford. Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53295.

Previous studies have demonstrated in several species that the lungs are a source of tonic inhibitory input to vasomotor centers. In anesthetized, sinoaortic denervated dogs with heart removed and lungs intact, 2-3 minutes of cervical vagal cooling resulted in substantial increases in BP and decreases in RBF (Circ. Res. 37:275,1975). It is not known if loss of pulmonary input influences BP or RBF in otherwise intact dogs. BP, left RBF and HR were measured in 5 pentothal anesthetized, baroreceptor intact, open chested dogs before and after selective surgical denervation of the lung (LDX). Efficacy of LDX was verified by loss of the Hering-Breuer reflex. Data are mean ± SEM.

	CONTROL	5 MIN POST LDX	30 MIN POST LDX
BP (mmHg)	117±8	124±8	126±4
RBF (ml/min)	109±36	111±33	107±27
HR (b/min)	146±7	146±8	150±6

BP, RBF and HR were not altered at 5 or 30 min post LDX, which suggests that acute loss of pulmonary input has little effect on cardiovascular control in otherwise intact dogs. In a separate group of 6 conscious dogs, BP and HR were measured before and after chronic LDX. Before LDX, BP was 108±4 mmHg. BP was not altered at 18 days (106±3) or 43 days (106±2) after LDX. LDX also did not alter HR. These results imply that under resting conditions, redundant cardiovascular control mechanisms compensate for the loss of pulmonary inhibitory input to central vasomotor neurons. However, this does not preclude a role for lung reflexes in cardiovascular control under other physiological conditions. Supported by VA and NIH.

27.20

FUNCTION OF CORONARY ARTERIES IN DOGS WITH LUNG ALLOGRAFTS. D.A. Lewis, A.J. McLarty, C.G.A. McGregor and V.M. Miller, Mayo Clinic and Foundation, Rochester, MN 55905

The vascular smooth muscle of canine coronary arteries are hyper-responsive to potassium chloride, angiotensin$_1$ and endothelin$_1$ in animals with rejecting lung allografts. This study was designed to determine whether these changes are due merely to the operative procedure, i.e. denervation of the lung during surgery. Single lung autotransplantation was performed in dogs. Rings with and without endothelium from left circumflex coronary arteries were suspended for the measurement of isometric force in organ chambers. Coronary arteries from unoperated dogs were used as controls. Contractions to potassium chloride, angiotensin$_1$ and endothelin$_1$ were not different between coronary arteries from autotransplanted and control dogs. Serum levels of angiotensin converting enzyme which were reduced during acute rejection of a lung were not affected by autotransplantation. There were no differences in circulating catecholamines between the rejecting and autotransplant groups. These data indicate that increased response of coronary arterial smooth muscle in animals with a rejecting lung allograft is not due to denervation and therefore must be due to the rejection process, to the immunosuppression regime, or a combination of the two.

27.21

ANGIOTENSIN II REVERSIBLY INCREASES CALCIUM CURRENTS IN CULTURED RAT NODOSE NEURONS. Kira Bacal*, Morris A. Priddy* and Diana L.Kunze, Baylor College of Medicine, Houston, TX 77030

Angiotensin II (AII) is a neuropeptide known to affect the activity of the baroreflex, but the mechanism(s) of its actions have yet to be fully characterized. The baroreceptor is innervated by nerves whose cell bodies are contained within the nodose ganglion. Fibers from these cells travel from the baroreceptors to synapse in the medial nucleus of the tractus solitarius (mNTS). Working in a thin-slice preparation from the mNTS, Yang and Andresen recently reported (FASEB Journal 5:A677, 1991) that administration of AII to this region caused an increase in EPSP amplitude, and they suggested that a presynaptic mechanism was responsible. To examine this possibility, we studied the effects of AII on the calcium currents of cultured nodose cells using a whole-cell voltage clamp protocol. The nodose cells were isolated from neonatal rats (1-4 days old) and grown in culture for 4-7 days before use. An intracellular solution of (124mM CsCl, 11mM EGTA, 1mM $CaCl_2$, 2mM $MgCl_2$, 10mM HEPES) was used, to which nystatin was added for a final concentration of 50ug/ml; this served to provide electrical access to the cell interior without washing out endogenous factors such as second messengers. The bath solution contained (139mM tetraethylammonium chloride, 2mM $CaCl_2$, 5.4mM KCl, 2mM glucose, 10mM HEPES, 5mM 4-aminopyridine). Under these conditions, perfusion with 100nM AII was found to increase the calcium current in 83% of the cells (5/6) where full current-voltage relationships were obtained. This increase was reversible and occurred within the range of -40 to 0 mV. This work was supported by NIH Grant No. HL36840.

27.22

DIFFERENTIAL INDUCTION OF TYROSINE HYDROXYLASE BY T3 AND NGF IN CULTURED ADRENAL CHROMAFFIN CELLS: CORRELATION WITH MECHANISMS OF HORMONE ACTION. Emmanuel U. Nzekwe* and Paola S. Timiras. Department of Molecular and Cellular Biology, University of California, Berkeley, California 94720 USA

The activity of the adrenergic enzyme, tyrosine hydroxylase (TH), from rat adrenal chromaffin cells, normal and neoplastic (PC-12), is differentially stimulated by nerve growth factor (NGF) and the thyroid hormone, triiodothyronine (T3). In "normal" chromaffin cells, NGF exerts its well-known stimulatory action, and T3, as well, stimulates TH activity in a dose-dependent manner but to a lesser (although statistically significant) degree than NGF. In "neoplastic" PC-12 cells, NGF is the only substance to possess a stimulatory action. These differing responses may correlate with the specific molecular mechanism of action of each hormone as well as with neoplastic transformation. Increased TH activity is associated with higher TH amounts after NGF and T3 in chromaffin cells and after NGF in PC-12 cells. The absence of higher synthesis despite higher TH amount after NGF in PC-12 cells suggests that NGF may act by preserving the enzyme from degradation.

Key words: Triiodothyronine-Tyrosine hydroxylase-Chromaffin cells

27.23

CATECHOLAMINE AND SEROTONIN EVALUATION BY HPLC AND AMPEROMETRIC DETECTION: STABILITY AND STORAGE. Stan'Willenbring* and William G. North* (SPON: S.W.T. Cheng). Dartmouth Medical School, Hanover, NH 03756

Amperometric detection following HPLC separation is now widely used to identify and quantify catecholamines, serotonin, their precursors and their metabolites in brain extracts. The extraction medium of choice has been a solution of 0.01 M perchloric acid (PCA) containing a reducing agent such as sodium bisulfite or cysteine. PCA is commonly employed because at higher concentrations this acid is an effective protein precipitant. Unfortunately, little information has been published regarding the stability of amines to storage in this and other acids. We therefore studied the changes with time in the electrochemical responses of norepinephrine (NOR), dopamine (DA) and serotonin (5-HT) in 0.01 M PCA or 0.1 M HCl with 10^{-4} M cysteine as reducing agent in the presence and absence of acid soluble proteins from brain. Values obtained by electrochemical measurement of these substances in solutions stored at -20°C and 2°C for 24 hours, 48 hours and 20 days were compared with those obtained when solutions were freshly prepared. Our results showed that NOR is stable under all of the conditions tested while DA and 5-HT remain unchanged at 2°C for up to 48 hours. However, there was a significant loss of amperometrically detectable 5-HT when solutions were frozen at -20°C and of 5-HT and DA after storage for 20 days at 2°C. No difference in stability of NOR, DA and 5-HT was found for solutions in 0.01 M PCA versus 0.1 M HCl. The results indicate that acid extracts of brain studied for catecholamine content by HPLC/amperometric detection should not be frozen, and can be stored at 2°C when processed within 48 hours.
(This research was supported by NIH Training Grant #HL07449)

27.24

STREPTOZOTOCIN-INDUCED DECREASES IN SEROTONIN TURNOVER ARE PREVENTED BY THYROIDECTOMY. Linda L. Bellush* and William N. Henley. Dpts of Psych. and Zool. and COM, Ohio University, Athens, OH 45701

Although characterized as hypothyroid, streptozotocin diabetic (STZ) rats have reduced serotonin turnover (5-HIAA/5-HT) in brainstem, while surgically thyroidectomized (TX) rats have increased 5-HIAA/5-HT. In the present study, the two treatments were combined to determine if they affected 5-HIAA/5-HT through the same mechanism. Adult male rats were thyroidectomized (TX) or subjected to sham surgery (SH). Two wks later, subgroups of TX and SH were made diabetic with STZ or given vehicle. Two wks later, the rats were killed and brain stem and spinal cord 5-HIAA/5-HT and plasma T_3 and T_4 were measured. STZ led to moderate reductions in T_3 and T_4, but the hormones were not detectable in TX rats. In TX-STZ rats, TX led to significant increases in 5-HIAA/5-HT in both regions while STZ caused reduced 5-HIAA/5-HT However, TX-STZ rats still had higher 5-HIAA/5-HT than SH-VEH controls. The results indicate that STZ and TX influence 5-HIAA/5-HT through separate mechanisms. Supported by the American Osteopath. Association.

27.25

P-CHLOROPHENYLALANINE (PCPA) CAUSES GREATER DEPLETION OF SEROTONIN IN THYROIDECTOMIZED RATS. William N. Henley, Margaret A. Notestine*, and Dennis V. Davis*. Dpt. of Zool.& Biomed. Sci. and Coll. of Osteo. Med., Ohio U., Athens, OH 45701

Adult male rats (n=37) underwent thyroidectomy (TX) or sham surgery. Catheterized, unanesthetized rats were infused with either a 3% mannitol-saline solution as vehicle (V) or V plus the serotonergic inhibitor, PCPA (2.5 hr infusion; 1.2 mg/kg-min). TX caused significant reductions in blood pressure, heart rate (HR), plasma [T_4], and elevations in urinary norepinephrine excretion (NE). PCPA caused significant depletion of 5HT and its principal metabolite, 5HIAA, in brain stem and spinal cord. Both were depleted to a greater extent in brain stem and spinal cord (5HIAA only) of Tx rats. PCPA caused an elevation in blood pressure and a drop in HR and NE. PCPA-induced changes in hemodynamic variables and NE did not differ statistically between TX and Sham rats. These findings provide additional evidence for increased turnover of serotonin in brain stem and spinal cord with TX. However, the data do not provide convincing support ($p=0.07$) for an association between 5HIAA/5HT and NE. Supported by the Am. Osteopath. Assoc.

27.26

ACTIONS OF SEROTONIN IN THE SOLITARY TRACT NUCLEUS. Peter D. Feldman and Julio C. Guillen, Jr.* Dept. of Pharmacology, LSU Medical Center, New Orleans, LA 70112

The nucleus tractus solitarius (nTS) is the site of the first synapse in central visceral sensory pathways, and, as such, is an important site for the modification of visceral information by afferent transmitter systems projecting to the nucleus. One afferent system arises from the medullary raphe nuclei. This study investigates the effects of the major transmitter of the raphe nuclei, serotonin (5T), in the medial nTS in isolated, superfused rat brainstem slices. Single unit recordings were made of spontaneous nTS neuronal activity and of synaptically-evoked responses to fixed-intensity electrical stimulation of primary visceral afferent fibers in the solitary tract. A total of 23 neurons were studied. Eleven of the 20 neurons (55%) that displayed only spontaneous activity showed dose-dependent decreases of firing rate (FR) during superfusion of 5T (50, 100, 250, 500, 1000 nM), with an EC_{50} of 449 nM. Three neurons (15%) showed graded increases of FR, with a calculated 50% increase ($ED_{50\%}$) at 363 nM. The remaining 6 neurons showed no response. Only 3 of the 23 neurons studied showed synaptically-evoked activity, one of which was silent at rest. This neuron showed a graded increase of responsiveness to tract input. Of the 2 evoked neurons with spontaneous activity, one showed a facilitation of synaptic responsiveness and no change of FR, while the other showed a depression of FR and no change of synaptic responsiveness. 5T-evoked changes of spontaneous and evoked activity could be blocked by 5Tergic antagonists such as mianserin (100-200 nM) or methysergide (200-500 nM) in all 5 neurons so tested. These data suggest that 5T may facilitate the synaptic responsiveness of one population of medial nTS neurons, and depress the spontaneous activity of another population.
Supported by NIH grant NS29458.

27.27

MU-OPIOID RECEPTORS IN NTS CONTRIBUTE TO COMPENSATORY RESPONSES TO HEMORRHAGIC HYPOTENSION. A. H. Hassen and E. P. Broudy*. West Virginia School of Osteopathic Medicine, Lewisburg, WV 24901

Hypotension (mean arterial pressure of 50 mmHg) induced by withdrawal of blood from anesthetized, artificially respired male Sprague-Dawley rats was followed by a gradual increase in blood pressure and heart rate when bleeding was terminated. Microinjection (10 nl) of artificial-cerebro-spinal fluid, kappa-, or delta-opioid receptor agonists into the Nucleus of Tractus Solitarius (nTS) had no effect on this response. However, administration of a mu-receptor agonist elicited greater increases in both blood pressure and heart rate. Compensatory responses were blocked following microinjection of the mu-receptor antagonist naloxone into the nTS. Attenuation of the compensatory response was not observed following microinjection of naloxone into regions lateral to the nTS. These observations suggest that mu-opioid receptors in the nTS contribute to the compensatory responses elicited by hemorrhage. Supported by the West Virginia School of Osteopathic Medicine.

27.28

SYMPATHETICALLY MEDIATED PRESSOR RESPONSES TO INJECTION OF ANG I IN THE ROSTRAL VENTROLATERAL MEDULLA OF SPONTANEOUSLY HYPERTENSIVE RATS (SHRS). Takuya Tsuchihashi* and David B. Averill Cleveland Clinic Foundation, Cleveland, OH 44195.

Angiotensin II (Ang II) endogenous to the ventrolateral medulla (VLM) makes a greater contribution to the level of blood pressure (BP) in SHRs versus Wistar Kyoto rats (WKYs) (Hypertension 17:422, 1991). The current study had two objectives: 1) to demonstrate that Ang I evokes pressor responses similar to those of Ang II; 2) to show that the pressor response of Ang I in the RVLM is sympathetically mediated. SHRs and WKYs were anesthetized with halothane and prepared for the measurement of BP and splanchnic sympathetic nerve activity (SpSNA). L-glutamate (L-Glu, 2 nmol) or Ang I was microinjected in the RVLM. Pressor responses to Ang I (100 pmol) were similar in both strains (SHR: 16 ± 2 (SEM) mm Hg; WKY: 16 ± 3 mm Hg). These pressor responses were similar to those observed for Ang II in a previous study (Am. J. Physiol. 1991 [In press]). Increases in BP were accompanied by similar increases in SpSNA in SHRs (+26 ± 7%) and WKYs (+17 ± 6%). In contrast, L-Glu evoked significantly larger pressor ($p<0.01$) and SpSNA ($p<0.05$) responses in SHRs (48 ± 3 mm Hg, +126 ± 17%) compared to WKYs (36 ± 2 mm Hg, +74 ± 12%). These results are consistent with the hypothesis that Ang I is a precursor to Ang II in the RVLM of SHRs and WKYs. Moreover, the changes in BP are more closely linked to changes in central sympathetic outflow and are not dependent upon possible differences in vascular reactivity between these two strains. Supported in part by NIH grant HL-6835.

27.29

ANGIOTENSIN II IMMUNOREACTIVITIES IN THE ROSTRAL VENTROLATERAL MEDULLA OF DEVELOPING NORMOTENSIVE WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS. T.M. Wong*, X.S. Qiu* and Y.S. Chan* (SPON: A.C. Brown). Department of Physiology, University of Hong Kong, Hong Kong.

Angiotensin II (Ang II) has been shown to exert a tonic influence on the cardiovascular neurons of the rostral ventrolateral medulla (RVL) in spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) as control. The influence was greater in SHR than in WKY. The results suggest that such an influence of Ang II on the RVL cardiovascular neurons may be involved in the genesis of hypertension. The present study attempted to correlate the Ang II activities in RVL with the development of hypertension in developing male and female SHR. WKY and SHR of 1, 2, 4, and 8 weeks of age were anaesthetized with pentobarbitol-sodium. RVL was microdissected and the position of microdissection was confirmed by histological examination. The Ang II immunoreactivities were determined by radioimmunoassay (RIA) using RIA kits from Amersham. It was found that Ang II immunoreactivites increased with age in both WKY and SHR, starting from 2nd weeks of age in the male and 4th weeks of age in the female. Such increases were significantly greater in SHR than in WKY of the same sex. In SHR, there was also a sex difference with the increase significantly greater in the male than in the female, starting from 4th weeks of age. The greater increases in Ang II immunoreactivities of developing SHR, particularly in the male, correlated well with the increases in blood pressure. The results suggest that the development of hypertension in SHR is closely related to Ang II levels in RVL. (The study was supported by The Croucher Foundation, C.R.C.G., Sun Yat Sen Foundation Fund and Lee Wing Tat Medical Research Fund. X.S.Q. was partly supported by Beijing-H.K. Exchange Centre and Academic Staff Exchange Scheme of H.K.U.)

27.30

IMMUNOHISTOCHEMICAL COLOCALIZATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) AND OTHER NEUROPEPTIDES IN THE INSULA MAGNA OF THE CANINE BRAIN. James C. McKenzie* and Jasiri Kennedy* (SPON: Vincent H. Gattone II) Department of Anatomy, College of Medicine, Howard University, Washington, D.C. 20059

ANP has been localized in the "Islands of Calleja Complexes (ICCs)", distinct structures in the olfactory tubercle with unknown function. The purpose of the present study was to define the type and distribution of ANP-positive neurons in the most medial ICC, the insula magna (ICM) and to characterize these neurons by identification of their afferents. Six dogs were deeply anesthetized with sodium pentobarbital and perfused with saline followed by 4% formaldehyde. Brains were removed, blocked, immersed in sucrose and sectioned at 50 µm on a freezing microtome. Sections through the septum were incubated in ANP antibody (1:10,000) for 72 h at 4° C and processed by the ABC technique. Serial sections were immunostained for ANP, Substance P (SP, 1:2,000) and metenkephalin (mENK, 1:10,000). Some sections were double-stained for ANP and either tyrosine hydroxylase (TH), SP, mENK or neuropeptide Y (NPY) using the ABC and glucose oxidase (GO) techniques. ANP was localized in large neurons of the granule-cell poor regions of the ICM. These neurons were similar in morphology to large ANP-positive neurons of the pallidum. ANP-positive neurons were enmeshed within networks of SP- and, to a lesser degree, mENK-positive fibers. The ANP-positive neurons of the ICM are similar to the large pallidal neurons in morphology, immunoreactivity and innervation, and they may represent the most ventral and medial portion of the pallidum. Supported by NIH grant HL 45241 and a grant from Howard University.

CELLULAR MECHANISMS OF CARDIOVASCULAR FUNCTION

28.1

Signaling by α_1-adrenoceptor subtypes in vessels of stroke prone spontaneously hypertensive rats. Chide Han, Jinling Li and Yongmei Chen, (SPON: F. Gordon) Cardiovascular Research Laboratory, The Third Hospital, Beijing Medical University, Beijing, 100083, China.

Two α_1-adrenoceptor subtypes can be distinguished by antagonists and chloroethyclonidine (CEC). The CEC-insensitive subtype (α_{1A}) may activate Ca^{2+} influx in smooth muscle. We characterized in rat that aorta contained primarily α_{1B}, renal artery primarily α_{1A} and mesenteric artery a mixture. We compared blood vessels between stroke prone spontaneously hypertensive (SP) and WKY rats. Pretreatment with CEC (50 µM) decreased the maximal contractions by NE to 35±2.9% and 31±8.3% (aortae); 80±7.3% and 68±8.3% (renal arteries); 55±7.0% and 68±8.2% (mesenteric arteries) of control in the WKY and the SP rats, respectively. None of the differences between WKY and SP were statistically significant. In contrast, 10 µM nifedipine reduced the maximal contractions induced by NE to 56±4.8% and 3±1.5% ($p<0.01$, aortae); 23±3.5% and 9±4.1% ($p<0.05$, renal arteries); 28±0.8% and 6±2.5% ($p<0.01$, mesenteric arteries) in the WKY and the SP rats, respectively. Phasic contractions induced by 10 µM NE were decreased to 75±8.3% and 52±9.6%, while tonic contractions were decreased to 54±10.3% and 11±2.8% of controls in WKY and SP with nifedipine. These results suggest that in SP rats the composition of α_{1A} and α_{1B} subtypes in blood vessels were not changed. However, signal transduction of α_1-adrenoceptor subtypes could be changed in pathological situations.

28.2

Molecular characterization of alpha-adrenoceptor gene expression in intact and cultured vascular smooth muscle. JAMES E. FABER, PEIPEI PING AND *JOHN W. REGAN Department of Physiology, University of North Carolina, Chapel Hill, NC 27599. *Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

Pharmacological and physiological studies have demonstrated the existence of both alpha1 and alpha2 adrenergic receptors on vascular smooth muscle cells. Three different genes coding different alpha1 adrenergic receptor subtypes have been cloned. However, northern blot analysis of rat aorta have detected mRNA for only one subtype, the alpha1A adrenergic receptor, leading to the suggestion that only this gene is expressed by rat vascular tissue. On the other hand, recent studies suggest that aortic tissue possesses receptors with the pharmacological characteristics of the alpha1B cloned receptor. We applied a more sensitive method, polymerase chain reaction, to differentiate alpha1 adrenergic receptor gene expression on vascular smooth cells. Total cellular RNA was extracted from fresh and cultured rat aorta and vena cava smooth muscle cells. Unlike previous studies, endothelial cells were removed from tissue samples because they also possess adrenergic receptors. Oligonucleotide primers homologous to conserved regions of the cloned alpha1 adrenergic receptor genes were synthesized. The polymerase chain reaction was used to probe for the presence of alpha1 adrenergic receptor cDNA sequences after reverse-transcription of total cellular RNA. PCR products were distinguished among the alpha1 genes based on both the size of amplified DNA fragments and specific restriction endonuclease cleavage products. Our results indicate that there are two alpha1 adrenergic receptor genes expressed by rat aorta and vena cava. One of them is identified as the alpha1B subtype, and the other is the putative alpha1A subtype. Both the alphaIB and the putative alpha1A subtype were also detected in cultured smooth muscle cells, indicating that alpha1 adrenergic receptor gene transcription is preserved in cultured (passage two) smooth muscle cells.(Support: HL-38783 and -02377).

28.3

NEGATIVE INOTROPIC ACTION OF STAUROSPORINE, A PROTEIN KINASE INHIBITOR, IN CONSCIOUS β-BLOCKED DOGS. R.A. Buchholz, M.L. Volberg*, P.F. Pratt*, and P.J. Silver*. Sterling Research Group, Rensselaer, NY 12144

We previously reported that staurosporine (S) caused a dose-dependent reduction in MAP in conscious, normotensive dogs that was unaccompanied by a change in left ventricular (LV) dP/dt (FASEB J 4:A747, 1990). In contrast, an equihypotensive dose of nitrendipine (N) caused a marked increase in LV dP/dt. Thus, we examined the mechanism(s) responsible for the difference in the inotropic response to equihypotensive doses of S and N in conscious dogs (n=6). A single iv injection (100 μg/kg) of S or N was given with or without β-adrenergic blockade with atenolol (1 mg/kg,iv) using a cross-over design. S and N caused similar maximum reductions in MAP (-11 mmHg). N caused a greater fall in TPR and increases in HR and CO than S. LV dP/dt increased 53% ($p<0.01$) after N, but was unchanged after S. After β-blockade, S and N caused a -21 mm Hg fall in MAP. The increases in HR and CO after N were reduced 60% after β-block and the increase in LV dP/dt was eliminated. Atenolol also blunted the smaller but significant increases in CO and HR caused by S. Moreover, β-blockade unmasked a negative inotropic action of S. S decreased LV dP/dt 59% below baseline after atenolol. The increase in CO caused by N appears to be due, in part, to reflex increases in HR and LV dP/dt mediated largely by enhanced sympathetic drive to the heart. The smaller increase in CO after S also appears to be due to a reflex increase in HR. However, a negative inotropic action of S opposes the sympathetically-mediated increase in inotropy and limits the increase in CO. The negative inotropic action of S may be due to inhibition of protein kinase C, myosin light-chain kinase and/or cAMP kinase.

28.4

EFFECTS OF VASCULAR CELLS ON MEMBRANE PROPERTIES FROM HYPERTENSIVE AND NORMOTENSIVE RATS' NEURONS IN VITRO. Bruno C. Jubelin* and M.S. Kannan (SPON:E.M. Gallant). Dept. of Veterinary Biol., University of Minnesota, St. Paul, MN 55108.

Membrane electrical properties of superior cervical ganglia neurons from neonatal spontaneously hypertensive (SH), Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats were studied in co-culture with aortic (A) and mesenteric (MA) vascular smooth muscle cells (VSMC), using intracellular microelectrodes. Neurons were cultured either with VSMC from their own strain or one of the two others. Cultured SH neurons were previously shown to be unable to accommodate during long-duration depolarizing current pulses (1). The main result was the inhibition of neuronal firing in the presence of their respective A VSMC: >90%, SH, WKY; 41%, SD. SD A VSMC were equally less effective in inhibiting SH (26%) and WKY (18%) neurons firing. MA VSMC from normotensive strains did not affect neuron firing properties. Only SH MA VSMC shifted SH neurons multiple firing frequency toward double spikes. Most membrane electrical properties remained unchanged, and there was no correlation between the changes noted and the modifications of the firing properties. It is concluded that aortic tissue can specifically prevent neuron firing without preventing their survival, and that vascular tissue demonstrates a combination of general (vascular), specific (aorta vs. mesenteric artery) and strain related effects on neuronal firing properties. Further studies are needed to determine the factor(s) involved in these inhibitions (1) Jubelin and Kannan, AJP, 259:C389-C396, 1990. Supported by Am. Heart Assn. (MN Affl.) and CHF.

28.5

IDENTIFICATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II IN VASCULAR SMOOTH MUSCLE. C.M. Schworer and H.A. Singer. Weis Center for Research, Geisinger Clinic, Danville, PA 17822

Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase II) has been implicated as a mediator of signal transduction events associated with elevated levels of intracellular Ca^{2+}. We have characterized CaM-kinase II in arterial vascular smooth muscle (VSM), and our results suggest that the kinase may be compartmentalized, i.e., there is both a minor, readily soluble fraction that binds to DEAE-Sephacel (eluted by 0.1-0.2 M NaCl), and a major, myofibril-associated fraction that can be solubilized with 15 mM $MgCl_2$, after which most of the kinase does not bind to DEAE. The minor soluble pool is characterized by: 1) similarity to brain CaM-kinase II in elution properties from several chromatographic supports; 2) Ca^{2+}/CaM-dependent phosphorylation of autocamtide-2, a synthetic peptide substrate specific for CaM-kinase II; 3) generation of Ca^{2+}-independent kinase activity after Ca^{2+}/CaM-dependent autophosphorylation, a hallmark of CaM-kinase II; 4) appearance of a ^{32}P-labeled, 62-kDa band after autophosphorylation using [γ-^{32}P]ATP; and 5) absence of cross-reactivity after immunoblotting with either of two antibodies specific for the α or β subunits of rat brain CaM-kinase II, indicating that the major species of brain CaM-kinase subunits are absent in VSM. Experiments are in progress to define the myofibrillar pool more definitively and to identify the subunit species of VSM CaM-kinase II. (Supported in part by American Heart Association, Pennsylvania Affiliate.)

28.6

REVERSIBLE INHIBITION OF CALCIUM TRANSIENTS IN CULTURED CARDIAC MYOCYTES FROM NEONATAL RATS BY 1,1,1-TRICHLOROETHANE. Peter Hoffmann* and Mark Toraason. Cellular Toxicology Section, ETB, DBBS, CDC-NIOSH, Cincinnati OH 45226

1,1,1-Trichloroethane (TCE) is a widely used solvent that is annually linked to several cases of sudden death, presumably from cardiac arrhythmia, following accidental exposure or abuse. Although the subcellular action of TCE has not been elucidated, it is known that TCE acts directly on the heart to produce myocardial depression. We investigated the mechanism of myocardial depression by assessing the effect of TCE on intracellular calcium ($[Ca^{2+}]_i$) transients in isolated cardiac myocytes using fura-2. Cells were exposed to TCE in Hank's BSS (aliquoted as 0.2% DMSO solution) by a single-pass suffusion in an environmentally controlled chamber. TCE (0.25 - 8 mM) reduced the magnitude of electrically (1 Hz, 60 V, 10 msec) induced $[Ca^{2+}]_i$ transients in a concentration-dependent manner with no effect on diastolic (basal) $[Ca^{2+}]_i$. TCE did not significantly alter the time course of $[Ca^{2+}]_i$ transients (time to peak and half-time for relaxation). Upon washout of TCE, $[Ca^{2+}]_i$ transients returned to normal or increased to levels greater than those recorded during pre-exposure. Experiments with potassium-induced depolarization (90 mM), verapamil (5 uM), caffeine (10 mM), or ryanodine (10 uM) indicated that TCE acts on sarcolemmal calcium entry and sarcoplasmic reticulum calcium release.

28.7

ENHANCED ERYTHROCYTE CALCIUM PUMP ACTIVITY IN A KINDRED WITH FAMILIAL BENIGN HYPERCALCEMIA. Nelson Escobales, Carlos Fariñas, and Lillian Haddock. Univ. of Puerto Rico Sch. of Med., San Juan, P. R., 00936-5067.

The hypothesis that a global defect in cellular calcium transport may be critical in the development of Familial Benign Hypercalcemia (FBH) was investigated. To that effect calcium pump activity in intact red cells was determined using the method developed by Dagher and Lew (J. Physiol. 407:569, 1988.). Twelve patients from a kindred with FBH (9 hypercalcemic and 3 normocalcemic) and 9 normal subjects were evaluated. For comparison purposes, 3 patients with primary hyperparathyroidism (PHPT) were also studied. Our results indicate that calcium pump activity in the FBH kindred was significantly higher (about 33%, $p<0.005$) when compared to normal subjects irrespective of their calcium status (normocalcemic or hypercalcemic). Calcium pump activity was similarly increased in patients with PHPT. The increased pump activity observed in the FBH kindred is not secondary to an enhanced calcium entry into the red cell as this parameter was not affected in these subjects.

Evaluation of the mineral status of these subjects indicated normal or slightly high bone mineral density, normal levels of serum thyrocalcitonin and $1,25(OH)_2D$, low urinary calcium and high renal calcium reabsorption. PTH levels were increased two fold in hypercalcemic subjects with FBH when compared to normocalcemics, and phosphorus levels, although within normal range, were higher in the latter group. Since PTH levels were similar in normocalcemic (FBH) and control subjects, the increased calcium pump activity in FBH does not appear to be a direct result of PTH action.

Altogether these findings indicate that although increased calcium pump activity exists in FBH patients, this alteration in itself cannot be responsible for the hypercalcemia of this condition. Our results support the notion that in these patients an abnormal function of the parathyroid glands may be a significant factor in the development of the hypercalcemia. Partially supported by USPHS Grant RR-08224.

28.8

ATHEROGENIC RABBITS TREATED WITH SUPEROXIDE DISMUTASE. E. L. BEARD, J. C. MENA, AND A. L. VANBLARICOM. Loyola University, Dept. Biological Sciences, New Orleans, LA 70118-6195.

Superoxide dismutase by scavenging free radicals in the mammalian cells protects them from the cytotoxic effects caused by peroxidation of membrane lipids. Oxidation of LDL's by free radicals may be a prime cause of atherogenesis. By scavenging superoxide radicals in cells, superoxide dismutase may counteract the altered cell membrane behavior which allows cholesterol invasion into the arterial wall and the deterioration of arterial smooth muscle cells leading to atherogenic plaque formation. Here SOD-PEG (1500 units halflife of 36 hrs donated by Enzon Labs and Sterling Drug, Inc.) was injected intraperitoneally into male New Zealand strain rabbits. The rabbits were fed the atherogenic diet for two months prior to SOD treatment in one experiment. In a second study, atherogenic dieting started simultaneously with SOD treatment. The rabbits were sacrificed after ten weeks of SOD treatment. At autopsy, the aortae of the SOD treated rabbits were found to have been protected by SOD in that atherogenic plaque development was low in comparison to non SOD treated atherogenic dieted rabbits. Blood cholesterol and phospholipid levels were somewhat lower but plasminogen activator activity was greater in SOD treated rabbits than in non SOD controls. SOD treatment improved uptake of glucose by insulin treated blood platelets.

28.9

ZAPRINAST REVERSES NITROGLYCERIN-INDUCED TOLERANCE IN VITRO AND IN VIVO E.D.Pagani, P.J.Silver*, L.de Garavilla, G.Van Aller*, B. O'Connor* and R.A.Buchholz. Sterling Research Group, Dept. of Cardiovascular Pharmacology, Rensselaer, N.Y. 12144

To determine if zaprinast (ZAP), an inhibitor of vascular smooth muscle (VSM) cGMP phosphodiesterase (PDE), could reverse nitroglycerin (NG)-induced tolerance in vitro and in vivo, we evaluated NG and ZAP + NG for 1) vasorelaxant activity in aortic VSM from normotensive rats (NTR) and spontaneously hypertensive rats (SHR) pre-exposed to NG (550 uM, 1 hr, in vitro) and on cGMP levels in NTR VSM and for 2) the effect on mean arterial pressure (MAP) in SHR pretreated with NG (100 mg/kg s.c.) t.i.d for 3 days. Tolerance developed in vitro in VSM: NG EC_{50} values were greater in both NTR (20 nM control vs 246 nM NG-treated) and SHR (9 nM vs 34 nM) VSM. Preincubation of VSM with a vasoinactive concentration of ZAP (10 uM) reduced the NG EC_{50} values in tolerant NTR (12 nM) and SHR (7 nM) VSM to control values. NG (300 nM) increased cGMP levels to 12.5 pmol/mg protein in non-tolerant VSM but only to 3.5 pmol/mg in tolerant VSM; however, NG (300 nM) + ZAP (10 uM) increased cGMP levels to 11.8 pmol/mg in tolerant VSM and to 51.8 pmol/mg in non-tolerant VSM. In tolerant SHR, a bolus injection of 30 ug/kg NG did not affect MAP while this dose reduced MAP 25 mmHg in non-tolerant SHR. In contrast, 10 mg/kg ZAP (nonhypotensive dose) + 30 ug/kg NG reduced MAP by about 40 mmHg in tolerant and non-tolerant SHR. These results demonstrate that a cGMP-PDE inhibitor can reverse tolerance to NG in isolated VSM and in SHR, presumably by maintaining VSM cGMP levels.

28.10

AGE-RELATED ALTERATIONS IN BETA ADRENERGIC MODULATION OF cAMP LEVEL AND PROTEIN PHOSPHORYLATION IN INTACT RAT MYOCARDIUM. Ming-Tao Jiang*, Margaret P. Moffat* and Njanoor Narayanan* (SPON: J. Kraicer) Univ. of Western Ontario, London, Canada N6A 5Cl.

Diminished contractile response of the aging heart to beta adrenergic stimulation has been documented in previous studies. To understand the mechanism(s) underlying this age-related deficit, the present study compared beta adrenergic mediated contractile response, cAMP accumulation and protein phosphorylation in isolated perfused hearts from adult (6-8 months) and aged (28-30 months) Fisher 344 rats. In isometrically contracting hearts (4Hz), beta adrenergic stimulation with isoproterenol (ISO, 0.1 μM) evoked positive inotropic and lusitropic responses both of which were significantly lower (15-20%, P<0.05-0.001) in the aged compared to adult. Under similar experimental conditions, ISO-induced increase in tissue cAMP content was also lower (13-18%, P<0.05) in the aged heart. ISO-induced phosphorylation of the sarcoplasmic reticulum protein phospholamban (PL) and the myofibrillar protein troponin-I (TNI) were significantly diminished (25-35%, P<0.05-0.001) in the aged compared to adult heart. No age-related difference was, however, evident in ISO-induced phosphorylation of C-protein of myofibrils. These data suggest that age-related decrements in beta adrenergic mediated cAMP accumulation and phosphorylation of PL and TNI contribute to the diminished contractile response of the aging heart to beta adrenergic stimulation. (Supported by Heart and Stroke Foundation of Ontario).

TEMPERATURE REGULATION: HYPERTHERMIA AND HYPOTHERMIA

29.1

EFFECTS OF VARIOUS ELECTROLYTE BEVERAGES ON HYDRATION STATUS DURING EXERCISE IN A HIGH HEAT ENVIRONMENT. K.M. Wilmore*, B.L. Bennett*, J.H. Heaney*, P.K. Griffith*, R.S. Pozos*, and S.R. Newmark, Naval Health Research Center, San Diego, CA 92186; GEO Centers, Inc., Fort Washington, MD 10903; Humana Hospital Sunrise Diabetes Center, Las Vegas, NV 89109

The purpose of this study was to determine the effectiveness of different electrolyte beverages for maintaining hydration during prolonged exercise in the heat. Nine male U.S. Marine personnel performed four tests in a hot, dry environment (46.2°C, 20% rh) under the following fluid conditions: 1) distilled water (W) - colored and flavored; 2) CHO/low-level electrolyte (LE); 3) CHO/moderate-level electrolyte (ME); and 4) CHO/high-level electrolyte (HE). The total Na^+, K^+, and Cl^- contents for LE, ME, and HE were 175mg, 255mg, and 365mg per 240 ml, respectively. 230 ml were consumed every 15 minutes over four hours. The subjects sat for the first and fourth hours, while the second and third hours consisted of a 40/20 min work/rest cycle of treadmill walking (3.0 mph, 0% grade). The subjects were dressed in standard utilities with a total load carriage of 20.45 kg beyond nude body weight. Plasma volume changes (PVΔ) from pre to post for W and LE were significantly greater than for ME and HE. Weight change (WtΔ) and sweat rate (Swtrt) showed no significant differences between fluid trials. Urine output was similar between LE, ME, and HE, while W was significantly greater. No significant differences were found between fluids for mean heart rate (109.1 ± 1.8), rectal temperature (37.8 ± 0.08°C), or skin temperature (37.2 ± 0.06°C). There were also no significant differences found for serum electrolytes and osmolality.

Fluid	PV Δ (%)	Wt Δ (kg)	Swtrt (L/hr)	Urine (L/hr)
W	-8.73 ± 5.5†	-0.540 ± 0.61	0.850 ± 0.12	0.825 ± 0.49*
LE	-6.95 ± 7.5†	-0.202 ± 0.70	0.876 ± 0.16	0.372 ± 0.29
ME	-1.96 ± 5.8	-0.172 ± 0.74	0.849 ± 0.15	0.486 ± 0.34
HE	-2.47 ± 5.7	-0.020 ± 0.64	0.839 ± 0.16	0.385 ± 0.29

Values are means ± SD. † significantly > ME, HE (p<0.05); * significantly > LE, ME, HE (p<0.05).

The PVΔ results indicate that ME and HE were more effective in maintaining hydration than W and LE. This suggests that a threshold concentration of electrolytes is necessary to prevent body fluid loss during prolonged exercise in the heat.

29.2

PHYSIOLOGICAL RESPONSES FOLLOWING FLUID HYPERHYDRATION DURING HIGH HEAT EXPOSURE. B.L. Bennett*, J.H. Heaney*, K.M. Wilmore*, P. K. Griffith*, R.S. Pozos*, and S.R. Newmark, Naval Health Research Center, San Diego, CA 92186; GEO Centers, Inc., Fort Washington, MD 10309; and Humana Hospital Sunrise Diabetes Center, Las Vegas, NV 89109

This study examined the efficacy of ingesting different hyperhydration (HH) solutions on expanding and maintaining plasma volume prior to high heat exposure. Nine U.S. Marine Corps personnel were randomized into one of four treatments: 1) non-fluid condition (NF); 2) carbohydrate electrolyte sloution (CE); 3) CE and 1% glycerol solution (CG); and 4) colored/flavored water and glycerol (1gm/kg BW) solution (GS). Once a week each subject hyperhydrated with a total of 2000 ml (500 ml/ 15 min), rested for two hours then entered the heat chamber (46.1°C, 22% rh). In the heat subjects wore field utilities and carried a load equaling 22.8 kg greater than nude body weight, and consumed up to 2000 ml water ad lib. The first and fourth hour each subjected rested in a chair. The second and third hour subjects walked (3 mph/0% grade) 40/20 min work/rest protocol. Plasma volume (PV1) increased (p>0.05) after the HH; however, a moderate decrease (p>0.05) occurred at the end of heat exposure (PV2). Urine ouput (Ur1) in the HH period and in heat (Ur2) showed similar values in each of the three fluid treatments. The rate of change in rectal temperature (ΔTr), skin temperature (ΔTsk) and body weight loss (ΔBW) were similar (p>0.05) in all treatment conditions. Values are means±SD. * significantly < CE,CG,GS ; † < CE ; (p<0.05)

Drink	PV1(%)	PV2 (%)	Ur1 (ml)	Ur2 (ml)	ΔTr (°C)	ΔTsk (°C)	Δ BW(kg)
NF	0.9±5.4	-9.4±7.1	182±173*	178±138†	2.1±0.5	2.0±0.8	2.1±0.9
CE	5.6±5.0	-7.6±2.9	1091±388	589±365	1.9±0.5	2.1±1.0	1.9±0.7
CG	2.6±4.2	-7.1±4.4	1267±331	443±287	1.9±0.6	1.5±0.7	1.7±0.7
GS	5.9±3.8	-7.9±6.3	1266±281	442±416	1.9±0.5	1.6±0.6	2.1±0.7

In conclusion, the results indicate that hyperhydrating before heat exposure and ingesting up to two liters of water in heat do not appear to be more effective for maintaining PV than solely ad lib water consumption. Reported thermoregulatory benefits derived from fluid induced hypervolemia may be solution and protocol dependent. This may partially explain the inter-study differences on the efficacy of hyperhydration on thermoregulation.

29.3

ADRENAL HORMONAL RESPONSES IN SUBJECTS WEARING A CHEMICAL DEFENSE ENSEMBLE DURING SHORT DURATION HEAVY WORKLOAD. S. Newmark, R. Hesslink*, A. Sucec*, D. Trone*, G. Banta*, R. Pozos* Humana Hospital Sunrise Diabetes Center, Las Vegas, NV 89109; Naval Health Research Center, San Diego, Ca 92186

Twenty five male subjects carrying a 34Kg load over nude weight and wearing a chemical protective suit exercised on a treadmill at 3.0 mph and 2% grade in a chamber maintained at 120°F 20% relative humidity. In addition to monitoring body temperature and cardiac response, blood analyses before and after the exercise session were conducted for cortisol (Cort), Aldosterone (Aldo), electrolytes, BUN, glucose, and albumin levels. Body weights were also measured pre and post exercise. The average exercise duration for the subjects was 28 minutes ± 6 minutes.

	Cort (ug% ± sd)	Aldo (ng% ± sd)
Pre Exercise	17.2 ± 5.6	14.4 ± 4.6
Post Exercise	34.8 ± 8.8	48.5 ± 21.4

Both Cort and Aldo demonstrated significant increases (p<0.05). There were no significant differences in the other measured blood components. After exercise there was a significant (p<.05) 1.3% decrease in body weight. The elevated levels of Cort indicate a major stress imparted by the exercise protocol in a high heat environment. The high levels of Aldo and body weight loss although associated with a short exercise time, indicates a rapid shift in hydration status in these subjects.

29.4

THE EFFECTS OF MICROCLIMATE COOLING ON TOTAL BODY SWEAT RATE AND VASOACTIVE INTESTINAL PEPTIDE. Jay H. Heaney*, G.R. Banta*, M.J. Buono*, R. Bulbulian*, T. L. Sopchick* and R.L. Hesslink. Naval Health Research Center, San Diego, CA 92186; San Diego State University, San Diego, CA 92182 and University of Kentucky, Lexington, KY 40506.

The purpose of this study was to investigate the effects of repeated high heat exposure with use of microclimate cooling (Ice Vest) on sweat rate (SWR) and vasoactive intestinal peptide (VIP) production. U.S. Navy engineroom personnel (N=7) volunteered to stand a simulated 4-hr engineroom watch (EW) with mean ambient temperatures (°C) of globe=45.5, dry bulb=43.3, wet bulb=32.2, relative humidity=46%, and partial vapor pressure=31mmHg. Subjects were tested on 4 consecutive days alternating Ice Vest (Ice) and No Ice Vest (No-Ice) conditions (2 days each). VIP samples were obtained from pre/post nude body weight and corrected for fluid exchanges. Skin and forearm blood flow (laser doppler and plethysmography, respectively) were sampled at 3 intervals during the EW (hours 1,2,3); heart rate (HR), rectal (Tre) and skin temperatures (Tsk) were recorded continuously. Post-VIP (67%), SWR (.42 l/hr), and HR (94 bpm) results were all significantly higher in the No-Ice condition compared to the Ice condition results (5.4%, .21 l/hr and 86.8 bpm, respectively. Mean skin and forearm blood flow values were slightly higher in the No-Ice vs. Ice conditions (7% and 15% respectively). These results support the hypothesis that VIP production and sweat rate are linked in the thermoregulatory process and indicate that microclimate cooling can reduce thermal strain when working in high heat.

29.5

PHYSIOLOGICAL PARAMETERS RELATING CARDIOVASCULAR DRIFT IN HIGH HEAT LOW HUMIDITY CONDITIONS.

D. Trone,* A. Sucec,* R. Pozos,* R. Hesslink,* C. Bischoff* and E. Labranch* (SPON: M. Buono) Naval Health Res. Ctr., San Diego, 92186

Cardiovascular drift (HR_d) is characterized by an increase in heart rate at steady rate work. An extreme cardiovascular drift (HR_d), defined here as the slope of heart rate by exercise time, was observed while wearing chemical defense gear. The HR_d at 34°C WBGT and at 25°C WBGT was 0.82 and 0.66 bts/min. Eight euhydrated unacclimatized male Marines (mean age, weight, and %fat of 21yrs, 78.7kg, and 14%) completed a counter balance designed testing protocol to exhaustion at 80 m/min 2% grade at WBGT 34°C and WBGT 25°C with 34kg of gear. HR, rectal temperature (T_r), and mean skin temperature (T_{msk}) were recorded continuously in one minute intervals. Multiple regression analysis was performed on four variables in order to determine their contribution to HR_d. The equation follows :

$HR_d = 4.42x_1+1.87x_2-0.00074x_3+0.0095x_4-1.184$

$HR_d=(HR_{peak\ ex}-HR_{5min\ ex})/ExTime$; $x_1=(T_{msk\ peak\ ex}-T_{msk\ 5min\ ex})/ExTime$; x_2=ratio of gear mass to lean mass; x_3=fitness (product 3mi run time with %fat); x_4=WBGT.

Sixty % (R^2=0.60) of the HR_d variability is accounted for by these four parameters. By controlling the parameters which contribute most to HR_d, one could increase work performance time.

29.6

EVIDENCE OF A LIMULUS AMEBOCYTE LYSATE DETECTABLE LPS-INHIBITOR IN PLASMA OF RABBITS TOLERANT TO LPS, BUT OTHERWISE HYPERSENSITIVE TO INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR.

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In vivo responses to bacterial endotoxin depend among others considerably on the immunological status of the host. Tolerance to endotoxin has been induced either by repeated injections of minute doses of endotoxin or by a single large one. Cytokines have been shown not only to be mediators of the toxic activities of endotoxin, but also to elicit beneficial effects as resistance to infections and endotoxin tolerance. To explore this phenomenon we injected rabbits i.v., after a control period of 45 min, either 1 μg/kg E. coli Lipopolysaccharide (LPS, O113, Cape Cod), or 1 μg/kg recombinant human (rh) Interleukin-1β (IL-1), or 10 μg/kg rh Tumor necrosis factor-α (TNF), and the same doses again 24 hours later. Body temperatures and respiratory rate were followed up for 3 hours. An ear artery, cannulated under local anesthesia, served for recording blood pressure and heart rate, and blood sampling. To the 1st dose of LPS, all rabbits responded with a biphasic fever and, with a latency of 45 min, with tachycardia. After the 2nd LPS dose, the fever response was attenuated and transformed into a monophasic one, with diminished tachycardia. When control serum of the 1st and 2nd day was spiked with various doses of LPS, about 5 to 10 times more LPS had to be added to 2nd day serum to reach identical optical absorbance values in the chromogenic Limulus amebocyte lysate (LAL) assay. In other rabbits IL-1 induced a continuous rise of core temperature, which was steeper and higher with the 2nd dose, while the immediately rising heart rate increased on both days by the same amount. LPS added to 2nd day control serum also exhibited the 5 to 10-fold sensitivity reduction to LPS in the LAL assay. The fever reactions were considerably augmented when IL-1 was given 24 hours after LPS, but also when LPS was given 24 hours after IL-1, indicating that reduced LAL-sensitivity after IL-1 is not related to LPS-tolerance. TNF induced a biphasic fever, which was higher on day two, however, significant changes of heart rate and blood pressure did not occur on both days. About two times more LPS had to be added to day two control serum in the LAL assay to get the optical absorbance values of day one serum. TNF given 24 hours after LPS induced a monophasic fever of short duration followed by rapid defervescence. Tolerance to LPS can, thus, be explained by attributing the primary mediator function to TNF rather than to IL-1.

29.7

COMPUTER-BASED MONITORING OF THERMOREGULATORY RESPONSES OF ANESTHETIZED RATS EXPOSED TO RADIOFREQUENCY RADIATION.

Rick E. Berger*, Melvin R. Frei*, and James R. Jauchem. U.S. Air Force Armstrong Laboratory, Directed Energy Division, Brooks AFB, TX., 78235-5301.

The consequences of incidental biological exposure to radiofrequency radiation (RFR) due to commercial and military applications are, in part, unknown. Conventional techniques for monitoring real-time cardiovascular and respiratory responses to the thermal stress produced by RFR are generally unsuitable due to interaction of transducers with RFR. Earlier in-house developmental work has produced a successful protocol that overcomes previously encountered difficulties in physiological monitoring in RF fields by using specialized instrumentation (*Journal of Microwave Power and Electromagnetic Energy*, *23*, 1988, 81-84). This protocol has now been PC computer automated in a menu-driven modular software system that includes modules for automated experimental control, dosimetry calculation, instrument calibration, and statistical analysis of experimental data. The experimental module allows the real-time monitoring and graphical display of ECG, respiratory rate, blood pressure, and temperature from five body sites in anesthetized rats during exposure to RFR. Heart rate is derived from real-time software analysis of ECG QRS complexes sampled via nylon-covered fluorocarbon leads that show a similar conductivity to biological tissue and do not affect the specific absorption rate in tissue. Respiration is monitored via a rubber bulb with Teflon® fitting connected to a semirigid polyethylene tube which is positioned under the rat's diaphragmatic area. This tube connects to a pneumatic pressure transducer which interfaces with an electrosphygmograph coupler. Respiratory waveforms are digitally smoothed and respiration rate is determined by counting peaks and troughs. Body temperature (left and right side subcutaneous, tympanic, colonic and tail) is sampled using Bowman type carbon-loaded Teflon®-coated probes. Five experimental series have thus far been completed utilizing this new technology.

29.8

CIRCULATORY SHOCK DURING SUPERFICIAL HEATING WITH MILLIMETER WAVES.

Melvin R. Frei*, James R. Jauchem, and Rick E. Berger*. Trinity University, San Antonio, TX., 78212, and U.S. Air Force Armstrong Laboratory, Directed Energy Division, Brooks AFB, TX., 78235-5301.

Recent development of hardware systems capable of generating millimeter waves (MMW) has spawned interest in the possible bioeffects and health hazards of incidental exposure. At present, no studies of the patterns of heat distribution and attendant physiological responses during whole-body irradiation in the MMW frequencies (30-300 GHz) have been reported. To gain knowledge of the effects of MMW electromagnetic radiation on whole-animal physiology, ketamine-anesthetized rats were exposed to 35 GHz continuous-wave radiofrequency radiation (RFR) at an average specific absorption rate of 13 W/kg (power density=75 mW/cm^2). During left lateral irradiation, colonic (T_c), left subcutaneous (T_s) temperature, ECG, arterial blood pressure, and respiratory rate were continuously monitored by a PC computer-based system. Upon initiation of irradiation, the T_s began to increase immediately, however, there was a 3-4 minute delay before T_c began to increase, indicating that circulatory transfer of heat from the periphery was responsible for internal heating. During the course of exposure, the T_s increase was significantly greater than the T_c increase (36 to 50°C vs. 36 to 39°C, respectively). Heart rate increased monotonically during irradiation, while mean arterial blood pressure increased initially and then decreased dramatically. Such a reaction is characteristic of circulatory shock seen during heat stroke and terminal exposure to RFR. However, these reactions normally occur at colonic temperatures of 41.5°C or higher, while in the present study they occurred at 38°C. These results suggest that extreme peripheral heating, such as occurs during MMW exposure, as well as core heating, can initiate circulatory shock.

29.9

DIFFERENCES IN THE COLD-INDUCED HEAT DEBT DETERMINED BY PARTITIONAL CALORIMETRY VS CONVENTIONAL METHODS.

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Core temperature (T_c) measurements at different sites sometimes produce different cooling curves in cold-exposed humans, suggesting that corresponding heat debts (S_{Tb}) would be different, when calculated by conventional methods [via the change in mean body temperature (T_b)]. We have also compared those S_{Tb} values to the heat debt obtained by partitional calorimetry (S). Nine subjects who showed similar initial but different final T_c [rectal (T_{re}) and tympanic temperatures (T_{ty})] during nude cold exposure (2h at 1°C at rest) were used. Using various well-known T_b weighing coefficients, S_{Tb} (from T_{re}) varied from 266 to 1409 kJ and from 404 to 1479 kJ with S_{Tb} (from T_{ty}). In contrast, S corresponded to 504±79 kJ, a level that could be reproduced with thermoneutral/cold T_b weighing coefficients of .818/.818 for T_{re} and .865/.865 for T_{ty}. The results demonstrate that conventional methods can in some cases greatly overestimate or underestimate heat debt. Since about the same effect was observed using either T_{re} or T_{ty}, these differences could not be explained by the site used as T_c. It is concluded that S_{Tb} in the cold does not appear, in some cases, as accurate and reliable as the S of partitional calorimetry.

29.10

SEASON AFFECTS SOCIAL & METABOLIC HABITUATION OF DEER MICE

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Prairie deer mice responded to pairing by increasing their metabolic rates during social encounters which followed placement of pairs in a novel environment. Markedly increased metabolic rates accompanied various intensities of agonistic behaviors during initial cohabitation, but declined from peak activity levels to 1/2 maximum levels at differing rates depending on the photoperiodic backgrounds to which mice had been conditioned. After social order was established through behavioral interactions, resting metabolic rates (RMR) further declined to new, lower steady states during amicable huddling. Achievement of the lower steady states of RMR by huddling pairs of deer mice was evident within 1 day of pairing if they were conditioned to Winter photoperiods; 3-4 day spans were required for habituation of mice which were conditioned to Summer light-cycles. The effects of placing solitary mice or habituated pairs of mice into new environments resulted in metabolic responses of lower amplitudes and shorter durations than expressed during novel mouse encounters. Rates of metabolic habituation to social stimulus provide measures of social compatibility between individual animals even when they suppress their agonistic behaviors. *Supported in part by an NIH-BRSG (RR0533901).

29.11

THE ROLE OF CATECHOLAMINES IN THE CARDIAC DYSFUNCTION OF SHOCK DUE TO SEVERE HYPOTHERMIA. H.I. Miller, M.E. Giaimo and T. Hughes. Dept. of Physiology, LSU Medical Center, New Orleans, LA 70112.

Severe, acute hypothermia of very short duration (15 min) produces a shock-like state after rewarming back to normal temperature (38.5°C). Some of the symptoms persisted up to 48 hr. A cardiac dysfunction was observed at 4 hours after return to normal temp and was seen up to 24 hr after. Guinea pigs with indwelling carotid artery and jugular vein catheters and a microthermistor bead maneuvered into the arch of the aorta, were anesthetized with Brevital®, a fast acting barbiturate. The anesthetized animals were immersed in an ice-distilled water bath and were removed when the core body temperature fell to 25°C. The guinea pigs were dried and wrapped in a heating pad. The core body temperature (BT) fell another 1.5°C before starting to rise. It rose rapidly, returning to normal in about 45 minutes. Our experimental period started at that time. Heart rate, blood pressure and cardiac output were measured. A small blood sample was taken at various time periods and blood gases, ph, hematocrit, free fatty acids, glucose, lactic acid and catecholamines were determined. Some of the animals were sacrificed at 4, 8, 24, and 48 hours. After being anesthetized, the hearts were excised, and mounted on a perfused working heart apparatus. By varying the left atrial pressure and cardiac output or stroke volume, Starling Curves were generated. Chilled animals developed flat starling curves while control animals had normal curves. During cooling and after, when catecholamines were extremely high (10x) heart rate and contractility increased modestly. It would appear that the shocked animals were less sensitive to catecholamines. (Supported by a grant from the Naval Medical Research and Development Command).

29.12

LIPOPROTEINS IN PLASMA OF FED VS FASTED POLAR BEARS G.Edgar Folk, Jr.*, Terry L.Kaduce, and Arthur A. Spector. Dept. Physiology & Biophysics, and Dept.of Biochemistry, The Univ. of Iowa, Iowa City IA 52242

Polar bears select blubber from the seals they kill, then discard the muscle. We obtained plasma from two captive polar bears living on self-selected seal fat; we compared this with plasma from two other bears apparently fasting for four months. Earlier we reported on plasma lipid; here lipoprotein data are presented, always comparing fed and fasted bears. In the earlier data lipids were higher in fasted bear plasma (for example, total cholesterol: fed 306mg/dl, fasted 381mg/dl). Some results from isolated lipoprotein fractions:

Serum Fraction	Phospholipids mg/dl		Triglycerides mg/dl		T.Cholesterol mg/dl	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
VLDL	7±1	10±3	30±7	49±10	9±2	27±12
HDL	308 ±19	421±15	5±1	16±6	170 ±6	165±18

1)HDL in both fed and fasted animals were much higher than both VLDL and LDL in phospholipidsand total cholesterol (TC), as in healthy human subjects. 2) The HDL triglyceride was very low as in human subjects. 3) Lipoprotein of fasted bears was much higher (with one exception) than fed bears. A plausible explanation is the presence of omega-3FA in the diet of fed bears. Supported by NIH (HL-39308).

29.13

COLD EXPOSURE DURING MILITARY MANEUVERS: EFFECTS OF PHYSICAL ACTIVITY LEVEL - SLEEP DEPRIVATION ON PERFORMANCE AND HYDRATION INDEXES. J.A. Hodgdon, R. Hesslink Jr., and A.C. Hackney. NHRC, San Diego, CA 92138.

This study examined the effects of physical activity level (high [Hi] and low [Lo]) and sleep (S) deprivation (2 h vs. 6 h) on soldiers conducting field maneuvers (3 d) in the arctic polar region of Norway during winter. Subjects (n=33) were divided in HiS2, HiS6, LoS2, and LoS6 groups. Performance (2 km snow shoe) and select hydration indexes (blood; Hct, Hb, % plasma volume change: urine; specific gravity, osmolality) were measured before, daily, and 2 days into recovery from the maneuvers. The largest performance decline ($p<0.01$) occurred during the first 24 h of the maneuvers, and was greater in the Hi than Lo groups ($p<0.01$). Blood measures showed activity level vs. time, and activity level vs. sleep deprivation interactions. These interactions suggested the Hi activity and 2 h sleep groups had greater degrees of hemo-concentration than the other groups. Urine measures were variable, but displayed changes ($p<0.01$) suggesting that renal concentrating occurred in all groups. Analysis of change values (experimental minus baseline) revealed the initial decline in performance was significantly related to % plasma volume reductions (rho = -0.41, $p<0.03$). We concluded that in the cold, high levels of physical activity and sleep deprivation are associated with apparent hypohydration. Furthermore, this hypohydration impacts performance capability in soldiers operating in cold weather.

37.1

MODULATION OF AORTIC SMOOTH MUSCLE CELL PROLIFERATION IN DIABETES MELLITUS. C. Alipui, K. Ramos and T. E. Tenner Jr. Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

The purpose of the current study was to determine if experimental diabetes mellitus modulates the proliferation of dispersed vascular smooth muscle cells (VSMC) in culture. VSMC were harvested from thoracic aortic segments excised from naive and diabetic rabbits to establish individual cell lines for each animal. The proliferative capability of cultures was determined by measuring [3H] thymidine uptake into cultures and cell growth was monitored. Serum-dependence of cultures was also evaluated. Culture morphology was similar in naive and diabetic groups. Diabetic cultures demonstrated enhanced proliferative capabilities relative to naive cultures. While the efficiency of cell attachment was not different between the two groups, diabetic cells grew faster and with a shorter population doubling time (41.08 +/- 4.15 hrs) than naive cells (58.08 +/- 6.79 hrs). Both culture groups required serum for maintenance of viability and the initiation of growth. Diabetic cultures were more severely affected by low serum concentrations (0.5% or less) than naive cultures but they outgrew naive cultures in medium containing 3% serum or more. These findings suggest that diabetes induces changes in VSMC proliferation consistent with atherogenesis.

37.2

HYPERINSULINEMIA, INSULIN RESISTANCE AND HYPERTRIGLYCERIDEMIA: RELATIONSHIP TO THE DEVELOPMENT OF HYPERTENSION IN RHESUS MONKEYS. Noni L. Bodkin, Judy S. Hannah, Heidi K. Ortmeyer, and Barbara C. Hansen.* Univ. of Maryland, Baltimore, MD 21201.

Considerable correlational evidence suggests the possibility that a fundamental defect may be common to hyperinsulinemia, insulin resistance, hypertriglyceridemia, and hypertension. The sequence of development and potential link(s) between these disorders are unknown. Twenty four rhesus monkeys (4.5-25.7 yrs; 5.8-23.8 kg) were studied. Measurements of plasma glucose and insulin; lipoprotein fractions, glucose tolerance (IVGTT-KG), blood pressure, and insulin resistance (M-euglycemic clamp) were obtained. In all subjects combined insulin resistance was significantly associated with plasma insulin ($r=-0.77$), triglyceride levels ($r=-0.62$), HDL cholesterol ($r=0.61$), and systolic ($r=-0.56$) and diastolic ($r=-0.42$) blood pressure (all p's <0.05). Weight-matched hyperinsulinemic subjects showed 2 distinct groups: hyperinsulinemic insulin resistant monkeys with normal blood pressure (111±6/58±6) and normal triglyceride levels (X±SD: 63±26 mg/dl) vs hyperinsulinemic, insulin resistant monkeys with hypertension (143±17/79±17) and normal or elevated triglyceride levels (X±SD: 168±127 mg/dl). These data show that hyperinsulinemia and insulin resistance, while generally associated with hypertriglyceridemia and hypertension, can occur independently, suggesting the lack of a common underlying defect.

37.3

ENDOGENOUS DIGITALIS LIKE SUBSTANCE (EDLS) MAY BE INVOLVED IN THE HYPERTENSION (HT) ASSOCIATED WITH STREPTOZOTOCIN (STZ) INDUCED INSULIN DEPENDENT DIABETES MELLITUS (IDDM) IN RATS.

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We have previously developed a consistent model of chronic HT associated with STZ induced IDDM in normotensive Wistar rats with 25% reduction in renal mass (RRM). In this study, we examined the role of EDLS in the development of HT in this model. Three groups of rats were studied, namely: 1) 25% RRM with STZ induced IDDM (25-DM), 2) normal rats with STZ induced IDDM (2K-DM), and 3) 25% RRM with vehicle treatment (25-V). Systolic blood pressure (SBP) progressively increased in 25-DM rats from 120 ± 2.1 to 152 ± 4.3 mmHg by the 3rd week after STZ treatment, which was associated with concurrent microalbuminuria, very low plasma renin activity and left ventricular hypertrophy. In contrast, 2K-DM and 25-V rats remained normotensive throughout. Furthermore, plasma levels of EDLS determined by RIA for digoxin (Baxter, MA) were significantly higher in hypertensive 25-DM rats relative to those in 2K-DM or 25-V rats. In the latter EDLS was undetectable. This increase in the EDLS in 25-DM rats was associated with a significant decrease in Na^+,K^+-ATPase activity in microsomes prepared from left or right ventricles. The EDLS level was inversely correlated (r=0.86, p<.001) with myocardial Na^+,K^+-ATPase activity and positively correlated with SBP (r=0.68, P<.005). The decreased Na^+,K^+-ATPase activity was not due to the changes in membrane area to cell volume ratio as a result of ventricular hypertrophy, because the 5'-Nucleotidase was unchanged. These results suggest that increased level of EDLS by inhibiting cardiovascular muscle cell Na^+,K^+-pump activity may play a role in the mechanism of hypertension associated with IDDM in rats.

37.4

THE CARDIOVASCULAR RESPONSE TO INSULIN AND 2-DEOXYGLUCOSE INDUCED GLUCOPENIA IN NORMAL AND DIABETIC RATS. Susan Schultz-Klarr*, Joyce Wright-Richey* and Joseph C. Dunbar. Wayne State University, Detroit, MI 48201.

We induced glucopenia using insulin-induced hypoglycemia or 2-deoxyglucose (2-DG) and monitored their effects on cardiovascular responses in normal and diabetic rats. Acute hypoglycemia induced by insulin infusion (2.0 U/kg) resulted in a 27% decrease in mean blood pressure (BP) in normal rats. However, in diabetic rats the decrease in BP in response to insulin-induced glucopenia was more pronounced (41% BP) when compared to normal controls. Diabetic rats were bradycardic when compared to controls but the heart rates were not significantly altered by insulin-induced hypoglycemia in either group. When glucopenia was produced by 2-DG in normal rats the counter-regulatory response was evident by a 200% increase in plasma glucose. No significant increase in plasma glucose occurred in diabetics. 2-DG initially decreases the mean BP in normals but was subsequently increased after 30 minutes. The heart rates were also increased 24%. The diabetic animals had a 40% decrease in mean BP as well as a 10% decrease in heart rate. These studies suggest that diabetic rats have an enhanced hypotensive response to insulin-induced glucopenia as well as 2-DG induced glucopenia. These results support the concept that insulin and glycemia may have an effect in mediating cardiovascular responses independent of well established sympathetic counter-regulatory mechanisms.

37.5

MODIFICATION OF THERAPY OF TYPE II DIABETIC MELLITUS FROM INSULIN TO SULFONYLUREAS FAILS TO DECREASE BLOOD PRESSURE IN SPITE OF CONCOMITANT WEIGHT LOSS. **JK Schmitt* Dept of Medicine, VA Medical Center, Richmond, Va 23249**

Insulin has been reported to cause weight gain and sodium retention. Furthermore, it has been postulated that the hypertension of type II diabetes mellitus is, in part, due to insulin resistance, which may be reversed by sulfonylureas. These factors suggest the possibility that modification of therapy of type II diabetes from insulin to sulfonylureas decreases blood pressure. To investigate this phenomenon, the records of 1140 type II diabetics seen sequentially over 4 years were reviewed. Forty-five patients were found whose medication had been changed to 1st generation sulfonylureas for at least 3 months (Mean Interval 12±1 mos). Body weight index (%IBW), systolic BP (SBP) and diastolic BP (DBP) follow: (Mean ± SEM).

Rx	%IBW	SBP(mmHg)	DBP(mmHg)
insulin	131±4	135±3	81±1
sulfonylurea	125±3	140±3	80±1
P	<.001	.06	NS

There was no significant difference in diabetic control on the two treatments.

In conclusion, altering medication from insulin to sulfonylureas fails to reduce blood pressure, even though weight loss occurs. In fact, systolic BP tends to increase. This would suggest the possibility that sulfonylureas have a clinically significant hypertensive action.

37.6

HEPATIC GLUCOSE PRODUCTION AND INTRACELLULAR CALCIUM MOBILIZATION DURING SEPSIS. Subir R. Maitra, Wansong Pan, Evan R. Geller. Division of Trauma, Department of Surgery, School of Medicine, State University of New York, Stony Brook, New York.

Severe sepsis is associated with hypoglycemia and hyperlacticacidemia. Hypoglycemia may be due to depressed hepatic glucose production(HGP) in response to lactate as a gluconeogenic substrate. Since gluconeogenic pathways involve several calcium sensitive steps, the present study evaluated HGP in response to lactate and intracellular free Ca^{++} concentration $[Ca^{++}]_i$ in hepatocytes during this hypoglycemic phase of sepsis. Sepsis was induced in fasted, anesthetized rats by cecal ligation and puncture(CLP). 18 hours after surgery, CLP(n=10) and control(C)(n=10) rats were anesthetized and blood samples were obtained for determination of plasma glucose(PG) and plasma lactate(PL). Liver perfusion in vivo was employed to measure HGP. Hepatocyte $[Ca^{++}]_i$ was determinted using the Ca^{++} selective indicator Fura-2, under basal and epinephrine stimulated conditions.

	PG	PL	HGP(uM/gm/hr)			$[Ca^{++}]_i$(nM)	
	mg/dl	mM	Lactate Perfusion(mins)			Basal	Epi.10^{-5}
			0	5	10		
C	115.7	1.4	16	17.7	20.1	217	335
	±4.9	±0.1	±1.3	±1.2	±1.5	±39	±57
CLP	81.8*	3.7*	8.5	9.4	11.9*	391	424
	±9.0	±0.4	±0.9	±10.9	±1.3	±125	±147

*p<.05 compared to C

CLP was associated with hypoglycemia and hyperlacticacidemia. In CLP rats, HGP in response to lactate and hepatocyte $[Ca^{++}]_i$ in response to epinephrine is attenuated. We conclude that substrate specific stimulation of gluconeogenesis and hormone mediated mobilization of $[Ca^{++}]_i$ is altered in the livers of rats subjected to CLP.

37.7

LEAN (LBM) AND FAT (FBM) BODY MASS MEASUREMENTS USING EM-SCAN MODEL SA-2. L.L. Bellinger and F.E. Williams, Dept. of Physiology, Baylor College of Dentistry, Dallas, Texas 75246.

The SA-2 measures LBM directly and indirectly calculates FBM in the anesthetized rat. There have been problems with the initial prediction equation; however, a new equation has been supplied for rats tested in the supine position and the peak mode. In the present study nine Sprague Dawley rats were anesthetized, weighed, length measured and placed in the SA-2. To simulate increases in LBM the rats (171±4g body weight, [BW]) were injected, at multiple sites, with 5, 10, 15, and 20 ml of 0.9% NaCl. A week later the above was repeated (182±5g BW), but the rats were injected with oil to simulate changes in fat mass. SA-2 predicted LBM and FBM were compared to calculated changes. FBM at the start of each trial was 8.4±0.5 and 9.0±1.3% of BW. SA-2 and calculated total LBM were similar (SA-2 vs calculated (g): 0 ml, 157±3.0; 5 mls, 164±2.8 vs 164±3.0; 10 mls, 172.9±2.4 vs 171.1±3.0; 15g, 182.0±3.5g vs 177.9±3.0; 20 mls, 192.0±3.6 vs 184.7±3.0, all nonsignificant). Δ LBM showed a slight tendency of SA-2 to over predict LBM, but r = 0.88, P<0.001. When oil was added LBM did not change (LBM [g]: 0 ml, 166±5.8; 5 mls, 166±5.0; 10 mls, 165±5.4; 15 mls, 165±4.8; 20 mls, 166±4.6). SA-2 predicted and calculated Δ FBM were similar, r = 0.79, P<0.001. SA-2 could show (P<0.001) a 5g change in LBM (157±3.0 vs 164±2.8, P<0.01) or FBM (16.1±2.2 vs 22.2±2.0g, P<0.01). Although within group variation is a concern with SA-2, the data indicate it could be a useful research tool. Supported in part by funds from Baylor College of Dentistry and NIH #DEO9725-01.

37.8

FASTED SERUM INSULIN IS A KEY DETERMINANT OF HDL-CHOLESTEROL (HDLC) IN FIT YOUNG MEN AND WOMEN. Louis J. Marchitelli & Karl E. Friedl, Occup Physiol Div, US Army Res Inst Environ Med, Natick, MA 01760-5007.

A variety of determinants of HDLC have been identified in populations usually characterized by a wide range of age, health, and fitness habits. In order to test the strength of these associations with HDLC earlier in life, we examined these factors in a group of nonsmokers which was homogeneous for young age (19-22 yrs), high fitness, and high activity. Blood samples were obtained following an overnight (10 h) fast in a select sample of 108 male (BMI=24.9±2.4 kg/m^2; %BF= 14.1±3.3) & 83 female (BMI=22.6±1.8 kg/m^2; %BF=23.5±3.2) West Point cadets. HDLC was measured by dextran-sulfate precipitation, with standardization in a Lipid Research Laboratories program. Testosterone (T), estradiol-17b, insulin (INS), & DHEAS were measured by RIA; SHBG was measured by IRMA. These endocrine parameters, %body fat from 4 skinfolds, waist/hips girth, subscap/triceps skinfolds, cholesterol, triglycerides, and family medical history were analyzed in stepwise multiple regression models. HDLC and cholesterol means were 45.0±9.3 (22-71), 56.1±11.3 (35-93) mg/dl & 153.3±29.8 (76-248), 157.5±11.3 (100-240) mg/dl for men & women, respectively. For men, INS, %body fat, cholesterol, & family history of hypertension were independently associated with HDLC, accounting for 25% of the variance (r=0.496); for women, only INS & cholesterol emerged as significant factors (r=0.499). Regression models of the combined male & female samples yielded INS & cholesterol, but subscap/triceps skinfold & T/SHBG ratios emerged as gender discriminators of HDLC. The strength of the relationship between INS and HDLC suggests a key role in the hepatic regulation of HDLC by INS; truncal and abdominal fatness, which may play a role in elevating INS, show weaker trends and are replaced in the male regression by INS, while fatness is not related to HDLC in the women.

37.9

PROXIMAL TUBULAR DYSFUNCTION AS A CAUSE OF DIABETIC MICROALBUMINURIA. H. Ha*, A. Tojo* and H. Endou* (SPON: K. Kim). Yonsei University, Seoul, Korea and University of Tokyo, Tokyo, Japan

We developed a new fractional micropuncture method which effectively exclude contamination of extratubular proteins. Renal tubules were punctured using an outer puncture pipette, into which an inner collection pipette was repeatedly inserted to collect tubular fluid usually up to the fourth fraction. Utilizing this new method, the possible mechanisms for diabetic microalbuminuria was investigated. Streptozotocin treated rats (STZR) with uncontrolled diabetes exhibited significantly increased urinary excretion of albumin (Alb), when studied 2 weeks after injection of STZ (50 mg/kg, i.v.). The glomerular filtration (%) of plasma Alb and low molecular weight proteins (LMWP) were 0.023 and 62.6 for STZR and 0.062 and 98.9 for age-matched control rats (CR), respectively. The tubular reabsorption of Alb and LMWP, especially in early proximal tubules of STZR, were prominently inhibited. Respective values for percentage reabsorption of glomerular filtrated proteins in early PCT, late PCT and DCT were 22 , 22 and 37 for Alb and 19, 45 and 0 for LMWP in STZR and 37, 34 and 23 for Alb and 54, 28 and 5 for LMWP in CR. These data provide evidences that microalbuminuria in early phase of diabetic nephropathy is caused by the inhibition of tubular protein reabsorption without increment of glomerular protein filtration. This functional abnormality was further supported by morphological observation which revealed deformed microvilli such as clubbing formation, and reduced microvilli and endocytic vacuoles in proximal convoluted tubules prior to glomerular leison in STZR. In addition, the extremely increased urinary excretion of thiobarbituric acid adductable malondialdehyde, as an index of lipid peroxidation (9.3±1.8 vs. 0.25±0.04 umol/24 hr, STZR vs. CR, p<0.0001) was originated from not only twice increment in plasma but also early proximal tubules.

37.10

ENHANCED CELLULAR AMYLASE CONTENT AND SUPPRESSED EXOCRINE PANCREATIC FUNCTION BY NICOTINE IN RATS. P. Chowdhury, R. Doi*, A. Tangoku*, and P.L. Rayford, Dept. of Physiol & Biophys, Univ. of AR for Med. Sci., Little Rock, AR 72205.

Studies indicate that nicotine has an adverse effect on pancreas. In the current study, we examined the effect of nicotine on cellular amylase content and exocrine pancreatic function in rats. Method: Two groups of rats were used in the study. Control group received ad-lib food and water; the nicotine group received ad-lib food and water containing 0.77 mmoles of nicotine. During the 28 day treatment period, the rats were monitored daily for body wt gain, food and fluid intakes, and then sacrificed. Panc. wet wt. was measured and acinar cells were isolated by collagenase digestion. The total amylase content and the ability of acinar cells to release amylase in response to CCK-8 (10^{-10}M) were determined.

Results: (Mean ± S.E.)	Control	Nicotine	
Body wt (g)	334 ± 11.6	298 ± 6.5	p<0.05
Panc wt (g)	1.37 ± 0.06	1.31 ± 0.03	
Amylase content (mU/mg)	47.7 ± 1.68	57.3 ± 3.29	p<0.05
Amylase release (mU/mg)	12.9 ± 0.05	6.2 ± 0.4	p<0.05

Nicotine ingestion significantly enhanced cellular amylase content; however, cellular amylase release was significantly decreased in nicotine treated rats. Conclusion: Increased enzyme (amylase) storage may result from impairment of secretory processes due to nicotine.

37.11

CASTRATION AND/OR TESTOSTERONE PELLETS AFFECT SPLEEN WEIGHT AND LYSOSOMAL ENZYME FUNCTION IN SYRIAN HAMSTERS. M.K. Vaughan, A. Menendez-Pelaez*, J.P. Chambers* and R.J. Reiter. Univ. Texas Health Sci. Ctr., San Antonio, TX 78284 and Univ. Texas at San Antonio, San Antonio, TX 78285

Sex differences in spleen weight and hematopoiesis can be modulated by castration and/or gonadal regression (Ravines, Lab. Invest. 10: 341-353, 1961; Vaughan et al., Amer. J. Anat. 179: 131-136, 1987).. In the present study, groups (7-8/group) of intact male and female hamsters and a group of castrated males were implanted sc with testosterone pellets (TP; 4 mg TP/24 mg beeswax) at 30 days of age; intact or castrated hamsters served as controls. Females received a second implant 2 weeks later. All animals were killed at 60 days of age. Castrated males ($p < 0.01$) and intact females ($p < 0.001$) had elevated spleen weights compared to intact males and this increase was totally prevented by TP. Females treated with TP had higher hexosaminidase enzyme activity in their spleens than did control females ($p < 0.001$) or intact males ($p < 0.05$). Castrated males ($p < 0.025$) and control females ($p < 0.05$) had higher acid phosphatase enzyme activity compared to intact control males and this was not influenced by TP treatment. Control female hamsters had lower ß-glucuronidase activity than did intact males ($p < 0.05$). However, TP treatment elevated ß-glucuronidase activity in intact males ($p < 0.001$), castrated males ($p < 0.01$) and females ($p < 0.01$) compared to their respective non-implanted controls. In conclusion, castration and/or TP treatment significantly affected spleen weight over a 30 day period; thus, the Syrian hamster spleen should be considered an androgen-dependent organ. Since administration of testosterone often induces an increase in ß-glucuronidase activity in androgen-dependent organs (e.g. mouse kidney), the increased ß-glucuronidase activity of all TP-treated groups provides further evidence for the androgen-sensitivity of the hamster spleen.

HYPERTENSION

38.1

INSULIN (I) DIFFERENTIALLY MODULATES SYSTEMIC AND SKELETAL MUSCLE VASCULAR RESISTANCE IN MAN. IMPLICATIONS FOR INSULIN RESISTANCE AND HYPERTENSION. Alain D. Baron, M.D. and Ginger Brechtel, R.N.*, Indiana University and VA Medical Center, Indianapolis, IN 46202-5124

To study the acute metabolic and hemodynamic effects of I, 3 groups (n=8 each) of age and weight matched lean healthy men were studied at baseline (I level ~8 μU/ml) and during a 3 hour euglycemic hyperinsulinemic clamp at [I] (mean ±SEM) of 35±4 (low I), 86±8 (mid I) and 2240±290 μU/ml (high I). Glucose uptake (GU) in whole body (WBGU) and leg muscle (LGU), leg blood flow (LBF, thermodilution), cardiac output (CO, dye dilution), mean arterial pressure (MAP, invasive monitoring) were measured at each [I] and during a 3 hour saline control study. LGU=LBF x arteriovenous glucose difference. Mean percent changes from baseline (Δ±SEM) are shown:

[I]	%ΔMAP	%ΔCO	%ΔLBF	%ΔSVR⁺	%ΔLVR⁺
Low	-4.7±1.6*	5.9±1.6	88±18**	-11±2*	-46±5**
Mid	-5.3±1.2*	12.3±4.4*	64±11**	-15±5**	-40±4**
Hi	-10±3**	26±9**	73±23**	-26±6**	-42±6**

*p<0.05, **p<0.01 vs basal ⁺SVR/LVR: Systemic/leg vascular resistance

LBF and MAP were unchanged from baseline during saline alone. I produced a greater relative fall in LVR than in SVR at all levels. ΔCO and ΔLBF were highly correlated r=0.73, p<0.0001. Thus, physiological I increases and redistributes CO to SKM. LVR and ΔMAP were correlated with WBGU r= - 0.54, p<0.0001 and r=0.44, p<0.02, respectively. LGU was also correlated with ΔSVR, r = 0.6 p<0.001. Since >80% of WBGU occurs in skeletal muscle (SKM), I's effect to increase SKM blood flow by modulating CO and regional vascular resistance is an important mechanism for increasing WBGU. Impaired ability of I to increase SKM blood flow, as seen in the I resistant state of obesity (JCI, 85:1844-52, 1990) may be an important mechanism for 1) the higher incidence of hypertension in I resistant states, and 2) I resistance in essential hypertension.

38.2

ALTERATIONS IN INSULIN-SENSITIVE (GLUT4) GLUCOSE TRANSPORT PROTEIN IN DEOXYCORTICOSTERONE (DOC)/SALT HYPERTENSIVE RATS. S.R.Chipkin, G. Thoidis*, W. Mamuya*, N. Kotliar*, L.Coderre*, P. Pilch*, Boston University School of Medicine, Boston, MA 02118

Insulin resistance has been postulated to play a role in the pathophysiology of hypertension (HTN). We investigated whether the DOC/SALT model of HTN produces a decrease in GLUT4 similar to those found in other models of insulin resistance. Wistar rats underwent uninephrectomy (UNI) and were compared with uninephrectomized rats receiving deoxycorticosterone (UNI/DOC) and uninephrectomized rats that received deoxycorticosterone plus 0.9% saline in their drinking water (UNI/DOC/SALT). Total cellular membranes were prepared from four different insulin sensitive tissues: heart, fat, red gastrocnemius and white gastrocnemius muscle. GLUT4 levels were determined by Western analysis using the monoclonal antibody 1F8.

	BP	Heart Wt/BodyWt	GLUCOSE
UNI	115 ± 4	3.00 ± .04	180 ± 15
UNI/DOC	146 ± 10	3.51 ± .06	198 ± 17
UNI/DOC/SALT	183 ± 7	4.15 ± .09	201 ± 17

Western analysis of left ventricle demonstrated a 40% decrease in GLUT4 in UNI/DOC/SALT compared to UNI. The differences between each of the treatment groups and UNI rats were statistically significant. Red gastrocnemius did not significantly differ in either treated group compared with UNI. In white gastrocnemius, levels of GLUT4 were equivalent between UNI and UNI/DOC but increased by two-fold in UNI/DOC/SALT. We conclude that the regulation of GLUT4 in DOC/SALT HTN is tissue specific. Changes in heart are consistent with insulin resistance and may have implications in cardiac fuel metabolism. Supported by NIH# DK01901, DK30425, Howard Hughes pre-doctoral fellow (WM), Canadian Diabetes Foundation (LC).

38.3

INSULIN'S EFFECT ON BLOOD PRESSURE IN HYPERTENSIVE RATS.
Deborah A. Martin* and R. James Barnard. UCLA, Los Angeles. CA 90024

The Spontaneously Hypertensive rat (SHR) is hyperinsulinemic and insulin resistant. The role of insulin in systolic blood pressure (SBP) regulation in the SHR is presently investigated. In Part I, SBP was measured in SHR and Sprague Dawley (SD) using the tail-cuff method after streptozotocin treatment (STZ) and following subsequent daily insulin injection to normalize blood glucose. (*p<.05 vs. control;(n))

SBP mmHg	control	STZ 45mg/kg	STZ 75mg/kg	insulin
SHR	182±5(10)	165±4(12)*	145±3(7)*	183±4(8)
SD	119±3(10)	125±4(9)	121±2(7)	124±3(12)

In part II, direct SBP was monitored while insulin or control vehicle was infused intravenously for 75 minutes under euglycemic clamp in STZ-treated (45mg/kg), unanesthetized SHR and Wistar Kyoto (WKY) rats. After noting ΔSBP with insulin infusion, hexamethonium (HM) was then injected (40mg/kg), and its effect was noted (HMSBP). Pilot studies showed no SBP change with maximum volume infusion of control vehicle.

SHR	0mu/kg.min	10mu/kg.m	WKY	0mu/kg.m	15mu/kg.m
Δ SBP	1±2.3(4)	20±4(4)*	Δ SBP	1±1.1(5)	1±2.5(4)
HMSBP	81±9(4)	71±1(4)	HMSBP	63±3(5)	66±4(4)

SHR showed a significant increase in SBP with insulin infusion or long-term insulin treatment in contrast to normotensive controls. Sympathetic nervous system activation is implicated as the mechanism of SHR pressor action since HM following insulin infusion reduced SBP in the SHR to a similar level as observed in HM-treated WKY. Making special note of the abnormal insulin metabolism in SHR, it is possible that its dramatic SBP response to insulin is involved in the mechanism of SHR hypertension.

38.4

INSULIN RESISTANCE PREDIATES HYPERTENSION (HTN) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).
Mohsen Lachaal, MD* and Chan Y. Jung, Ph.D. VA Medical Center, SUNY at Buffalo, New York 14215

The hypothesis that HTN is an insulin-resistant state has gained support and more acceptance over the last few years. This hypothesis was best demonstrated in the animal model as evidenced by impairment in insulin-stimulated glucose uptake and hyperinsulinemia shown to be present in the SHR. In order to understand this relationship we studied 3-O-methyl-D-glucose (3-OMG) flux into adipocytes isolated from SHR and their control group Wistar-Kyoto rats (WKY). We looked into basal and insulin stimulated glucose uptake in these rats at different ages starting at 4 weeks of age prior to the development of HTN in the SHR up to 50 weeks. We found that insulin stimulated 3-OMG flux is reduced by 50% in the adipocytes from SHR compared to WKY rats. This reduction occurred at the age of 4 weeks at its maximum extent prior to the development of HTN and persisted thereafter up to 50 weeks. We conclude that a defective glucose transport system exists in SHR and predates the development of HTN.

38.5

PRESSOR RESPONSE TO INHIBITION OF ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) IS AMPLIFIED IN HYPERTENSION.
William H. Beierwaltes, D'Anna L. Potter*, Charles S. Kiell*, Alfredo A. Pegoraro*, David H. Sigmon* and Oscar A. Carretero. Henry Ford Hospital, Detroit, MI 48202

Inhibition of EDRF increases blood pressure (BP). Since endothelial dysfunction has been associated with hypertension *in vitro*, we studied whether the response to EDRF inhibition *in vivo* would be altered in different models of hypertension. We used adult male anesthetized rats. BP was monitored by a femoral catheter. EDRF was inhibited with 10 mg/kg bw N$^{\omega}$nitro-L-arginine (N-Arg). We studied the BP response to N-Arg in: 9 anephric rats (NX), 20 control rats (C), 6 Dahl Salt-sensitive rats (Dahl SS), 6 2k,1c renal hypertensive rats (2k,1c) and 8 rats acutely hypertensive by carotid occlusion (C-O):

GROUP	NX	C	Dahl SS	2k,1c	C-O
basal BP (mmHg)	104 ±5	101 ±2	151 ±5	157 ±5	135 ±4 (SE)
Δ+N-Arg (mmHg)	11 +3	22 +1	33 +3	47 +6	58 +7

The pressor response to EDRF inhibition is not consistent with the basal BP, but does seem to follow the expected angiotensin- (and in C-O, catecholamine-) dependence of each model. Our results suggest the BP response to EDRF inhibition is amplified in hypertension but not as a function of basal BP. These responses may reflect an interaction between EDRF and endogenous vasoconstrictors associated with hypertension. (HL28982)

38.6

CHANGES IN THE NET WHOLE-BODY TRANSCAPILLARY FLUID FLUX DURING ANGIOTENSIN II HYPERTENSION.
Jorge Valenzuela-Rendón and R. Davis Manning, Jr. Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi 39216-4505.

The objective of this study was to investigate the chronic roles of the net whole-body transcapillary fluid flux and lymph flow in the distribution of extracellular fluid volume (ECFV) during angiotensin II (ANG II) hypertension. Hypertension was induced by constant intravenous infusion of ANG II (25 ng/kg/min), and the data was collected from 11 conscious dogs. By the second day of ANG II infusion, the net whole-body transcapillary fluid flux had increased 45% above control, the thoracic duct lymph flow had increased 58%, and plasma volume, ECFV (sulfate space), and interstitial fluid volume remained close to control. Also, by the second day of ANG II infusion, the fluid transport by a peripheral subcutaneous lymphatic in a hindlimb was not increased, and the permeability-surface area product of this region decreased 40%. The origin of the increased thoracic duct lymph flow probably was from the splanchnic bed. In conclusion, during ANG II hypertension the net whole-body transcapillary fluid flux increased. However, edema formation was prevented by the increased lymph flow. (Supported by NIH Grant HL11678 and Universidad Autónoma de Nuevo León, México.)

38.7

RELATIONSHIP OF PLASMA RENIN ACTIVITY TO CARDIOVASCULAR PARAMETERS IN THE RHESUS MONKEY.
K. A. Gruber and S. Opava Stitzer. Univ. of Puerto Rico, San Juan, PR 00936

Previous reports have found no relationship between plasma renin activity (PRA) and arterial pressure in rhesus monkeys (RM). We have been studying the relationship of endocrine factors to cardiovascular (C-V) parameters (systolic, diastolic, and mean arterial pressure, and heart rate) in a troop of RM. Studies were conducted under ketamine anesthesia with oscillometric monitoring of indirect arterial pressure and drawing venous blood for endocrine assays. When we compared PRA and plasma aldosterone (ALDO) to C-V parameters we could find no relationship. However, we noted a rather wide range in ALDO levels (3-131 ng%), raising the possibility that some of our subjects might be responding to the stress of trapping. We divided our population into low and high ALDO groups, using a cutoff of 20-25 ng% (effectively dividing the population in half). The two groups did not show differences in C-V parameters. However, when we analyzed our data with a Principal Component procedure, we found a direct correlation (0.7-0.8) between PRA and C-V parameters only in low ALDO RM (n=5). To confirm these observations we repeated these studies in a second group of low ALDO RM (n=6). We again found a strong correlation (0.6-0.8) between PRA and C-V parameters. The results suggest that a subset of RM with ALDO < 20-25 ng% may have renin-dependent arterial pressure regulation.

38.8

EFFECT OF CHRONIC TREATMENT WITH CLONIDINE AND SPIRONOLACTONE ON COLD-INDUCED ELEVATION OF BLOOD PRESSURE.
Melvin J. Fregly, Andreas Baron* and Anne Riesselmann*. Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610.

The present study was designed to determine whether antihypertensive agents known to affect the renin-angiotensin-aldosterone (RAA) system might affect the elevation of blood pressure induced by chronic exposure to cold. Spironolactone, a mineralocorticoid receptor blocker, was added to food and administered to rats chronically exposed to cold. In addition, clonidine, an alpha$_2$-adrenergic agonist and inhibitor of renin secretion, was administered to another group of cold-exposed rats by daily intraperitoneal injection. A warm-adapted and a cold-treated control group were also used. Chronic administration of spironolactone prevented the development of hypertension, but failed to prevent other adaptive physiological changes characteristically occurring during exposure to cold and seen in the cold-treated control rats. Thus, increased weight of the heart, kidneys, adrenals, and brown adipose tissue; increased dipsogenic responsiveness to angiotensin II; increased urinary outputs of norepinephrine and epinephrine, and increased food and water consumption were observed in all rats, treated and untreated, during exposure to cold. Similarly, daily injection of clonidine attenuated the elevation of blood pressure, but also failed to prevent the other adaptive physiological changes characteristic of cold. These results are consistent with the hypothesis that the RAA system plays a role in the development of the cold-induced elevation of blood pressure. (Supported by grant HL39154-04 from the National Heart, Lung, and Blood Institute, Bethesda, MD)

38.9

THE EFFECT OF ESTROGEN AND FOOD RESTRICTION ON BLOOD PRESSURE IN DOCA/NaCl RATS. Andra DeCarlo, O. Shechtman and M.J. Katovich. Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 36210.

There is a documented sex difference in the development of DOCA/NaCl hypertension in rats which may be associated with sex steroids. It has also been suggested that estrogens (E_2) are protective for cardiovascular diseases. We have previously shown that both castration and E_2 treatment reduced blood pressure in DOCA/NaCl male rats. However, E_2 treatment was also associated with a significant reduction of body weight (BW). The purpose of the present study was to determine whether E_2 treatment or reduced BW is responsible for the observed decrease in blood pressure. Male rats were randomly divided into 4 groups. One group served as controls and the remaining animals were unilaterally nephrectomized, administered a 25-40 mg pellet of DOCA, and maintained on a 1% NaCl drinking solution. The second group of rats served as DOCA controls while the third group was administered a 0.5 mg E_2 pellet. The fourth group did not receive the E2 pellet, but was pair-fed with the E2-treated group. Blood pressures and BW were monitored for the next 5 weeks. Food and water intakes as well as urinary output of Na^+ were measured. Terminal blood was collected for determination of sodium, potassium and E_2 concentrations. DOCA/NaCl treatment resulted in an increased blood pressure, decreased BW, increased fluid intake, and elevated urinary excretion of sodium and potassium. Both E_2 treatment and food restriction resulted in a similar reduction in blood pressure. Also, BW, food and water intakes, and urinary excretion of sodium did not differ significantly between these two groups. Therefore, the results suggest that the antihypertensive effect of this dose of E_2 may be mediated by the restricted food intake or the corresponding lowering of BW. (Supported by American Heart Association, Florida Affilliate)

38.10

EFFECT OF DIETARY SALT ON THYROID FUNCTION IN THE DAHL RAT. L.J. Huffman*, M.A. Boegehold, J.M. Connors, and G.A. Hedge. Departments of Physiology and Medicine, West Virginia University Health Sciences Center, Morgantown, WV 26506

Alterations in thyroid hormone levels have been linked to abnormalities in cardiovascular function. The aim of this study was to evaluate the role of the thyroid in salt-sensitive hypertension by determining whether thyroid status differed among Dahl salt-sensitive (DS) and salt-resistant (DR) rats on low (0.45%) or high (7.0%) NaCl diets. Thyroid function was assessed by measuring thyroid weights, thyroid blood flows (by microspheres), and plasma thyroxine (T_4) and thyrotropin (TSH) levels (by RIA) in anesthetized rats after 4 weeks of dietary salt treatment. Mean arterial blood pressure (MABP) for DS on low NaCl intake was significantly higher than that for DR on low NaCl (124±5 *vs.* 104±3 mmHg). High NaCl intake had no effect on MABP in DR, but produced hypertension (145±1 mmHg) in DS. There were no differences in thyroid weights, thyroid blood flows, or plasma [TSH] among groups ($p>.05$). However, plasma [T_4] was significantly lower in DS rats given a low salt diet compared to levels in DR rats. Furthermore, ingestion of a high NaCl diet lowered plasma [T_4] in DR rats (2.75±0.58 μg/dl *vs.* 5.06±1.04 μg/dl in DR rats given a low NaCl diet), but did not result in further reductions in [T_4] in DS rats. These results may reflect the existence of a strain difference in plasma [T_4] between DS and DR rats or indicate that reductions in plasma [T_4] are related to elevations in MABP in DS rats. While the contribution of this phenomenon to the overall cardiovascular status of the DS rat is not clear, we speculate that the observed decrease in [T_4] in DR rats on a high salt diet may subserve a protective function since experimental reductions in thyroid hormones can prevent rises in blood pressure induced by some hypertensive stimuli (NIH HL-44012 and NSF DCB-8904470).

38.11

HIGH SALT DIET INDUCES RENAL HYPERPLASIA IN YOUNG BORDERLINE HYPERTENSIVE RATS (BHR). William R. Jacobs*, Brynn Jones*, Pamela Stott*, James E. Lawler and Eddie S. Moore*. Depts of Pediat. and Psychol. Univ. of Tennessee, Knoxville TN.

Increased dietary Na intake elevates blood pressure and alters renal function in adult BHR. The present study was undertaken to evaluate whether Na intake affects renal development in young BHR. Animals were weaned at 3 weeks of age to normal rat chow and divided into either control (1.0% NaCl) or high salt (8% NaCl) groups at 4 weeks of age. Blood pressure was monitored by tail cuff to 10 weeks of age when kidney weight, DNA and protein contents were determined. BP in the high salt group was elevated between 5-9 weeks of age, $P < 0.05$, with no increase observed 3 days after high NaCl intake. No difference in body weight was observed between the two groups. However, kidney wet weight was 14% greater in the high salt group (from 2.8 ± 0.1 to 3.2 ± 0.05 grams, $p<0.05$). Both total kidney protein and DNA were elevated in the high salt group. When corrected for kidney wt, DNA content was significantly elevated, 29%, in the high salt group (from 12 ± 0.6 to 15.5 ± 1.1 mg DNA / g wet wt, $p<0.05$) with no difference in protein content (from 173 ± 5 to 186 ± 11 mg protein / g wet wt). Preliminary measurements of glomerular filtration rate (24 hr creatinine clearance) suggest no difference between control and high NaCl groups. The increase in DNA/g kidney wt is interpreted as evidence for salt induced renal hyperplasia in young BHR.

38.12

CHARACTERIZATION OF THE CHRONIC PRESSURE-NATRIURESIS RELATIONSHIP IN GENETICALLY HYPERTENSIVE DOGS. Paula E. Papanek, Kenneth C. Bovee*, Meredith M. Skelton*, Allen W. Cowley, Jr. Med. Coll. Wis., Milw. WI. 53226

A genetic model of essential hypertension in the dog developed at the University of Penn. termed Penn. Hypertensive Dogs (PHD)was studied to determine the relationship between the mean arterial pressure (AP), hormone, and renal excretory response to 4 different levels of Na intake (5,40,120,240 mEq/day) delivered i.v. isotonically. AP and heart rate were recorded beat-by-beat, 24 hr/day in 8 dogs. Water and Na balances were determined daily for 4 days at each level of intake and blood samples taken on the last day of each salt step for the analysis of plasma renin activity(PRA), atrial natriuretic peptide(ANP), aldosterone(ALDO) and vasopressin (AVP). While Na was retained (37 ± 3 mEq) during the first day of each increase of salt intake, a return to balance was observed within 4 days. The steady state chronic pressure-natriuresis relationship was shifted upward from that seen in normal dogs but was not significantly changed over a large range of sodium intake. AP was characterized, however, by significant moment to moment lability throughout the day. Plasma hormone levels responded in a manner similar to normal mongrel dogs with reductions of PRA, ALDO, elevations of ANP and no change in AVP. The PHD is similar in many ways to a subset of humans with essential hypertension indicating an important role for PHD in future studies of genetic hypertension. Supported by NIH #HL35435 & #HL29587.

38.13

EFFECTS OF A NEUTRAL ENDOPROTEASE INHIBITOR(NEP), THIORPHAN, ON BLOOD PRESSURE (BP) AND URINE OUTPUT IN SHR MB Pamnani, FL Douglas, C Yuan, S Chen, JF Schooley, FJ Haddy, RD Ghai: Department of Physiology, Uniformed Services University, Bethesda, MD 20814 & Ciba Geigy Corp. Pharmaceutical Division, Summit, NJ. 07901

Thiorphan enhances the effects of atrial natriuretic peptide (ANP) *in vivo*. We have shown that iv infusion of thiorphan causes a similar decrease in BP in four different models of hypertension (HT) in rats, suggesting that the vasodepressor action of thiorphan is independent of the mechanism of HT. Thiorphan not only inhibits degradation of ANP *in vivo* it also inhibits conversion of AI to AII by inhibiting the angiotensin converting enzyme (ACE). We examined the role of the renin-AII (R-AII) system in the vasodepressor response to thiorphan in the SHR. We also studied the combined effects of thiorphan and C-ANP_{4-23}, a C-ANP clearance (C) receptor blocker. For the role of R-AII system, SHR were anesthetized with inactin and instrumented to record BP, and for collection of urine. All animals received iv thiorphan (0.5 mg/kg) for 60 min before or after blockade of ACE (Enalapril) or AII receptor (DuP 753). The AII receptor blockade was confirmed by AII injection. Blood samples were collected before and after administration of Enalapril, DuP 753, or Thiorphan for the measurement of PRA. For the combined effect of thiorphan and C-ANP_{4-23} in SHR, thiorphan (0.5 mg/kg) was infused iv for 60 min before and after blockade of C receptors with iv infusion of C-ANP_{4-23} for 30 min. Blockade of ACE or AII receptors in SHR decreased mean BP by 24 ± 3.2 and 23±3.2 mmHg, respectively. Thiorphan in these rats caused further decrease in BP of 39 ± 6.2 and 27±4.9 mmHg, respectively. Similar results were obtained after ACE inhibition or AII receptor blockade following thiorphan. ACE inhibitor increased PRA levels but thiorphan had no further effect on PRA. Urine output was increased after blockade of R-AII system or inhibition of NEP. In the second series of experiments, infusion of C receptor blocker and NEP inhibitor decreased BP by 23.0 ± 3.3 and 27.0 ± 4.9 mmHg respectively, causing a total decrease in BP of 50.0±8.1 mmHg. The same total fall in BP occurred when C receptors were blocked after infusion of the NEP inhibitor, except in this case the NEP inhibitor decreased BP 44 ± 2.9 and the C blocker decreased it 13 ± 4.6 mmHg. Cardiac contractility index (dp/dt/P) did not change. Urinary cGMP levels were significantly increased at 90 minutes after the start of C-ANP_{4-23} and thiorphan infusion; the same results were obtained when the order of infusion of blockers was reversed. These results show that a significant part of the vasodepressor effect of thiorphan is independent of the R-AII system. Furthermore, thiorphan and R-AII system blockers seem to have additive vasodepressor effects even in SHR, a model not considered to be renin dependent. Increased urine output in spite of a fall in BP suggests a renal effect for these two blockers. Inhibition of both metabolic degradation (thiorphan) and clearance of endogenous ANP (C-ANP_{4-23}) seem to produce an additive vasodilator and vasodepressor effect.

38.14

RELEASE OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN PERINEPHRETIC HYPERTENSIVE RABBITS. C.A. Courneya, K.A. King and J.R. Ledsome. Physiology, UBC, Vancouver B.C. V6T 1Z3.

In situ wrapping of the kidneys of rabbits with cellophane results in the development of sustained perinephretic hypertension which is associated with ventricular hypertrophy. Ventricular hypertrophy has been shown to be associated with ANF gene expression in the ventricles and elevated basal plasma ANF in rats. In the present study we measured both basal ANF and stimulated release of ANF (atrial pacing, 6hz) in anaesthetized perinephretic hypertensive (HT) and sham operated (C) rabbits. Following the experiment the hearts were removed and weighed (our index of hypertrophy). Arterial pressure was greater in HT (89.7 ± 3.7 mmHg) than C rabbits (63.7 ± 2.2 mmHg), however basal ANP was not significantly different (17.9 ± 3.7; 10.82 ± 1.17 pg/ml respectively). Atrial pacing increased HR and ANP in both groups. HR was increased to a similar degree in both groups (+109 ± 7.6 bts/min). The increase in ANP, however, was attenuated in the HT rabbits (+7.2 ± 1.8 pg/ml) compared to C rabbits (±21.8 ± 5.7 pg/ml). Total heart weight was greater in the HT rabbits (2.12 ± 0.08 gm/kg) than C rabbits (1.8 ± 0.07 gm/kg), and this increase was due predominantly to ventricular hypertrophy. These results suggest that in this model of hypertension ventricular hypertrophy was not associated with elevated basal plasma ANP, and that stimulated release of ANF was attenuated. This may be related to the rather mild hypertension and hypertrophy achieved by the rabbits in this study. Supported by HSFBCY and MRC.

38.15

DEVELOPMENT OF DOCA/NaCl HYPERTENSION IN THE WISTAR FURTH RAT. Michael J. Katovich, Kim Hanley and O. Shechtman. Dept. of Pharmacodynamics, University of Florida, Gainesville, FL 32610

It has been previously reported (Hypertension 10:176, 1987) that the Wistar Furth (WF) rat is resistant to mineralocorticoid but not renal artery stenosis-induced hypertension. We have attempted to compare the development of hypertension in the Sprague Dawley (SD) and WF strains by using two methods of DOCA treatment. Similarly aged SD and WF female rats were divided into 3 groups of 9-10 animals per strain. One group from each strain served as control. Remaining rats from each strain were unilaterally nephrectomized and provided with a 1% NaCl drinking solution. Induction of hypertension was performed by weekly injection of desoxycorticosterone pivalate in 1 group from each strain and by subcutaneous implant of a 25-40 mg pellet of desoxycorticosterone acetate in the remaining group from each strain. Indirect blood pressure (BP) was monitored weekly for 6 weeks. BP was significantly elevated in the SD, DOCA pellet group by the second week of the study and SD rats injected with DOCA developed an increase in BP during the 3rd week of the study. BP were similar between these 2 groups during the 5th and 6th week. However, BP was not elevated in the WF, DOCA groups until the 5th week, with pelleted animals demonstrating a higher blood pressure than injectable DOCA. The severity of BP was similar in the two SD, DOCA groups and the WF, DOCA pelleted group during the 6th week. Mortality rate was about 20% in both DOCA pellet groups in the 5th week and rose to 40% by the 6th week. No mortality was observed in the injected DOCA groups. Thus, the WF rat can develop hypertension; however, the development is of slower onset and less intense than observed in SD animals. Additionally, DOCA pellets produce a more intense hypertension than weekly injections of DOCA. (Support in part by American Heart Association, Florida

38.16

NEUROPEPTIDE Y IN PHEOCHROMOCYTOMA: CLINICAL IMPLICATIONS. Emmanuel L. Bravo and P. deS Senanayake*. Cleveland Clinic Research Institute, Cleveland, OH 44195

Plasma neuropeptide Y like immunoreactivity (NPY-LI) in adrenal pheochromocytoma patients was elevated (range; 338 - 6400 pg/ml; n=5) when compared to normal controls (range; 35-275 pg/ml; n=8). This is in sharp contrast to patients with extraadrenal pheochromocytoma whose plasma NPY-LI values were within the normal range (n=5, 38-274 pg/ml). Surgery reduced plasma NPY-LI levels to the normal range except in malignant pheochromocytoma (n=3) where they remained elevated. With surgical manipulation of extraadrenal tumors NPY-LI remained unchanged despite marked increases in circulating norepinephrine. NPY-LI concentration was lowest in extraadrenal tumors (39 ng/g wet weight) and highest in adrenal tumors (1070 ng/g). Fractionation of plasma extracts from pheochromocytoma patients by HPLC showed the predominance of human NPY (1-36); surgical removal of the adrenal tumor resulted in a peptide profile resembling the normal plasma profile where NPY (1-36) is a very minor component. These studies have for the first time characterized the effect of surgical intervention on the circulating levels of NPY in pheochromocytoma. Furthermore, our findings indicate that NPY may be useful in distinguishing between adrenal and extraadrenal pheochromocytoma, and as a marker of residual disease.

38.17

BLOOD PRESSURE RESPONSE TO ALDOSTERONE AND ANGIOTENSIN II IN CONSCIOUS DOGS ON NORMAL AND HIGH SODIUM INTAKE. Robert McCaa, Creekwood Publishing Company, Brandon, MS. 39042-9280.

Studies in man demonstrated that prolonged aldosterone infusion produced little or no effect on blood pressure, while angiotensin II infusion induced a marked increase in arterial blood pressure. These studies were conducted to determine the blood pressure response to aldosterone and angiotensin II in conscious dogs on normal (55 mEq Na+/day) and high (+300 mEq Na+/day) sodium intake. Aldosterone administration for 6 days increased blood pressure 14 mm Hg (99±4 to 113±7) in dogs on normal sodium intake and to a maximum level of 20 mm Hg above control in dogs on high sodium intake. Angiotensin II administration (0.1 µgm/kg) increased arterial blood pressure 39 mm Hg (99±4 to 138±5) in dogs on normal sodium intake and 48 mm Hg (113±7 to 161±8) in dogs pretreated with large amounts of aldosterone for 6 days. Continuous angiotensin II infusion into conscious dogs on normal sodium intake increased blood pressure 28 mm Hg (103±4 to 131±6) and to 45 mm Hg above control in dogs on high sodium intake. Two significantly different renal function curves were obtained with aldosterone and angiotensin II in conscious dogs on normal and high sodium intake. The aldosterone renal function curve was shifted only slightly to the right of the normal renal function curve, while the angiotensin II renal function curve was shifted far to the right of the normal and aldosterone renal function curves.

38.18

EFFECT OF 19-ACETYLENIC-DOC ON HYPERTENSION IN SALT-SENSITIVE RATS. Sami T. Azar*, J. O'Neal Johnston**, George T. Griffing, James C. Melby. University Hospital, Boston, MA 02118. ** Marion Merrell Dow Research Institute, Cincinatti, OH 45215

Urinary 19-nor-Deoxycorticosterone (19-nor-Doc), a potent mineralocorticoid, is elevated at the onset of hypertension in salt-sensitive rats. 19-acetylenic-Doc (19-ac-Doc), an inhibitor of 19-nor-Doc was evaluated for its anti-hypertensive effect in 20 salt-sensitive female rats.
At 60 days of age, the rats were started on a high salt diet; then, at 120 days of age, the survivors were divided into 2 groups: a) 5 animals had 19-ac-Doc implants (1mg released/10 days) and b) 5 animals underwent placebo implants (controls). At 130 days of age, another set of implants was applied to those remaining alive. Blood pressure readings were done at 90,120,130,140 and 150 days.

Age (days)	number alive		Mean BP (mmHg)	
60	20		-	
90	16		200	
	control	19-ac-Doc	control	19-ac-Do
120	5	5	204	208
130	0	4	-	165
140	0	4	-	160
150	0	2	-	200

This suggests that 19-ac-Doc can lower the blood pressure and improve survival in salt-sensitive rats possibly through the inhibition of 19-nor-Doc synthesis.

38.19

PRESSURE DEPENDENCY OF CHANGES IN VASCULAR REACTIVITY IN ALDOSTERONE-SALT HYPERTENSION. Cathy A. Bruner and Paula M. Dennerlein. Albany Medical College, Albany, New York 12208.

We designed experiments to determine the extent to which enhanced contractile sensitivity to vasoconstrictors seen in aldosterone (ALDO)-salt hypertension is a result of the high arterial pressure. Male Wistar rats received ALDO (0.15 ug/hr, s.c. for 4 wk) and 1% NaCl, 0.2% KCl drinking fluid. Some rats received hydralazine and hydrochlorothiazide (100 mg/l of each; H/H) or timolol (50 mg/l; TIM) in the drinking fluid. Helically cut strips of carotid artery were prepared for isometric force recording. ALDO infusion for 4 weeks produced a 60 mm Hg increase in systolic blood pressure (SBP) and increased vascular contractile sensitivity to KCl, norepinephrine (NE) and serotonin (5HT) when compared to normotensive controls. H/H treatment prevented both hypertension and the appearance of enhanced vascular sensitivity. TIM treatment only partially (50%) prevented ALDO-salt hypertension, and had no effect to prevent the enhanced vascular sensitivity. In rats receiving ALDO-salt for 2 weeks, SBP was elevated by only 10 mm Hg, yet the shift in contractile sensitivity had occurred to the extent seen in rats receiving ALDO-salt for 4 weeks. We conclude that changes in carotid artery contractile sensitivity to KCl, NE, and 5HT in ALDO-salt hypertension occur as a result of the elevated blood pressure, and that only small changes in pressure are necessary to produce changes in reactivity. **(Supported by NY State Heart 89-010G).**

39.1

ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM INHIBITS CONTRACTIONS TO ENDOTHELIN-1 IN CANINE CORONARY ARTERIES.
P. Aarnio, C.G.A. McGregor, V.M. Miller, Mayo Clinic, Rochester, MN 55905

Endothelin-1 contracts vascular smooth muscle and inhibits release of neurotransmitter from adrenergic and cholinergic neurons. The interactions of these mechanisms in a blood vessel which receives both adrenergic and cholinergic innervation is not known. Therefore, experiments were designed to determine whether or not neuronal activation to a blood vessel affects contractions to endothelin-1. Rings were cut from canine left anterior descending coronary arteries. The endothelium was removed and the rings suspended for the measurement of isometric force in organ chambers. Endothelin-1 caused concentration-dependent increases in tension in all rings. During electrical stimulation (1 Hz, 9V, 2 ms), the contractions to endothelin-1 were reduced significantly. This decrease was greater in the presence of atropine (10^{-6}M) and was eliminated by a combination of phentolamine (10^{-5}M) and propranolol ($5x10^{-6}$M). These results suggest that in canine coronary arteries contractions to endothelin-1 may be modulated by the level of sympathetic and parasympathetic tone. Therefore, endothelin-1 may have more profound affects on vascular resistance in denervated hearts, for example, those used for transplantation. Supported in part by the American Heart Association, AHA891222.

39.2

FEMORAL vs RENAL BLOOD FLOW RESPONSE TO INHIBITION OF ENDOTHELIUM-DEPENDENT RELAXING FACTOR (EDRF). David H. Sigmon*, Oscar A. Carretero and William H. Beierwaltes. Henry Ford Hospital, Detroit, MI. 48202

Inhibition of EDRF results in acute hypertension. We, and others, have found that EDRF inhibition decreases renal blood flow (RBF). We wished to compare the renal response to EDRF inhibition with a representative peripheral bed, the femoral vasculature. 12 anesthetized rats were treated with 10 mg/kg bw N^{w}-nitro-L-arginine (N-Arg) to inhibit EDRF. Blood Pressure (BP) was monitored by a femoral catheter. In 2 subgroups of 6 rats we determined either RBF or contralateral femoral blood flow (FBF) using flow probes. EDRF inhibition caused a 21 ± 1 mmHg increase in BP ($p<0.001$) and a 31 ± 7 bpm decrease in heart rate ($p<0.005$). RBF decreased by 32 ± 5% (5.9 ± 0.5 to 3.9 ± 0.3 ml/min/glkw; $p<0.005$) while FBF did not change (9.5 ± 0.4 vs 9.4 ± 0.4 ml/min). Renal vascular resistance (RVR) increased 97 ± 16% compared with only 24 ± 6% increase in femoral resistance (FVR; $p<0.001$). To eliminate the effect of increased BP, we returned organ perfusion pressure to pre-N-Arg levels (106 ± 2) by aortic constriction. The kidney autoregulated RBF by decreasing RVR 13 ± 5% ($p<0.05$) compared with no autoregulation of FBF. These results suggest that EDRF is more critical in maintaining RBF than FBF. We conclude that EDRF inhibition reveals a disproportionate reliance of the kidney on endothelial regulation of blood flow compared to peripheral vascular resistance beds. (HL28982)

39.3

GONADECTOMY ABOLISHES SEX DIFFERENCES IN ENDOTHELIAL MODULATION OF VASCULAR REACTIVITY TO VASOPRESSIN (VP).
L. I. Anderson* and J. N. Stallone. Dept. of Physiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Previously, we reported that VP-induced contraction of rat aorta is substantially higher in females (F) than in males (M), which results (at least in part) from greater release of endothelium-derived relaxing factor (EDRF) by M than by F aortae. To explore the role of the gonadal steroids in this sexual dimorphism in vascular function, the effect of gonadectomy (GNX) on vascular reactivity to VP was examined in thoracic aortae of M and F rats. Age-matched M and F Sprague-Dawley rats were GNX or sham-GNX (intact, INT) at 4 wks age. At 12-14 wks age, paired aortic rings were prepared for isometric tension recording (in Krebs-Henseleit-bicarbonate, 37°C, 2.50 g passive tension) in the presence of either N^{G}-methyl-l-arginine (NMLA, 0.25 mM) or l-arginine (control, C, 2.5 mM). After equilibration (2 hr), a cumulative dose-response curve to VP was obtained (10^{-11}-10^{-6} M). The data presented are means ± SE (n = 3-4 animals per experimental group). Maximal response of INT-C aortae to VP was markedly higher in F (4,406±310 mg) than in M (1,158±314 mg). In GNX-C aortae, maximal response to VP increased dramatically in M (4,167±324 mg), but was unchanged in F (4,162±252mg). Pre-treatment of aortae with NMLA markedly increased maximal response to VP in INT M (3,138±586 mg) but had no effect in INT F (4,493±355 mg), or in GNX M (4,117±616 mg) or GNX F (4,225±85 mg). Maximal response to 80 mM KCl did not differ among M, F, INT, or GNX (mean=2,819 ± 73 mg). These data suggest that the gonadal steroids are important modulators of endothelial function and are responsible for the prominent male-female differences in VP-stimulated release of EDRF by the rat aorta. (Supported by Am. Heart Assoc./Ohio Affiliate).

39.4

ENDOTHELIAL DEPENDENT CONTRACTING FACTOR RELEASED BY ARACHIDONIC ACID IN RABBIT PULMONARY ARTERY. Sandra L. Pfister*, Carol J. Buzzard* and William B. Campbell* (SPON: G.A. Ordway). UT Southwestern Medical Center, Dallas, TX 75235

Because recent evidence suggested that arachidonic acid (AA) releases an endothelial dependent contracting factor (EDCF), the aim of the present study was to characterize the EDCF released by AA in rabbit pulmonary artery. AA elicited endothelial dependent, concentration-related contractions (97.1 ± 4.6% of KCl contraction) that were blocked by the cyclooxygenase inhibitor, indomethacin. In addition, the thromboxane (TX) receptor antagonist, SQ 29548 and the TX synthetase inhibitor, dazoxiben both caused a 88% inhibition of the maximal AA-induced contraction. When segments of pulmonary artery were incubated with ^{14}C-AA, and the extracted metabolites resolved by reverse phase HPLC, a major radioactive peak that comigrated with 6-keto prostaglandin (PG) $F_{1\alpha}$, the stable metabolite of prostacyclin, and a minor radioactive peak that comigrated with TXB_2, the stable metabolite of TXA_2 were observed. The TXB_2 radioactive peak was rechromatographed on normal phase HPLC, and again comigrated with TXB_2. Using specific RIAs, AA stimulated both 6-keto $PGF_{1\alpha}$ and TXB_2 production. Indomethacin blocked the production of both 6-keto $PGF_{1\alpha}$ and TXB_2, whereas dazoxiben inhibited only TXB_2 production. In conclusion, these results suggest that pulmonary endothelial cells synthesize a product that may be TXA_2, and it is likely that this product mediates the endothelial dependent contraction elicited by AA.

39.5

ANGIOTENSIN AND VASOPRESSIN INDUCE PREPROENDOTHELIN-1 mRNA AND ENDOTHELIN-1 RELEASE FROM CULTURED BOVINE ENDOTHELIAL CELLS.
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2nd Dept. of Med.,Tokyo Med. & Dent. Univ., Tokyo, *1st Dept. of Pharmacology, Kyoto Univ., Kyoto, Japan

To elucidate the cellular mechanism of endothelin(ET)-1 production and release by vasoconstrictive hormones, we have studied the effects of angiotensin II (AII) and arginine-vasopressin (AVP) on cytosolic free Ca^{2+} levels ($[Ca^{2+}]_i$) by fura-2 method and phosphoinositide breakdown in cultured bovine endothelial cells (EC), and determined the agonists-induced immunoreactive ET-1 release and the expression of bovine preproET-1(ppET-1) mRNA by Northern blot analysis. Both AII and AVP dose-dependently stimulated immunoreactive ET-1 release, with concomitant increases in $[Ca^{2+}]_i$ and generation of inositol trisphosphate. ppET-1 mRNA was induced within 5 min by AII (10^{-8}M) and AVP (10^{-8}M) which lasted for 3 hrs. AII and AVP induced ppET-1 mRNA in a dose-dependent manner (10^{-8}-10^{-8}M). These effects were abolished by their specific receptor antagonists (salarasin and POMT-AVP), respectively. These data suggest that the release of ET-1 from EC stimulated by AII and AVP is mainly due to the induction of ppET-1 mRNA via the receptor-mediated phosphoinositide turnover and intracellular Ca^{2+} mobilization in EC.

39.6

AGING AND ELEVATED LIPID PEROXIDATION IMPAIR VASCULAR ENDOTHELIAL CELL FUNCTION. S.T. Davidge* and M.K. McLaughlin. Dept. Ani. Sci., UVM, Burlington, VT 05405 & Dept Pediatrics, CHMC, Cincinnati, OH 45267.

Oxidative damage has been implicated in many age-related diseases, including cardiovascular disorders. An elevation of lipid peroxides has the potential to alter prostaglandin synthesis and endothelial-derived relaxing factor(s)(EDRF), which are important for normal vascular function. The interaction between lipid peroxides and prostaglandins/EDRF on endothelial cell function during aging is unknown. We examined the effect of aging and elevated lipid peroxides on vascular function in the Sprague Dawley rat. Rats (n=20) were placed on a control(+) or vitamin E deficient(-) diet that resulted in equivalent increases in red blood cell hemolysis between age groups. Mesenteric arteries from 40 week old rats [Old;n=6(+), n=6(-),] and 20 week old rats [Young;n=4(+), n=4(-)] were studied in an isometric myograph system. Aging increased the sensitivity to phenylephrine vasoconstriction (.8μM vs 1.6μM, $p<0.05$). Diet had no effect. Aging decreased the sensitivity to the endothelium-dependent agonist, methacholine almost 2-fold (EC_{50}=.084μM (Old) vs .045μM (Young), $p<0.01$). Vitamin E deficient diet did not affect this response in the Young rats, but caused a further 2-fold decrease in the methacholine sensitivity in the Old rats (.084μM vs .164μM, $p<0.01$). Indomethacin potentiated the methacholine relaxation response only in the vitamin E deficient animals ($p<0.01$); the effect was greater in the Old (.067μM vs .164μM) vs the Young rats (.031μM vs .049μM). In summary, age resulted in an increase vasoconstrictor response and a decrease in EDRF vasorelaxant response. In a dietary antioxidant deficiency, both Young and Old rats demonstrated an increase in a cyclooxygenase-dependent vasoconstrictor that was more pronounced in the Old rats. In conclusion, age and elevated lipid peroxides are both important factors leading to an impairment of vascular endothelial cell function, which may contribute to age-related vascular diseases. PHS# HL40130.

39.7

SIMULTANEOUS INCREASE OF ENDOTHELIN-1 AND THROMBIN-ANTITHROMBIN III COMPLEX IN PLASMA AT OPEN-CHEST SURGERY.
M.Onizuka, T.Miyauchi*, S.Ishikawa*, K.Mitsui*, N.Suzuki*, K.Goto, T.Masaki and M.Hori. Institutes of Clinical Medicine and Basic Medical Sciences, University of Tsukuba, and Takeda Chemical Industries, Tsukuba, Ibaraki 305, Japan

We reported Endothelin-1 (ET-1), a potent vasoconstrictor, was released after open-chest surgery (Physiologist 33:1990). Enhanced production of ET-1 by thrombin has been demonstrated in cultured bovine endothelial cells (Biochem Biophys Res Commun 160:1989), in porcine aortic strips (Hypertension 15: 1990), and in isolated perfused guinea pig lungs (Proc Natl Acad Sci 86:1989). We measured plasma concentrations of ET-1 (pg/ml) and thrombin-antithrombin III complex (TAT, ng/ml) in patients who underwent open-chest surgery (n=8, 40 samples). The blood samples were collected from the median cubital vein. The data are summarized in the table (mean ± SEM).

	Time sampling				
	pre-ope	just-after-ope	6-hr-after	24-hr	48-hr
ET-1	1.3±0.1	3.5±0.8	2.8±0.4	1.6±0.1	1.3±0.2
TAT	5.8±1.2	31.1±12.4	24.3±9.1	12.5±2.5	12.4±3.7

Plasma concentrations of ET-1 and TAT were significantly increased after the surgery ($p<0.05$) and showed significant correlation between the two substances ($r=0.73$, $p<0.001$). Present findings suggest that accelerated clotting system by the surgery might stimulate the production of ET-1, or vice versa.

39.8

RESTING HEMODYNAMICS IN CONSCIOUS RATS WITH NEPHROTIC SYNDROME.
C. Hinojosa-Laborde and G. F. DiBona. Internal Med. Dept., Univ. of Iowa, College of Med. & VAMC, Iowa City, IA 52242

Nephrotic syndrome (NS) is characterized by renal sodium and water retention and edema formation mediated by neurohumoral systems. This study determined if activation of volume retaining mechanisms affected baseline hemodynamics in NS. Male Sprague-Dawley rats were given adriamycin (3.5 mg/kg,iv.) to produce NS (N=6). Vehicle control (VC; N=7) rats received isotonic saline (1.5 ml/kg,iv.). Three weeks later when NS rats have significant ($p\leq0.05$) proteinuria (369±26 vs.10±3 mg/24hr) and hypoalbuminemia (2.16±0.03 vs.2.85±0.09g/dl), the rats were instrumented with an electromagnetic flow probe on the ascending aorta and a femoral artery catheter for cardiac output and arterial pressure (AP) measurements. Due to differences in body weights (NS,303±5 g; VC,332±11 g), cardiac index (CI) and total peripheral resistance index (TPRI) were calculated.

	AP	CI	TPRI	Heart Rate
	mm Hg	ml/min/100g	mmHg•100g•min/ml	bts/min
NS	113 ± 4	27.6 ± 1.2	4.19 ± 0.16	465 ± 7
VC	116 ± 2	28.0 ± 1.4	4.22 ± 0.20	452 ± 12

There were no differences in hemodynamic variables between NS and VC rats. We conclude that the alteration in volume homeostasis in NS does not affect resting systemic hemodynamics.

39.9

Transcranial Doppler Evaluation of Syncope: An Application in aerospace physiology. Philip C. Njemanze * (SPON. Mary L. Ellsworth) Neurocybernetic Lab. Nigeria*, St. Louis Univ. Medical Center, St. Louis MO.63110

A non-invasive method which combines the simultaneous measurement of mean cerebral blood flow velocity (MFV), mean arterial blood pressure (MBP), and heart rate (HR) was used to monitor patients with history of syncope, in horizontal and vertical posture tilt at 80°. All subjects gave consent according to institutional guidlines. MFV in the right middle cerebral artery was measured using a transcranial Doppler instrument (TCD). MFV decreased concurrently with the onset of symptoms, and at the time of syncope reached an average of 68% below pretilt values. At the same time MBP showed an average decline of 25%, and HR increased by 38%. There was no correlation between MBP and MFV, at the onset of of tilt, presyncope and syncope. MFV correlated with MBP at supine recovery state. MFV but nor HR or MBP showed significant transition from one condition to the other. These data suggest that there may be a useful application of TCD measurements of MFV in the study of syncopal tendency. These measurements would necessarily be used in conjunction tilt table procedure. The possibility exists that MFV might be useful to preclude the actual occurrence of syncope in test subjects who are aircrew candidates. [1]

Reference: Njemanze PC Aviat Space Environ Med 62:569-72 1991.

39.10

BLOOD FLOW TO CIRCUMVENTRICULAR ORGANS DURING INFUSION OF CATECHOLAMINES. J. L. Williams, R. B. Page*, S. G. Shue*, S. C. Jones*, and R. M. Bryan, Jr. Cleveland Clinic Foundation, Cleveland, OH 44195 and Hershey Medical Center, Hershey, PA 17033

In contrast to other brain regions, circumventricular organs (CVOs) do not have a blood-brain barrier and are permeable to blood-borne hormones. In this study, we examined effects of catecholamines on blood flow to cerebral cortex and CVOs (SFO, subfornical organ; ME, median eminence; CP, choroid plexus; NH, neurohypophysis; AP, area postrema) in unanesthetized rats. Rats were anesthetized initially with halothane and N_2O in O_2 for cannulation of femoral arteries and veins. After recovery from anesthesia, one group of rats was treated with α-methyl-paratyrosine (AMPT; 250 mg/kg i.p.), which inhibits catecholamine synthesis. In 2 other groups, after AMPT treatment, dopamine (15.0 µg/kg/min) or epinephrine (1.0 µg/kg/min) was infused for 5 min. Control rats were not treated with either AMPT or catecholamines. Blood flow (ml/min/100g) was measured with ^{14}C-isopropyliodoamphetamine and quantitative autoradiography:

	Control(6)	AMPT(4)	Dopamine(4)	Epinephrine(4)
SFO	418±29	471±91	585±28*	370±73
ME	568±63	779±82*	849±23*	686±90
CP	702±57	728±51	989±60*†	706±55
NH	1036±42	1314±94*	1842±164*†	1212±80
AP	321±17	473±47*	551±23*	432±87
Cortex	180±20	168±40	241±45	169±60

*,† $P<0.05$ from Control, AMPT. Value = mean±SE. (Number) = n.

Cortical blood flow was not affected significantly by any treatment. AMPT increased blood flow to ME, NH, and AP, which suggests that catecholamines tonically vasoconstrict these CVOs. Dopamine produced large increases in blood flow to CP and NH. Our findings indicate that catecholamines can differentially affect blood flow to CVOs.

39.11

BLOOD LACTATE AND CATECHOLAMINE LEVELS IN THE CARBON MONOXIDE-EXPOSED RAT: THE EFFECTS OF ELEVATED GLUCOSE
Bharat B. Sutariya, David G. Penney, Joseph C. Dunbar, and Curtis J. Swanson. Wayne State University, Detroit, MI 48201

Previous studies have shown that elevated blood glucose is detrimental to outcome in acute CO poisoning. The present goals were to characterize the lactate and catecholamine changes, and to determine whether elevated blood glucose increases the levels of these substances. Two groups of adult Sprague-Dawley, Levine-prepared female rats (n=22 each) were exposed to 2400 ppm CO for 90 min: Group A received nothing; Group B was infused with 50% glucose solu. (4 ml/kg). The usual hypothermia, hypotension, bradycardia, and hemoconcentration associated with CO poisoning were observed. Survival rates were 77% and 68% in Gp. A and B, respectively. Arterial blood pressure tended to decline more in rats that died; the difference was signif. in Gp. B. In Gp. A, glucose was signif. lower after CO exposure in rats that died than in survivors (35+/-15 vs. 99+/-16 mg/dl), as previously reported. In Gp. B, glucose was signif. higher after 45 min in rats that died (d) than in survivors (s) (447+/-29 vs. 324+/-31 mg/dl). Elevated blood glucose in Gp. B failed to signif. increase blood lactate; however, lactate tended to be higher in rats that died in both groups [Gp. A; 175+/-17 (d) vs. 138+/-9 (s); Gp. B; 154+/-10 (d) vs. 143+/-8 (s)]. Epinephrine and norepinephrine increased approx. 6-10 fold and 2-6 fold in both groups, respectively. The results do not support the notion that elevated blood glucose signif. increases blood lactate during CO poisoning, and fail to unequivocally show that death is related to signif. higher blood lactate levels.

39.12

METHODOLOGICAL AND FUNCTIONAL CONSIDERATIONS OF GLUCAGON IN THE HEPATIC AND MESENTERIC VASCULAR BEDS.
Mark S. d'Almeida* and W. Wayne Lautt. Department of Pharmacology, Liver Unit, University of Manitoba, Winnipeg, Manitoba, Canada, R3E OW3.

Due to the dual supply of blood to the liver, and the relationship between these inputs, studying hepatic hemodynamics has been difficult and confusing. In part, by using the vasodilatory action of glucagon as a research tool, we have been able to develop better methods for studying the hepatic vascular beds in vivo. We assessed the effects of intravenous glucagon and raised portal blood flow (PBF) on hepatic arterial conductance and the resultant influence from the hepatic arterial buffer response (HABR) in the cat. We conclude that when studying hepatic hemodynamics, PBF must be monitored or controlled in accordance with the role of the HABR. In an opposite approach, we used the hepatic and mesenteric vascular beds as research tools to study the physiological and pharmacological characteristics of glucagon. Glucagon was confirmed to be a dilatory agent, but not an effective inhibitory modulator of nerve- and norepinephrine-induced constrictions. Comparison of the pharmacologic estimates of Rmax and ED_{50} indicated that the efficacy of intra-arterial glucagon in the mesenteric vascular bed was almost double that of the hepatic artery (HA) while the potency of glucagon in the HA was approximately 9-fold greater than in the mesenteric bed. This work was funded by MRC of Canada and the Heart & Stroke Foundation of Canada.

39.13

A THEORETICAL TREATMENT OF OXYGEN SHUNTING IN THE INTESTINAL VILLUS. J.W. Kiel and A.P. Shepherd. University of Texas Health Science Center, San Antonio, Texas 78284

This report describes a mathematical model of the counter-current shunting (CCS) of oxygen in the intestinal villus. The model divides the villus into four segments from base to tip. Steady-state equations describe the convective and diffusive fluxes of oxygen in the arteriolar, capillary and tissue compartments within each segment. Longitudinal diffusion is assumed to be negligible. Simulations with the model led to the following observations. 1) In the absence of CCS, the oxygen uptake (VO2) versus blood flow relation was linear at low flow when VO2 was delivery-limited. At high flow, VO2 was independent of flow. CCS shifted the VO2 versus blood flow curve down and to the right, slightly impairing VO2 at a given flow. 2) In the absence of CCS, the villus tip PO2 was lower than the base because of tissue O2 uptake; CCS further reduced the base-to-tip PO2 gradient. Reducing blood flow increased the base-to-tip PO2 gradient until the tip PO2 fell to zero, then the base-to-tip gradient fell with further flow reductions. 3) Lowering blood flow initially caused slight increases in shunting; further decreases in flow reduced shunting. 4) In the flow range in which VO2 was flow-independent, increasing VO2 or decreasing the arteriole-to-capillary distance increased shunting. 5) Lowering the hemoglobin's P50 to simulate fetal blood caused slight reductions in shunting and reduced VO2 at a given flow. In summary, the model confirms the potentially deleterious effects of CCS on intestinal oxygenation, and it shows that a base-to-tip PO2 gradient is not *prima facie* evidence of counter-current shunting. Supported by HL-36080 and AHA Grant-In-Aid 90G-343.

39.14

IMMUNOCYTOCHEMICAL LOCALIZATION OF ESTROGEN (ER) AND PROGESTERONE RECEPTORS (PgR) IN HUMAN AND MACAQUE VASCULAR TREE. S.R. Money* and L.P. Pertschuk* (Spon: J.B. Josimovich). SUNY HSC at Brooklyn and The Methodist Hospital, Brooklyn, New York 11203

An immunocytochemical assay, utilizing different, multiple monoclonal specific anti-ER and anti-PgR antibodies and a peroxidase-antiperoxidase detection system was used to study the vascular tree of humans and macaques. ER as well as PgR appeared limited to the nuclei of endothelial and smooth muscle cells of intima, media and vasa vasora. Positive staining was seen with 2 different anti-ER and 2 separate anti-PgR antibodies. Staining was limited to the thoracic aorta and its major branches in both species. Human umbilical cord vessels and ovarian and uterine arteries also demonstrated positivity. No staining was seen when nonimmune IgG was substituted for specific antibody. Positive staining was rare in other locations (extremities, mesenteric, iliac, renal arteries and the coeliac axis, etc.). We conclude that certain segments of primate vascular system are steroid hormone targets.

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39.15

ANDROGEN REGULATION OF PENILE ERECTION. T. Mills, V. Stopper* & V. Wiedmeier, Department of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA 30912-3000.

The direct involvement of androgenic steroids (AS) in the vascular aspects of penile erection is not fully established. A rat animal model has been used to determine whether reduction in AS leads to changes in the erectile process. The erectile response was quantified by measuring the maximal pressure achieved in the corpus cavernosum (CC) in response to stimulation of the autonomic innervation of the penile vasculature. Blood was also collected for the determination of circulating testosterone. The results show that within 24 hr of lowering plasma AS levels by castration, there was a decrease in the erection-induced maximal CC pressure with a progressively greater decline at 2-21 days after castration. AS replacement by implanting testosterone pellets prevented (or reversed) the decline in maximal CC pressure which followed castration. Castration also led to decreased sensitivity of the penile vasculature to vasoactive drugs whereas AS treatment maintained sensitivity suggesting that the androgen may act to regulate penile vascular responsiveness. These studies show that AS are essential for the maintenance of full penile erection and that AS may act to maintain the vascular receptor activity.

39.16

SEX DIFFERENCES IN VASCULAR REACTIVITY IN RAT AORTA. O. Shechtman, M.J. Katovich & E.E. Soltis. Dept of Pharmacodynamics, Univ of FL, Gainesville, FL 32605 & Dept of Pharmacol and Exprtl Therapeutics, Univ of KY, Lexington, KY 41005

There are documented sex differences in various cardiovascular diseases including hypertension. A difference in sensitivity of the vascular smooth muscle to vasoconstrictor and vasodilator agents may contribute to this sexual dichotomy. In the present study we measured the vascular reactivity of aortic and tail artery smooth muscle of male and female rats to various vasoconstrictor and vasodilator agents. The vascular responsiveness of male rats was significantly greater than that of the females to the lower doses of KCl (5,10 and 20 mM). However, the response to the higher doses of KCl (40, 60 and 80 mM) was significantly greater in the females. There were no differences in the vascular response to the vasodilator agents sodium nitrate ($NaNo_2$) and acetylcholine (Ach). However, the vascular responsiveness of aortic smooth muscle of males to both phenylephrine (PHE) and isoproterenol (ISO) was significantly greater than that of the females. On the other hand, there were no differences in vascular responsiveness of the tail artery between males and females to any of the vasoactive agents. Administration of phentolamine, an alpha-adrenergic blocker, prior to contraction with KCl, resulted in a significantly smaller contraction of the tail rings of both males and females but had no effect on aortic rings. This was probably due to the difference in adrenergic innervation between the two vascular beds and may account for the differences observed between the two vascular tissues for the adrenergic agents studied. The difference in aortic rings responsiveness to KCl may be due to sex-related differences in either calcium metabolism or calcium transport into the cell. However, the data for the adrenergic receptor-mediated response of the aortic rings indicate that male rats are more responsive to both alpha and beta adrenergic stimulation than female rats. This increased sensitivity of males to adrenergic challenge could explain in part the sex difference in cardiovascular disease. (Supported in part by the American Heart Association, Florida Affiliate).

39.17

$hCGRP_{8-37}$ COMPETITIVELY ANTAGONIZES VASODILATIONS PRODUCED BY HUMAN CGRP (hCGRP) IN PIG CAROTID ARTERIES. Ronald R. Fiscus, Huiqing Hao, Xian Wang, Steven F. Johnson+, Warwick A. Arden+, Sherry Ling, Robert K. Salley+ and David R. Gross+. Sanders-Brown Center on Aging, Dept. Physiol. & Biophys. and +CT Surgery Research Labs, Dept. Surgery, Univ. Kentucky, Lexington, KY, 40536

Calcitonin gene related peptide (CGRP) is found in nerves (mostly sensory afferent) within almost all organ systems. This neuropeptide is also found in the adventitial layer of almost all blood vessels. It is one of the most potent vasodilators known. An important first step necessary for the characterization of any physiological role that CGRP may play in the regulation of blood flow is to identify effective blocking agents. We studied the effects of $hCGRP_{8-37}$, a synthetically produced CGRP analog with the first 7 amino acids deleted. Dose-response curves to hCGRP were produced from carotid arteries harvested from 5, isoflurane anesthetized, crossbred, female pigs, approx. 25 kg. Typical log-dose-response curves were obtained for hCGRP using multiple vascular rings from each animal contracted with 3×10^{-8} M norepinephrine. The EC_{50} in the control specimens was 3.53×10^{-9} M. Rings prepared from the same animals, and tested at the same time, were treated with 1 μM $hCGRP_{8-37}$. Treated vessel dose-response curves were shifted to the right 7 X with a EC_{50} of 2.49×10^{-8}. The effects of both hCGRP and $hCGRP_{8-37}$ were similar in vessels with and without endothelium, indicating the observed responses are endothelium independent. We conclude that $hCGRP_{8-37}$ acts as a competitive antagonist for hCGRP in pig carotid artery and should serve as a useful tool for future studies.

39.18

FEMORAL ARTERIAL RING RESPONSES AFTER ENDOTOXIN (ETX). Z. Zhou*, J.M. Price, C.H. Baker, Dept. of Physiology and Biophysics, Univ. of South Florida, Tampa, Fl 33612

This study investigated the compensatory mechanisms of blood vessel responses to agonists after E. coli ETX administration. Femoral arterial rings were taken from anesthetized Wistar rats at three levels of mean arterial pressure (P_a): 1) control; 2) 100 mmHg and 3) 40 mmHg post ETX infusion (6mg/kg, 1hr). Dose-response curves were obtained with vasopressin (AVP) ($4\text{-}10 \times 10^{-8}$ M), KCl (5-105 mM) and phenylephrine (PHE) (10^{-8}-10^{-5} M). When compared at the same ring length (e.g. the length of maximal responses in control vessels, Lmax), no significant differences were found in EC_{10} and EC_{50} at three blood pressure levels, but maximal response was decreased to 62% for AVP, 52% for KCl and 53% for PHE (all $p<0.05$) when $P_a=40$ mmHg. Examining the smooth muscle length-tension curve with 5×10^{-6} M PHE showed that the Lmax of endotoxic rings at $P_a=40$ mmHg was 1.31 times of the Lmax at control P_a ($p<0.01$) and the maximal reponse at ETX Lmax was 1.59 times of the maximal response of control ring at Lmax ($p<0.01$). The data shows that smooth muscle contraction is not inhibited by endotoxin infusion and the comparison of response depends on the length chosen. We suggest that at $P_a=40$ mmHg vessel smooth muscles work in a different length-tension curve which is on the right of the control curve. (Supported by HL-33840)

39.19

ATRIAL NATRIURETIC PEPTIDE (ANP) INCREASES DISSIPATIVE BUT NOT CONVECTIVE ALBUMIN TRANSPORT. V.L. Tucker and E.M. Renkin. Univ. of California, Davis CA 95616.

Infusion of exogenous ANP increases transvascular protein escape. In the present study capillary solvent drag coefficients (1-σ) were estimated in hindlimb skeletal muscles of intact, anesthetized rats to determine the relationship between fluid and protein movement during ANP infusion. Synthetic rat ANP was infused i.v. at 0 (control, n=6) or 500 ng/kg/min (n=8) for 30 minutes. Tissue albumin transport was measured as 30 minute clearances of 131-I-BSA (Calb) using a double isotope technique. Water contents (W) were determined by wet-to-dry weight. Skeletal muscle fluid extravasation was varied within rats by elevating hydrostatic pressure (venous occlusion) of one hindlimb. The average (±SEM) increase in skeletal muscle filtrate (ΔW) in occluded limbs of control rats was 136±47 μl/g dry wgt and was associated with a small increase in Calb (ΔCalb=1.26±0.45 μl/g dry wgt) but no change in tissue plasma volume. Thus 1-σ, estimated as ΔCalb/ΔW, was 0.009. For ANP, fluid extravasation into occluded legs was similar to control: ΔW=116±16 and 1-σ=0.010. ANP significantly (P≤0.05) increased Calb in each leg: from 4.71±0..3 to 7.71±0.72 (non-occluded) and from 5.97±0.3 to 8.79±0.6 (occluded). If the estimates of sovent drag are correct, then the minimum amount of filtrate required to account for the elevation in Calb with ANP in the non-occluded leg was 300 μl (9% increase in W). However, W did not change with ANP (3224±23 vs 3220±11 μl/g dry wgt). We conclude that ANP increases tissue albumin uptake primarily by enhancing dissipative transport and not by increasing solvent drag of albumin. Supported by NIH HL07957 and HL18010

39.20

ARE ELEVATED PLASMA CATECHOLAMINES A REQUIREMENT FOR ARTERIOLAR VASOMOTION? S.P. Bruttig, J.A. Schmidt and P. Borgström. Letterman Army Institute of Research, Presidio of San Francisco CA 94129, UCSD-AMES-Bioengineering, and La Jolla Institute for Experimental Medicine, La Jolla, CA 92037

Vasomotion, the regular, rhythmic and repetitive constriction and dilation of terminal and pre-terminal arterioles, is frequently observed during periods of profound hypotension or hypoperfusion of skeletal muscle. In our laboratories, we study this hypotension/hypoperfusionas either massive hemorrhage (>30%), which results in systemic effects, or as partial occlusion of the infrarenal aorta, which results in local effects within the hindlimb tissue of anesthetized rabbits. Since vasomotion following hemorrhage can be associated with elevated systemic catecholamine levels, we wished to determine whether elevated systemic catecholamine levels were a pre-requisite for the onset of vasomotion following local pressure reduction of hindlimb skeletal muscle. Therefore, systemic plasma samples were analyzed by HPLC separation and electrochemical detection for the presence of epinephrine (A) or norepinephrine (NA). The data indicate that, following uncontrolled hemorrhage (aortotomy or venotomy), systemic plasma A levels, but not NA levels, rise to values 10x control levels, while following local pressure reduction within the hindlimb, systemic plasma catecholamines are unchanged from control. Consequently, the coincident rise in plasma A following systemic hypotension/hypoperfusion in profound hemorrhage is not a cause or necessarily supporting condition for the observed arteriolar vasomotion. Following local pressure reduction to the hindlimb musculature, the resulting arteriolar vasomotion does not require, nor appear coincident with a rise in either systemic plasma A or NA. However, these data do not rule out a supporting role for baseline levels of circulating catecholamine.

39.21

DIFFERENTIAL ACTION OF VERAPAMIL ON COLD INDUCED VASODILATATION IN THE INTERNAL MAXILLARY ARTERY BED. Klaus Pleschka, Ferenc Bari* and Olusegun Ariwodola*. W.G.Kerckhoff-Institut, 6350 Bad Nauheim, F.R.G.

Marked vasodilatation of arteriovenous anastomoses (AVA) and slight vasoconstriction of precapillaries (CAP) characterize cold induced vasodilatation (c.v.) of the canine internal maxillary artery (IMA). To find out whether thermal interference with calcium channels underlies c.v. in nasal AVAs, blood flow of the IMA (IMA-FLOW) was recorded electromagnetically together with perfusion pressure of the IMA (PP-IMA) in response to cold inspired air (c.i.a.:-35°C) during continuous unilateral infusion of Verapamil (200 nM/min) into the IMA in anesthetized dogs. Labelled microspheres were used to measure unilateral IMA-FLOW distribution. Vascular resistance (R-IMA) was derived from IMA-FLOW and PP-IMA. 1.) Verapamil significantly increased IMA-FLOW (+53%) due to a significant decrease in R-IMA (-40%). The latter affected mainly precapillaries as indicated by a marked increase in CAP-FLOW (+131%), while AVA-FLOW even decreased (-46%). 2.) During Verapamil infusion electrical stimulation of the cervical sympathetic chain (ST) induced a significant vasoconstriction in the IMA (IMA-FLOW: -16%, R-IMA: +19%), confined, however, only to CAPs (CAP-FLOW: -17%), while the usually stronger constriction of the AVAs beeing abolished. 3.) C.i.a. further enhanced the Verapamil induced IMA-FLOW elevation (+16%) due to a decrease of R-IMA (-14%), which was exclusively restricted to AVA-FLOW (+78%). 4.) During Verapamil and c.i.a., ST diminished c.v. of the IMA (-19%), again only due to a decrease in CAP-FLOW (-27%). The absence of an AVA-FLOW increase under Verapamil, but its presence during c.i.a., however, at an absent constrictor response to ST suggest dilator mechanisms independent from the usual calcium re-entry mechanism. The remaining constrictor response to ST in Verapamil dilated precapillaries may originate from incomplete blockade of calcium channels or unaffected intracellular calcium stores.

39.22

EFFECT OF HYPEROXIA AND EPIDERMAL GROWTH FACTOR ON FIBROBLAST INFILTRATION AND NEOVASCULARIZATION. D.W. Criswell* and W.J. Mehm. Armed Forces Institute of Pathology, Washington, DC 20306.

A polyvinyl alcohol sponge disc (13mm diameter) was implanted in mouse subcutaneous tissue to investigate two treatments [epidermal growth factor (EGF), and intermittent hyperoxia (100% oxygen for 90 mins twice a day at 250 kPa)] which may modulate neovascularization. Two conditions were established for treatment: exposure of animals to chronic hypoxia (12% oxygen for 23 hrs/day), simulating low oxygen tensions in problem wounds, and normoxia (21% oxygen). In experiments evaluating EGF, discs were implanted whose core contained EGF covered with a slow release polymer, the other group with placebo. Discs were harvested at 14, 25, or 32 days after implantation. The area of the disc infiltrated by fibroblasts was measured by planimetry and used as an indirect measure of neovascularization. When EGF (20 μg) was administered for 14 days under hypoxic and normoxic conditions no effect was seen on neovascularization. However, after 25 days under normoxic conditions EGF slightly increased (NS) neovascularization compared to placebo (24.8 mm^2, n=7, vs. 18.4 mm^2, n=10). Exposure of chronically hypoxic and normoxic animals to intermittent hyperoxia between 21-32 days of disc implantation without EGF did not affect of neovascularization. EGF may have more potential than hyperoxia in promoting neovascularization.

39.23

EFFECT OF PHORBOL-MYRISTATE ACETATE AND Ca^{2+} IONOPHORE A23187 ON THE PERMEABILITY OF THE EEL RETE CAPILLARIES. Eugenio A. Rasio and Carl A. Goresky. Notre-Dame Hosp., Univ. Montréal, Montreal General Hosp., McGill Univ., Montréal, Que. Canada, H2L 4M1

The possible role of protein kinase C activation and intracellular calcium changes on capillary endothelial permeability was tested in the countercurrent perfused rete mirabile of the eel at constant flow and pressure. Tracers were added to the perfusate at the arterial input and their concentrations were measured at the venous (V_o) and arterial (A_o) outputs. The diffusion capacity PS of the rete equals the flow x V_o/A_o and was used as a measure of permeability. In the first set of 8 perfusions, phorbol-myristate-acetate (PMA) was added to the medium at a concentration of 10^{-5} M. PS values for ^{125}I-albumin, ^{14}C-sucrose and ^{22}Na rose gradually from 6.9 ± 1.9, 19.5 ± 3.2 and 41.5 ± 7.9 $cm^3/sec \times 10^{-5}$ during the control period, to 13.3 ± 4.8, 46.2 ± 6.2 and 93.5 ± 11.9 $cm^3/sec \times 10^{-5}$, respectively, at 120 min. ($0.05 > p > 0.005$). PS values for ^{3}H-water were rapidly reduced from 2358 ± 554 $cm^3/sec \times 10^{-5}$ during the control period to a plateau of 1712 ± 238 $cm^3/sec \times 10^{-5}$, for 120 min ($p \sim 0.05$). In the second set of 8 perfusions, A23187 was added to the medium at a concentration of 5 x 10^{-6}M. PS values for ^{125}I-albumin rose from 6.0 ± 1.7 $cm^3/sec \times 10^{-5}$ to a plateau averaging 10.0 ± 1.3 $cm^3/sec \times 10^{-5}$, from 60 to 120 min ($p < 0.05$). Concurrently, PS values for ^{14}C-urea and ^{3}H-water were reduced from 74.8 ± 6.2 and 1953 ± 414 to 67.9 ± 6.0 and 1103 ± 219 $cm^3/sec \times 10^{-5}$, respectively ($p < 0.05$). It is concluded that PMA and A23187 increase paracellular permeation and decrease permeability to cellular probes. Supported by grants from MRC and QHF.

39.24

A PLATELET MODEL OF ATHEROSCLEROSIS, AND THE EFFECTS OF CHRONIC STRESS AND PSYCHOLOGICAL TRAITS ON PLATELET SENSITIVITY TO EPINEPHRINE-INDUCED AGGREGATION. H. Tracy, J. Patterson*, W. Patterson*, D. McGlasson*, and J. Marshall*. Armstrong Laboratory, Brooks AFB TX 78235 and Wilford-Hall USAF Medical Center, Lackland AFB TX 78236.

A model of stress-induced atherosclerosis, with platelet aggregation occupying a key position, has been developed. This study is investigating the sensitivity of platelets to epinephrine-induced aggregation, as a primary step in stress-induced atherogenesis, under conditions of chronic stress, including the effects of psychological traits on this sensitivity. Platelet aggregation with epinephrine was measured biweekly. Subjects are being categorized into three groups: 1) individuals whose platelets were epinephrine sensitive; 2) individuals whose platelets were epinephrine insensitive; and 3) individuals whose platelets exhibited variable epinephrine sensitivity. Preliminary findings indicate that, of the third group, 25% of the subjects exhibited epinephrine-induced aggregation at the beginning of the twelve week study, which rose to 80% after two weeks, and dropped to zero after five weeks. At the sixth week, 40% of the subjects exhibited aggregation, which remained relatively constant until the end of the study, when the number of subjects showing platelet reactivity decreased to 33%. These fluctuations correspond with the levels of stress experienced, as reported by subjective questionnaires. Scatterplots of platelet aggregation category versus psychological traits reveal a potential positive correlation with the driven trait (r=.4), the perfectionist trait (r=.4) and the job satisfaction trait (r=.6). These early findings are encouraging, but any conclusions must await analysis of a larger sample. The voluntary informed consent of the subjects used in this research was obtained in accordance with AFR 169-6.

40.1

NEW STATISTICAL METHODOLOGY FOR PHYSIOLOGY. R. Jevning*, M. Biedebach*, R. Anand, and J. Barson*. Calif. State Univ., Long Beach, CA 90840 and U.S. Inter. Univ., San Diego, CA 92340.

Concerns about probability and statistical methodology in physiology has increased markedly in the last 10 years. However, taken as a whole, the articles addressing this topic give a monolithic impression that the problem of statistical analysis of treatment effects is one of the adoption or non-adoption of particular statistical "tests" for achieving answers that are either correct or incorrect.

This impression logically derives from an almost exclusive emphasis on one statistical viewpoint-that based upon relative frequency, also known as classical or "objective" statistics. By a device known as "significance testing" whether a treatment effect exists is ascertained by comparing the relative frequency of the effect in an experiment to that theoretically expected in an infinite number of independent repetitions of that same experiment. In essence, the goal of objective statistics is thus to discover a property (treatment effect) of an idealized infinite number of experiments. "Acceptance or rejection of the null hypothesis" then denies or affirms this property, respectively. From these premises, a statistical problem, therefore, is only a problem of choice of the right significance test. Unfortunately, the neat idealized scheme of relative frequency statistics, most appropriate for physics, where properties of infinite collections are relevant, is of limited applicability in areas of science, such as physiology, where concern is with small, finite collections. For this reason it is important to consider other statistical viewpoints such as the neo-bayesian one, which despite its prominence in modern statistics, remains to be considered by physiologists.

Rather than giving final answers (accpetance or rejection), neo-bayesian statistics yields a probability of the existence of a treatment effect and asserts that no "test" or statistical method can create certainty in situations inherently uncertain, because of limited data.

40.2

A LABORATORY EXERCISE ON METABOLISM IN THE RAT. L.L. Bellinger, F.W. Williams, L.W. Frazier, and B.S. Wong, Dept. of Physiology, Baylor College of Dentistry, Dallas, Texas 75246.

This laboratory exercise shows students how various conditions of thyroid hormone status and food availability affect the animal's metabolism. Metabolism is measured using pentobarbital anesthetized rats with a Phipps and Bird, Inc. Small Animal Metabolic Apparatus Model 7122-410 from the change in O_2 volume in the metabolic chamber. The students are asked to calculate liters of O_2 consumed per day; to correct this to standard temperature and pressure; to calculate Kcal utilized per day using the mixed diet value of 4.83 Kcal/l O_2; and to correct Kcal/d for per unit surface area. Blood was then taken for radioimmunoassay of T_3. Six groups (Grp) are utilized: Grp 1, controls; Grp 2, thyroidectomized; Grp 3, T_3 injected; Grp 4, food restricted to 25% of normal intake for three days to simulate severe calorie restriction diets; Grp 5, same as Grp 4, but then fed *ad libitum* for one day to demonstrate the carry-over effect on metabolism; and Grp 6, *ad libitum* fed plus access to a 30% sucrose solution for one week (this has been reported to raise T_3 concentrations; a possible dietary thermogenesis defense mechanism). All statistical comparisons $P<0.05$ = *, 0.01 = **, were made against Grp 1. The following data represented O_2 consumption (l/d) and amount of T_3 present (ng/ml): Grp 1, 5.1±0.3, 0.8±0.06; Grp 2, 1.6±0.1**, 0.3±0.8**; Grp 3, 7.5±1.0**, 7.6±1.2**; Grp 4, 2.3±0.1**, 0.6±0.05**; Grp 5, 3.1±0.2**, 0.8±0.06; and Grp 6, 4.0±0.3, 1.2±0.1*. The exercise shows thyroid status and diet can have a profound effect on metabolism and T_3 plasma concentration.

40.3

SOFTWARE FOR TEACHING HEMODYNAMIC WAVEFORM RECOGNITION AND COMMON HEMODYNAMIC MEASUREMENTS. E Larry Combs*, Bernard J. Rubal, Joe M. Moody*, and James R. Bulgrin*. Brooke Army Medical Center, Fort Sam Houston, TX 78234

Cardiac catheterization studies representing a broad spectrum of cardiac diseases were selected from the hemodynamic tape library at Brooke Army Medical Center. Hemodynamic data included simultaneously recorded high-fidelity right and left heart pressures, ECG and selected right and left heart fluid pressures. Waveforms were filtered at 200 Hz and digitized at 500 Hz. Waveforms were displayed using commercially available X-windows software capable of simulating physiologic displays in most cardiac catheterization laboratories. Interactive teaching software allows users to select cardiac pathology of interest. The trainee is then prompted to identify waveforms, specific x or y values, or ranges of values. Fluid pressure measurements may be filtered and directly compared to high-fidelity pressures. Feedback is provided for correct and erroneous responses. Trainee may apply gain, bias or time skew to waveforms. Software provides an instructor interface to allow new waveforms to be incorporated into the student library. Using a simple procedural language instructors may generate new prompts for site specific training.

48.1

INCREASED INTRACRANIAL PRESSURE (ICP) IN HUMANS DURING SIMULATED MICROGRAVITY. Gita Murthy, Robert J. Marchbanks, Donald E. Watenpaugh, and Alan R. Hargens. Life Science Division (239-11), NASA Ames Research Center, Moffett Field, CA 94035-1000.

Microgravity-induced elevation of ICP may cause the headaches commonly reported by astronauts. The same symptom occurs in subjects during simulated microgravity (6° head-down tilt, HDT) and may be caused by vascular and extravascular fluid translocation from the lower to the upper body. Microgravity and HDT increase hydrostatic pressures in blood vessels above the heart and therefore, both conditions may elevate ICP. We noninvasively monitored ICP using the tympanic membrane displacement (TMD) technique in six normal male subjects during upright, 0° supine, 6° HDT, and 15° HDT postures. Postural transitions from sitting to: (1) 0° supine, (2) 6° HDT, and (3) 15° HDT produced negative TMDs of : (1) 317 ± 112, (2) 403 ± 114, and (3) 474 ± 112 nl, respectively, showing significant TMD decrements for each transition towards greater HDT ($p < 0.05$). Previous studies indicate that ICP is inversely related to TMD. Therefore, our results suggest that simulated microgravity (HDT) increases ICP, which may be responsible for the headaches and other fluid-shift problems associated with exposure to microgravity (Supported by NASA grant 199-14-12-04 and by the Ames Research Center Director's Discretionary Fund).

48.2

CEREBRAL BLOOD FLOW VELOCITY INCREASES WITH ACUTE HEAD-DOWN TILT OF HUMANS. Yasuaki Kawai, Gita Murthy, Donald E. Watenpaugh, and Alan R. Hargens. Life Science Division (239-11), NASA-Ames Research Center, Moffett Field, CA 94035-1000.

Microgravity causes a cephalad shift of blood and tissue fluids which may alter cerebral circulation and fluid homeostasis. The purpose of this study is to compare blood flow velocities in the middle cerebral artery before, during, and after exposure to acute head-down tilt (HDT). We hypothesized that the increase of local arterial blood pressure with HDT also elevates cerebral blood flow velocity significantly. Right middle cerebral artery (MCA) blood flow velocities were measured in 6 healthy males, aged 22 to 47 years, by 2 MHz transcranial Doppler (Transpect, Medasonics, Inc.). Baseline control measurements were obtained during upright sitting position with feet hanging over the side of a tilt table. Then the subjects were positioned supine (0°), tilted head-down at 6 and 15 degrees (5 min each), and then returned to upright sitting. Blood flow velocities were averaged for 30 sec just before posture was changed. MCA blood flow velocity increased significantly by 11 ± 4% ($p=0.040$), 14 ± 3% ($p = 0.007$), and 9 ± 3% ($p = 0.017$) at 0°, 6° HDT, and 15° HDT, respectively, compared to velocity during upright posture. During sitting recovery, flow velocities approached baseline levels. Our results suggest that transcapillary filtration and edema formation may be promoted in the brain during simulated microgravity (HDT) due to elevated intracranial blood pressure and flow (Supported by NASA and an NRC Fellowship to YK).

48.3

GAS EXCHANGE AND CARDIOVASCULAR RESPONSES TO TILTING DURING GRADED EXERCISE. Y. Haruna*, D. Linnarsson and B. Tedner*. Environmental Physiology Laboratory, Department of Physiology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Ten healthy male subjects were studied as they exercised at 50, 100 and 150 W. Breath-by-breath ventilation O_2 uptake and CO_2 elimination were recorded together with beat-by-beat heart rate (HR) and non-invasive arterial blood pressure (AP) in a finger. For each steady-state work load the subjects were tilted repeatedly from upright to −10° head-down tilt (HDT) and the reverse at 2 min intervals. AP at the level of the carotid showed a rapid increase with an initial overshoot in the upright – to HDT transition and HR displayed a reverse transient with a delay of a few seconds. HR responses to the HDT to upright transitsion were less marked. With increasing exercise intensity the high-frequency component of the HR/AP variations was reduced, whereas the basic response to tilting was preserved. Pulmonary capillary O_2 and CO_2 transfer showed short-lasting (10 sec) increases during the upright – to HDT transition and equally short-lasting reductions during the reverse transition. Our data suggest that the HR response of the arterial baroreflex is not attenuated with graded exercise. Furthermore the major component of the change in pulmonary blood flow during a sudden shift of body position in the exercising man seems to be of very short duration.

48.4

PRESENCE OF ATRIAL NATRIURETIC FACTOR IN SALIVA. COMPARISON OF PLASMA AND SALIVARY CONCENTRATIONS DURING A HEAD DOWN TILT. G. GAUQUELIN*, C. GHARIB*, A. MAILLET*, A. GUELL*, D. VOROBIEV** and A.I. GRIGORIEV**
*Lab.Physiol., Fac.Med. Grange-Blanche, 8 Av. Rockefeller, 69373 Lyon Cedex 08 , France, ** IBPM Moscow, USSR.

Using a specific and sensitive radioimmunoassay involving separation on Centripor and extraction with C18 Sep-Pak Cartridges, we measured the concentration of salivary and plasma ANF before and during a head-down tilt (HDT) at -6°. An increase of concentrations in saliva and plasma was found after 1h30 of HDT. Salivary ANF values closely correlated with plasma ANF. This is the first time that ANF is shown in saliva. This methodology will be used during the Soviet French Space Flight planned for 1992 (ANTARES PROJECT).

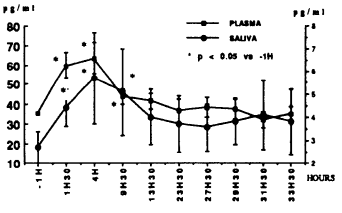

Figure : Modifications of plasma (left scale) and saliva (right scale) concentrations during a head down tilt (-6°). Results are expressed as mean ± SEM for each para-meters.

48.5

DEFORMATION OF THE CATORID ARTERIAL WALL & BAROREFLEX RESPONSE DURING SHIFTS OF EXTRA-CELLULAR FLUID VOLUME (ECFV). J.L.Cuche*, B.Pannier* S.Laurent* B.Levy* P.Maisonblanche* & M.Safar*. Dept Medicine & INSERM Unit-337, Hôpital Broussais, Paris, France.

In 6 volunteers, ECVF was shifted during 24-hours bed rest with -5° head-down tilt (24-BR with HDT), and during -40 mmHg lower body negative pressure (LBNP). Systolic tangential tension of the carotid artery was slightly increased during HDT (+9.5%, NS), and decreased during LBNP (-12.3%, $p<0.01$). 24-BR with HDT has no significant effect on sytemic hemodynamics, forearm blood flow and spectral analysis of heart rate (SA of HR); plasma dopamine only was significantly increased. During LBNP, expected effects on systemic hemodynamics were observed, with a reduction of the high/low frequency ratio (-61%, $p<0.01$) in SA of HR, and a significant increase in plasma catecholamines. No alteration was observed when comparing LBNP responses before and after bed resting, suggesting an appropriate response of the baroreflex. However, the response of total peripheral resistances (TPR) seems to be altered: +11.6% $p<0.05$ before, +9.8%-NS, after 24-BR with HDT, and -2.1%-NS when LBNP was superimposed. This blunted response of TPR appeared in the presence of a larger increase of catecholamines. Thus 24-BR with HDT has no altering effect on the baroreflex but seems to alter TPR mechanisms uncompletly counterbalanced by an increased activity of the sympathetic system. Whether BR with HDT induces a down-regulation of peripheral adrenergic receptors remains to be demonstrated. This could be an interesting hypothesis when BR with HDT is used as a ground-based model of weightlessness.

48.6

EFFECT OF A 28-DAY CONFINEMENT ON BLOOD PRESSURE, BLOOD VOLUME REGULATING HORMONES, AND ELECTROLYTES. A. MAILLET*, H.C. GUNGA**, G. GAUQUELIN*, K. KIRSCH** and Cl. GHARIB*. *Lab. Physiol., Fac.Med. Grange-Blanche, 8 Av. Rockefeller, F-69373 Lyon Cedex 08, France. **Dept. Physiol., Free Univ., Arnimalee 22, D-1000 Berlin, Germany.

Prolonged bed-rest induces hormonal modifications which are linked to a cephalad fluid shift as in microgravity. Confinement and inactivity induce large physiological and psychological modifications. Thus, some of the hormonal modifications described in bed-rest studies could be related to confinement, inactivity and stress. Our aim was to study some volume regulating hormones (Renin, Vasopressin, Aldosterone, Atrial Natriuretic Factor) and their modifications during a 4-week confinement. This study was completed by other analyzes (Na^+, K^+, creatinine, urea). For the hormones analyzes, 2 blood samplings were made before (control), 1 blood sampling each week during the confinement period (D2, D8, D16, D22, D27) and 2 blood samplings after (recovery). Urines were collected during the whole study with a day/night separation. Several points are interesting: 1/ The systolic blood pressure was higher at the beginning of the confinement ($p<0.01$ D2 vs PRE 2) We observed an increase in active renin ($p<0.05$ D8 vs PRE 2) and vasopressin ($p<0.05$ D2 and D8 vs PRE 2) during confinement , which was comparable to the increase observed during a 4 week bedrest. Several factors could account for this : stress, but also a decrease in extracellular fluid volume. 2-/ The increase in plasma creatinine could be related to hypohydration and decrease in plasma volume. But it is possible also to explain this increase by inactivity which involved a loss of muscular mass. If these hypotheses are confirmed, confinement could be a valuable analogous situation to simulate some aspects of space flights.

48.7

WATER INTAKE, URINE VOLUME, Ca INTAKE, AND URINE Ca DURING 10-DAYS BED -REST IN YOUNG WOMAN. K. YOKOZAWA, S. TORIKOSHI, J. NAGANO, Y. SUZUKI
(Lab. of Human Physiol., Tokyo Woman's Christian Univ. JAPAN)

To investigate the relationships between water intake(WI) and urine volume(UV), and between Ca intake(Cal) and urine Ca(UCa) during 10-days bed-rest(BR) in young woman. 5 female students were studied in 24hs' WI, Cal, 24hs' UV, its' UCa at every day and plasma volume(PV) and blood Ca(BCa) at 1st, 3rd, 11th day of BR. WI was gradually decreased, but UV was declined for the 3 days and then altered to increase, so that passing 8th day the difference between WI and UV was below 200 ml. Despite of a food program ruled by intaking a 850mg Ca. Cal was about 850mg for the first 2 days and then decreased into between 550 mg to 750mg. UVwas gradually increased by the 6th day and then altered to slightly decreasing until the final day. PV and BCa were significantly decreased at the 3rd days and indicated a restoring tendency at the 11th day. Body weight decreased an average 780g, in which the decreases in body fat and iean body mass were 480g and 300g, respectivly. From the results presented by 10-days BR study in young woman, UV and UCa are gradually increased although WI and Cal are decreased.

48.8

EFFECTS OF 10-DAYS BED-REST AND MODERATE TRAINING ON LBNP TOLERANCE IN YOUNG WOMAN. S. Torikoshi, K. Yokozawa, J. Nagano, Y. Suzuki, Tokyo Woman's Christian Univercity, 2-6-1 Zenpukuji Suginami-ku Tokyo

5 female students were studied LBNP tolerance after 10 days bed-rest(BD) and then the effects of a training program with 300Kcal/45min load per day and 20days/4wk on restoring the LBNP tolerance during post-BR. The subjects were devided into two groups, traning group(TG) and control group(CG) who did normal daily life activity but not the training. LBNP tolerance time was dropped over 20% from the control level due to BR, and it's decrease was significantly($p<0.01$) correlated to each of the decrease in left ventiricular end dyastolic dyameter(LVEDd), stroke volume(SV), and plasma volume (PC) due to BR. During post-BR, LBNP tolerance was restored in TG, but not in CG. Although LVEDd, SV, PC were similarly reached closely by each of the control values in both of TG and CG. On the other hand, changing manner of LBNP tolerance was similar to peak VO_2 not only at post-BR but post-training period. The present study probably suggests that LBNP tolerance reduced by BT is resulted from the decrease in blood volume, but as returning normal life activity at post BR it restored by recovering blood volume regardless of training.

48.9

Effects of moderate physical training after 10 days horizontal bed-rest on peak VO_2 and cardio-respiratory functions during submaximal supine and sitting exercise in young subjects

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Dpt. of Health Administration, Faculty of Medicine, Univ. of Tokyo

8 young subjects (female 5 + male 3) were studied for effects of 4 weeks upright bicycle exercise training (300 Kcal/day) after 10 days horizontal bed-rest (BR) on peak VO_2 and on cardio-respiratory functions during 100 Watts cycling in supine (sup.E) and sitting positions (sit.E), respectively. The subjects were divided into two, training group (TG, n=5) after BT and control group (CG, n=3). Peak VO_2 at BR was significantly ($p<0.05$) decreased about 10% from the control given before BR, while each of them returned to the control in TG but not in CG. After BR, measurements of cardio-respiratory functions during sit.E were not changed, while HR and Double Product were significantly increased ($p<0.05$), and O_2 removable was significantly decreased ($p<0.05$) from the control levels during sit.E. However, the changes in the measurements were returned the control levels not only in TG but also CG. A possiblitys from the presented results in young subjects is the peak-VO_2 reduced - by 10 days BR is restored by moderate training for 4 weeks , but not by only daily life activity, and that cardio-respiratory functions during Sup.E is affected by 10 days BR.

48.10

INTERSTITIAL SPACE DYNAMICS. SIMULATED RESPONSE TO LBNP. S Daiqui* L Beck*, F Baisch* DLR, Institute for Aerospace Medicine, Linder Höhe, 5000 Köln 90, FRG.

During step-wise LBNP test which consisted of -20 mbar for 15 min (Phase A) and -40 mbar for 5 min (Phase B) global volume changes for the thigh and calf could be regularly registered compared to steady state control. Average volumes of calf and thigh (measured by ultrasound plethysmography) respectively increased 3% and 9% by the end of Phase A and 5% and 14% by the last minute of LBNP. To better understand interstitial fluid spaces (IFS) dynamics, a computer model (running on a Xerox 1186) was developed using previous work by Guyton and coworkers. It was assumed that externally applied pressure was completely and instantaneously transferred to the interstitial spaces. IFS was analyzed with regards to its gel (G) and free fluid (FF) components. This included evaluation of protein concentration, colloid osmotic pressure, fluid pressure and volume for each of these compartments and for the tissue itself. By the end of Phase A IFS increased 8 % and 13.3% by the end of Phase B, primarily due to changes in G and FF. Fluid that remained in the IFS expanded G volume 22% during Phase A and 24% during Phase B. FF increased 177% by the end of Phase B and resulted in swelling of G and a decrease in muccopolysacchride concentration. The ability to derive magnitude and time course of these changes at each second of LBNP allows development of a dynamic model for fluid compartment change and suggests improved methods for countermeasure development against the decreased orthostatic tolerance regularly seen following spaceflight.

48.11

PERIPHERAL HAEMODYNAMICS ASSESSMENT during LBNP for the EVALUATION of the VASCULAR DECONDITIONING.

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ABSTRACT : The degree of vascular deconditioning was evaluated using a haemodynamic Doppler measurement during a 20 mn LBNP test (-20 to -60 mmHg). By recording simultaneously the cerebral and the femoral flow during LBNP on subjects disadapted and controls, we have identified a specific response on the population of disadapted subjects. As the LBNP depressure becomes lower, the femoral blood flow (mean frequency) decreases and the vascular resistances in the inferior limbs increase on both group, but the amplitude of the leg vascular resistance changes is higher on the control (non disadapted) group. By the same time, we observed on the two groups a slight increase of the heart rate, a decrease of the pic velocity and an increase of the diastolic velocity in the cerebral arteries, which confirms that the cerebral flow regulation is still working. The cerebral flow response to LBNP was quite similar for the two groups except that after the end of the test the increase of the cerebral systolic and mean frequency, and the vascular resistance was higher in the control group. Finally the regulation of the blood flow in the inferior limb is significantly disturbed in case of vascular deconditioning, and the leg vascular resistance response to LBNP could be used as an indicator of the vascular disadaptation in flight. In case of inflight countermeasure treatment this parameter will probably be helpfull to ajust the sequence of the countermeasures according to the amplitude of the deconditioning.

48.12

MATHEMATICAL MODELING OF CARDIOVASCULAR RESPONSE TO LBNP. F.M. Melchior * and S. Srinivasan * (SPON : S.M. Fortney)
LAMAS, CEV, F-91228 Brétigny Cedex.

A mathematical model was developped in order to better understand how the human cardiovascular response to Low Body Negative Pressure (LBNP) is related to blood volume redistribution, Starling law of the heart, and the effectiveness of the control elements. Blood volume redistribution was modeled by the association of three compartments including the upper body, the abdominal region, and the lower limbs. Starling law was modeled by the (Central venous pressure, Left ventricular end-diastolic volume) curve. The cardiovascular system was described by a three-element Windkessel model. The left ventricle was modeled as a time-varying elastance. Heart rate and total peripheral resistance were modulated through arterial and cardiopulmonary baroreceptors. The LBNP response of a test from 0 to -50 mmHg was simulated. Results of various hemodynamic parameters such as heart rate, central venous pressure, mean and pulse arterial pressure, total peripheral resistance, left ventricular end-diastolic volume, and cardiac output agree fairly well with experimental data. The LBNP response appears to be primarly dependent on the Starling law and less on leg compliance. This study shows that a simple mathematical model can accurately simulate the human cardiovascular response to LBNP. Secondly, this model provides a usefull tool to evaluate the importance of the relevant parameters on the cardiovascular response to LBNP.

48.13

PHYSICAL TRAINING AND THE REGULATION OF ATRIAL NATRIURETIC FACTOR RELEASE DURING ORTHOSTATIC STRESS. Charles-Yannick Guezennec*, Francis Louisy* and Francis Jouanin* (SPON : A. GÜELL). Centre d'études et de recherches de médecine aérospatiale, C.E.V, 91228 Brétigny sur Orge Cedex, France.

Plasma levels of atrial natruretic factor (pANF) and plasma norepinephrine (pNE) were measured in 30 men (10 endurance exercice trained - ET, 10 untrained - UT and 10 weight trained - WT) during stepwise decreasing lower body pressure. Blood was drawn from each subject at rest and after 20 mn exposure to -15 mmHg, then to -30 mmHg of LBNP. pANF was measured by radioimmunoassay and pNE by high-performance liquid chromatography with electrochemical detection. Results showed a gradual decrease in pANF levels in UT during LBNP (-15% at -15 mmHg, $p < 0.05$; -33% at -30 mmHg, $p < 0.05$) while in ET and WT, pANF only decreased at the -15 mmHg level of depressurization (-17% in ET, $p < 0.05$; -20% in WT, $p < 0.05$). At -30 mmHg, pANF remained constant for ET or slightly increased for WT when compared to levels observed at -15 mmHg. pNE increased in parallel with increasing negative pressure in the 3 groups with a greater increase in WT and UT (+66% and +55% respectively at -30 mmHg, $p < 0.05$ in both cases) than in ET (+32% at -30 mmHg, $p < 0.05$). There was no correlation between changes in pNE and changes in pANF in either group. This suggests that the volemic stimulus is the most important factor determining plasma ANF changes during LBNP. The smaller decrease in pANF observed in this modeling of physiologic response to gravitational stress is explained by a greater plasma volume (ET) and/or by a better venous return to the heart (ET and WT).

49.1

SPLENECTOMY DOES NOT ALTER THE NATRIURETIC RESPONSE TO HEAD-OUT WATER IMMERSION. J.L. Sondeen, J. R. Claybaugh, S. K. Hong, J. A. Krasney. Letterman Army Inst. Res., Presidio of San Francisco, CA, Tripler Army Med. Ctr., Honolulu, HI, Dept. Physiology, SUNYAB, Buffalo, NY.

Previous studies showed variable natriuretic responses to head-out thermoneutral water immersion (WI) in conscious dogs. In one study using intact (INT) dogs, the increase in urine flow (V) was due mostly to increased fractional sodium excretion (FENa) and in another study in splenectomized (SPX) dogs, was due to increased free-water clearance (CH2O). The present experiment was designed to test whether SPX altered the natriuretic response to WI. Six chronically catheterized, conscious female dogs were subjected to WI before and after SPX. In both the INT and the SPX state, dogs responded with similar increases in V (INT +505 ± 111%; SPX +660 ± 245%), FENa (INT +275 ± 13%; SPX +318 ± 56%), CH2O (INT +12 ± 3%; SPX +10 ± 2%), arterial pressure (INT +13 ± 3%; SPX +14 ± 3%), right atrial pressure (INT +90 ± 18%; SPX +70 ± 18%), and atrial natriuretic peptide (INT +99 ±13%; SPX +95 ± 9%). Similar decreases in plasma vasopressin concentration (INT -41 ± 10%; SPX -35 ± 6%) and plasma renin activity (INT -74 ± 3%; SPX -67 ± 4%) were seen with both treatments. We therefore conclude that the spleen does not alter the natriuretic and diuretic responses to WI. NIH Grant HL28542.

49.2

VASOPRESSIN, RENIN, ALDOSTERONE, AND ATRIAL PEPTIDE RESPONSES TO UPRIGHT TILT AT SEA LEVEL AND 450 M SEA WATER. J.R. Claybaugh, Y.C. Lin, J. Holthaus, H.G. Schafstall, and P.B. Bennett, Tripler Army Med. Ctr., HI 96859, Univ. Hawaii, HI 96822, GKSS Forschungszentrum, Geesthact, FRG, Duke Univ., NC 27710.

We assessed vasopressin (AVP), plasma renin activity (PRA), aldosterone (pALDO), and atrial peptide (AP) responses to upright tilt at 450 M in a He-N2-O2 environment. Tilt tests, 10 min supine followed by 15 min 90° passive standing, were performed at sea level (SL, 2 days), 450 M (2 days), 360 M (1 day) and near SL (NSL, last 2 days of decompression). Plasma AVP increased from 0.24 to 0.48 uU/ml ($p<0.005$) and AP decreased from 20.0 to 15.6 pg/ml ($p<0.025$) during SL tilt tests. Increases in PRA and pALDO during tilt at SL were not significant. Responses of AVP and AP to tilt were eliminated during hyperbaria and NSL. Both supine and upright AP values were reduced ($p<0.005$) in hyperbaria, but only upright AVP values were reduced at all times after SL ($p<0.005$). PRA increased during upright tilt at all periods after SL ($p<0.005$), and both PRA and pALDO responses were enhanced at NSL compared to SL ($p<0.005$). Although the accompanying dehydration during hyperbaria could explain the PRA, pALDO, and AP responses, the explanation for the reduced AVP response to tilt is not clear. (Supported by GKSS Forschungszentrum, US Army Health Services Command, NIH, HL 28542, NOAA Sea grant NA85-D-SG082 R/HP-6.)

49.3

CEREBRAL NATRIURESIS MEDIATED THROUGH URODILATIN.
C. Emmeluth*, C. Drummer*+, R. Gerzer*+ & P. Bie.
Department of Medical Physiology C, University of Copenhagen, Denmark. + Labor für Klinische Pharmakologie, Medicinische Klinik Innenstadt der Universität Munchen, Federal Republic of Germany.

The role of the Na^+ concentration of the head in the regulation of renal Na^+ excretion was evaluated in conscious dogs in which body water and Na^+ were controlled by separate servo-controlled infusions. A selective 2% increase in the Na^+ concentration of the carotid blood caused a marked increase in renal Na^+ excretion (from 3.2±0.4 µmol min^{-1} to 109±19 µmol min^{-1}) concomitant with a substantial increase in the rate of urinary excretion of urodilatin (from 21±2 fmol min^{-1} to 93±35 fmol min^{-1}). During infusion of the same amount of NaCl as an isotonic solution sodium excretion increased from 3.7±0.6 µmol min^{-1} to 50±9 µmol min^{-1}, i.e. the peak increase was significantly higher during the hypertonic infusion ($P<0.02$). The excretion of urodilatin did not change during the isotonic infusion (preinfusional level of excretion was 35±5 fmol min^{-1}, while the level at the peak sodium excretion was 31±5 fmol min^{-1}). Thus cephalic Na^+ concentration receptors seems to participate in the physiological regulation of the rate of excretion of sodium. Further, the results are compatible with the hypothesis that urodilatin is causally involved in this process.

49.4

SUSTAINED INTRAVASCULAR VOLUME DEPLETION DURING 2-DAY INFUSIONS OF ATRIAL NATRIURETIC FACTOR (ANF) IN HUMANS
Thomas Ebert, Peter Foley*, Leanne Groban*, Michael Muzi* and Allen Cowley, Jr. Med. Coll. of Wisconsin and VAMC, Milwaukee, WI 53295

Our previous work in humans demonstrates that brief (2-3 hr) low-dose infusions of ANF do not alter BP or renal function, increase capillary permeability and lower central venous pressure (CVP)(AJP:255:H685,1988; 256:F780, 1989; 259:H258, 1990). After informed consent, we evaluated hemodynamics, renal function, and plasma hormones (renin, aldo, vasopressin, norepi) in 8 subjects on fixed diets receiving 2-day infusions of ANF (2.5 ng/kg/min) or placebo (randomized and blinded).

	← ANF infusion →				Recovery
	Control	Hour 2	Day 1	Day 2	Hour 2
CVP, mm Hg	9.1 ± 1	7.1 ± 1*	6.6 ± 1*	5.8 ± 1*	7.8 ± 1
SV, ml	67 ± 4	56 ± 3*	55 ± 5*	58 ± 5*	60 ± 4
MAP, mm Hg	87 ± 2	90 ± 3	86 ± 3	83 ± 3	91 ± 3
Norepi, pg/ml	205 ± 38	198 ± 26	301 ± 47*	320 ± 44*	240 ± 62
ANF, pg/ml	40 ± 6	250 ± 56*	260 ± 62*	227 ± 83*	27 ± 5

X±SEM. *=$p<0.05$ vs. control and placebo response. SV=stroke volume.

Heart rate, blood pressure, renal function (GFR, $\dot{V}$, $U_{Na}\dot{V}$, $U_K\dot{V}$), renin, aldo and vasopressin did not change during either infusion. However, CVP and SV decreased and hematocrit and norepi increased throughout the ANF infusion period. In physiologic doses ANF's primary effect is to reduce intravascular volume by shifting fluids to extravascular sites. ANF also appears to limit the normal renal and hormonal response to intravascular volume depletion.
Supported by a NIH First Award to TJ Ebert.

49.5

ATRIAL NATRIURETIC PEPTIDE EXERTS PROTECTIVE EFFECTS ON CARDIAC FUNCTION. J Gu* and Z Tan*. Deborah Research Institute, Browns Mills, NJ 08015 (Spon: A.Malik)

A wealth of information about the newly discovered cardiac hormone atrial natriuretic peptide (ANP) has been obtained, however,knowledge about its actions on the heart itself has been very fragmentary. We examined the hearts of adult Wistar Kyoto rats (n=20) with immunocytochemistry and in situ hybridization and found ANP to be present in the cytoplasm of Purkinje cells in the subendocardium and myocardiocytes surrounding the bifurcation of the coronary arteries in the ventricles. In surgically overloaded and hypertrophic ventricles of rat hearts (n=30), ANP and its mRNA were also detected in more than 50% of the ventricular myocardiocytes. ANP perfusion ($3.26x10^{-8}$M) in an isolated working rat heart model produced no effects on the heart, however if noradrenalin ($8x10^{-5}$M) was administered prior to ANP perfusion, the marked chronotropic, inotropic, and vascular effects of the latter were reversed by ANP (n=5). The heart rate was reduced from 328±7.58 to 285±6.12/min; and coronary vascular resistance from 4.16±0.26 to 3.21±0.08mmHg.min/ml ($P<0.001$ for both parameters). We hypothesize that ANP exerts protective effects on cardiac function, which may not be apparent until abnormalties such as tachycardia and heart failure develop.
Supported by: Deborah Research Foundation DRG-1990-07

49.6

EFFECTS OF ANP ON BLOOD PRESSURE–HEART RATE BAROREFLEX IN CONSCIOUS DOGS AND RATS.
Robyn L. Woods*, Carol-Ann Courneya and Geoffrey A. Head*. Baker Medical Research Institute, Prahran, Victoria 3181, Australia.

The observation that ANP lowers blood pressure (BP) in conscious animals without eliciting reflex rise in heart rate (HR) has led to the suggestion that ANP interferes with the BP–HR baroreflex. We studied the effects of ANP on baroreflex responses that had differing proportions of input from the arterial and cardiopulmonary afferents by using the "steady–state" drug technique (predominantly arterial), in conscious dogs and rats, and the "rapid–ramp" drug technique (additional cardio–pulmonary) in conscious rats. "Steady–state" sigmoid MAP–HR curves were constructed from injections of pressor (phenylephrine or methoxamine) and depressor (nitroprusside) agents. HR responses (over 5–10 s) for the "rapid–ramp" technique were measured after rapid infusions of methoxamine. αhANP at 120 ng/kg/min in dogs and 150 ng/kg/min in rats did not affect any of the steady–state baroreflex parameters. However, αhANP caused an 80 ± 20% increase of the gain (slope) of the bradycardic response using the "rapid–ramp" technique. These results suggest that ANP may alter cardiopulmonary rather than arterial baroreflexes. Therefore, the lack of tachycardia in response to ANP–induced hypotension does not involve inhibition of the arterial baroreflex response.
Supported by the National Health and Medical Research Council of Australia.

49.7

EFFECT OF BUFFER NERVE SECTION ON ATRIAL DYNAMICS AND ATRIAL NATRIURETIC FACTOR (ANF) IN RESPONSE TO CHANGES IN BLOOD VOLUME. J.R. Ledsome and K. King* Dept. of Physiology, Univ. of B.C., Vancouver, B.C., Canada V6T 1Z3.

After section of the buffer nerves there is potentiation of the increases in plasma ANF which occur in response to changes in blood volume (BV). To determine the mechanism involved in the potentiation rabbits anesthetized with chloralose/urethane were subjected to changes in BV before and after section of the vagus, aortic depressor and carotid sinus nerves. BV was expanded to +20% then decreased at 1% BV per min to -20% BV or until arterial pressure (MAP) fell to 40 mm Hg. Plasma samples were taken at 5 min intervals and subjected to radioimmunoassay for ANF. We measured right and left atrial pressures and right and left atrial diameters, the latter using sonomicrometry. Right and left atrial systolic and diastolic wall stress (SRAS, SLAS, DRAS and DLAS) were calculated as the product of pressure and diameter divided by wall thickness (1 mm) at the peak of the a-wave and v-wave respectively. After nerve section, in 4 of 8 animals, there were large increases in IR-ANF and DLAS and SLAS. Data from these animals showed a linear relationship between IR-ANF and DLAS and SLAS over the range of BV tested. Data from all animals analyzed at +20% BV showed a similar relationship. There were no significant changes in DRAS or SRAS. The results suggest that the potentiation of the ANF response to changes in blood volume by buffer nerve section can be accounted for by changes in left atrial wall stress.

Supported by Heart and Stroke Foundation of B.C. & Yukon, and Medical Research Council of Canada.

49.8

THE EFFECT OF ANGIOTENSIN II, PHENYLEPHRINE AND NORADRENALINE ON PLASMA ATRIAL NATRIURETIC FACTOR IN ANESTHETIZED RABBITS. K.A. King* and J.R. Ledsome Dept. of Physiology, Univ. of B.C., Vancouver, B.C. Canada V6T 1Z3.

Although pressor agents release atrial natriuretic factor (ANF) from atrial myocytes, it remains unclear whether ANF release is mediated by a direct action or is secondary to the hemodynamic changes. Consequently, the effect of angiotensin II (ANG), phenylephrine (PHE) and noradrenaline (NOR) on atrial wall function and plasma IR-ANF was investigated in chloralose/urethane anesthetized rabbits. Left and right atrial dimensions were measured by sonomicrometry. Left and right atrial pressures were measured with cannulae inserted into the tip of the left atrial appendage and right jugular vein, respectively. Systolic left and right atrial wall stress (SLAS, SRAS) were calculated by multiplying peak a wave pressure by the corresponding atrial dimension and dividing by 4. Diastolic left and right atrial wall stress (DLAS, DRAS) were derived using v wave pressure and dimension. Plasma IR-ANF was determined by RIA. Animals were divided into 3 groups; each group was given only 1 drug, at 3 different infusion rates which increased arterial pressure (MAP) by 10, 25 and 50 mmHg. Group I (n=8) received ANG (0.1, 0.45 and 2.5 mg/kg/min for 5 min), Group II (n=8) received PHE (1, 7.5 and 30 mg/kg/min for 10 min) and Group III (n=7) received NOR (1, 7, 30 mg/kg/min for 10 min). Plasma IR-ANF was increased only after the highest dose of the three agents; MAP, SLAS and DLAS were also elevated. Thus extremely large increases in MAP appear to be necessary for ANF release to occur. This suggests that the release of ANF in response to ANG, PHE and NOR is secondary to hemodynamic changes, causing increased SLAS and DLAS. Supported by MRC and HSFBCY.

49.9

INHIBITION OF Jv BY ANF IN RAT PROXIMAL STRAIGHT TUBULE REQUIRES NOREPINEPHRINE (NE) OR ANGIOTENSIN (A II). Jeffrey L. Garvin. Ford Hospital, Detroit, MI. 48202.

The effects of ANF in the proximal nephron have proved controversial. In vivo experiments have provided evidence both for and against a direct effect of ANF in this segment. Reports for such an effect seem to require either high circulating levels of A II or that A II be infused into peritubular capillaries. Our hypothesis is that ANF inhibits Jv in the proximal nephron, but only after Jv has been stimulated by other agents. To test this hypothesis, we first measured the effect of 10^{-8}M ANF in rat isolated, perfused proximal straight tubules. There was no significant reduction in Jv. We next tested the ability of ANF to inhibit Jv while maintaining NE (10^{-7}M) in the bath. Initially, Jv was 0.50 ± 0.02 nl/mm.min. Fifteen minutes after 10^{-9}M ANF was added to the bath, Jv fell to 0.28 ± 0.04 nl/mm.min, an inhibition of 44%. We next tested the ability of ANF to inhibit Jv while maintaining A II (10^{-10}M) in the bath. Initially, Jv was 1.13 ± 0.06 nl/mm.min. Fifteen minutes after 10^{-10}M ANF was added to the bath, Jv fell to 0.65 ± 0.05 nl/mm.min, an inhibition of 43%. We conclude: 1) ANF alone does not inhibit Jv in the proximal nephron; 2) ANF inhibits Jv after it is stimulated by NE or A II; 3) the permissive role of NE and A II may involve diacylglycerol and activation of protein kinase C, since this pathway is common to both.

49.10

ANGIOTENSIN II ATTENUATES ATRIAL NATRIURETIC PEPTIDE-INDUCED CHANGE IN HYDRAULIC CONDUCTIVITY OF FROG MESENTERIC CAPILLARIES. Mary K. McKay* and Virginia H. Huxley
Dept. of Physiology, UMC Medical School, Columbia, MO 65212.

Previous work in our laboratory has demonstrated that the vasoactive hormone atrial natriuretic peptide (ANP) elevates the water conductivity (L_p) of true and venular capillaries. At present there is no known antagonist for ANP; it has been suggested that another vasoactive peptide, angiotensin II (AII), may antagonize the actions of ANP. In situ measurements of L_p were obtained using the modified Landis technique in individually perfused frog (*R. pipiens*) mesenteric vessels. In the present study we tested two hypotheses. First, we anticipated that AII would lower capillary L_p; Second, we expected that AII would abolish the rise in L_p induced by ANP. Control L_p (L_p^C) measurements were obtained during perfusion with frog Ringer's and 10 mg/ml bovine serum albumin for all experiments prior to perfusion with test solutions. To test hypothesis I, L_p was measured a second time during perfusion with 100nM AII. Median L_p^C= 1.9 x10^{-7}cm·s^{-1}·cmH$_2$0^{-1}(n=8). Addition of AII produced no significant change in L_p from control (L_p^{AII}/L_p^C = 1±0.2; mean±SEM). To test hypothesis II, L_p was measured a second time with 100nM ANP and a third time with 100nM ANP+1μM AII. Median L_p^C=1.2 x10^{-7} cm·s^{-1} cmH$_2$0^{-1} (n=7). In response to ANP, L_p rose 5.3±1.5-fold from control. ANP+AII perfusion decreased the ANP-induced rise in L_p by approximately 35%. These results suggest that while AII does not affect basal L_p, AII may attenuate the ANP-induced rise in capillary hydraulic conductivity. Supported by NIH HL42528; VHH is an AHA EI.

49.11

ATRIAL NATRIURETIC PEPTIDE REDUCES LEG TRANSCAPILLARY FILTRATION. DE Watenpaugh, SF Vissing, LD Lane, JC Buckey, BG Firth, W Erdman, AR Hargens and CG Blomqvist.
UT Southwestern Med. Ctr., Dallas, TX 75235, and Life Science Div., NASA Ames Research Center, Moffett Field, CA 94035-1000.

Atrial natriuretic peptide (ANP) commonly enhances transcapillary filtration (TCF) of fluid from the systemic vasculature. We measured TCF rate with prolonged venous occlusion plethysmography in legs of six supine subjects during infusion of ANP (48 pmol / kg x min for 15 min). Systemic hemoconcentration occurred during ANP infusion: mean hematocrit and plasma oncotic pressure increased 4.6% and 11.3%, respectively ($p < 0.05$). Mean leg TCF, however, was significantly reduced from 0.15 to 0.08 ml / 100 g x min with ANP. Heart rate increased significantly with ANP, while blood pressure was unchanged. Placebo infusion had no effects. Hemoconcentration indicates ANP-induced systemic capillary filtration took place, yet leg TCF was reduced with ANP. Increased plasma oncotic pressure partially explains reduced TCF in the leg, where the protein reflection coefficient may be relatively high. Therefore, pharmacologic ANP infusion appears to elicit TCF in the splanchnic circulation, where reflection coefficients are lower, while having the opposite effect in skin and skeletal muscle of the leg. (Supported by a NASA Graduate Student Research Fellowship to DEW).

49.12

EFFECT OF WEIGHTLESSNESS ON THE DISTRIBUTION OF ATRIAL NATRIURETIC FACTOR (ANF)-LIKE IMMUNOREACTIVITY IN THE BRAIN AND HEART OF THE FROG HYLA JAPONICA. M. Feuilloley, L. Yon, M. Okuno[1], S. Kikuyama[1], J. Gutkowska[2] and H. Vaudry (SPON: A. Dupont). Europ. Inst. Peptide Res., CNRS URA 650, Univ. of Rouen, Mt-St-Aignan, France; [1]Dept. Biol., School Education, Waseda Univ., Tokyo, Japan; [2]Clin. Res. Inst. Montréal, Canada.

Six treefrogs (Hyla japonica) were sent into space for 9 days in a "SOYUZ" spaceship and were kept in the space station "MIR". The distribution of ANF-like immunoreactivity was investigated in the brains and hearts of two "space frogs" and was compared to that observed in three control animals kept on Earth under similar conditions. In the telencephalon of the control frogs, the most prominent group of ANF-containing cells and fibers was observed in the amygdala. ANF-positive fibers and cells were also detected in the area of the nucleus olfactorius and the pallium mediale. In the "space frogs", the intensity of labeling of the amygdala and nucleus olfactorius was similar to that seen in the control animals; in contrast the rostral pallium of the "space frogs" was totally devoid of positive cell bodies. In the diencephalon, the preoptic nucleus of control and "space frogs" contained numerous ANF-immunoreactive perikarya. In all the animals, immunoreactive cells and fibers were noted in the anterior thalamus, infundibulum, median eminence and, caudally, in the nuclei tegmenti mesencephali and the nucleus cerebelli. Conversely, positive cell bodies were only observed in the lateral forebrain bundle of the control frogs and in the posterior nuclei of the thalamus of the "space frogs". In the hearts of all the frogs, atrial myocytes exhibited intense ANF-like immunoreactivity. The intensity of labeling in the ventricles was variable but apparently lower in the "space frogs" than in the control animals. These data support the concept that the biosynthesis and/or release of ANF-related peptides in the brain and heart may be affected by space flight and low gravity. Supported by grants from CNRS (URA 650), the Conseil Régional de Haute-Normandie and Tokyo Broadcasting System.

49.13

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AND ATRIAL NATRIURETIC PEPTIDE DURING CHRONIC EXPOSURE TO ALTITUDE. G.Opocher*, F.Mantero, D.Noventa,* A.Bussanich*, A.Daniele*, F.Pedini*, M.Zaccaria* Institute of Semeiotica Medica, University of Padua, 35100 Padua, ITALY

While the association of natriuresis with exposure to altitude and hypoxia is well known, less concordant are the data on the renin-angiotensin-aldosterone system (RAAs) and atrial natriuretic peptide (ANP) in this condition. We have studied the effect of a prolonged exposure to altitude during the EVK2-CNR Italian expedition in Nepal. Seven trained subjects, aged between 22 and 29 have been studied at sea level (sl1), after 1 (py1) and 3 (py 3) weeks at 5.050 m in the "pyramid lab" and back at the sea level (sl2). Blood samples for plasma renin activity (PRA), Aldosterone (aldo) and ANP had been drawn in upright position, centrifuged and frozen "in loco".

	ur. Na	ur.volume	PRA	pl.aldo	ANP
	mEq/24h	mL/24h	ng/mL/3h	ng/dL	pg/mL
sl1	166±34	1064±129	1.90±0.40	8.3±2.1	13.3±5.7
py1	427±46*	2450±255*	0.08±0.03*	3.9±0.5*	9.1±2.7
py2	257±34	1398±303	0.51±0.12*	4.1±0.1*	3.5±1.2*
sl2	228±33	1685±232	3.00±0.50	8.2±1.2	5.6±2.2

(* p< 0.01 versus sl1) Conclusions: our results indicate that, at least during the first period of adaptation to altitude, there is a great natriuretic and diuretic effect, a profound decrease of the RAAs while ANP remains unchanged and later tends to decrease. Natriuresis and inhibition of RAAs cannot be accounted to ANP changes which otherwise appear to be secondary to rather than the cause of altitude-induced natriuresis.

49.14

DEGRADATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) BY CPA 47 ENDOTHELIAL-DERIVED CELLS: EVIDENCE OF SOLUBLE AND CELL SURFACE PROTEOLYTIC ACTIVITIES. S.J. Frost, Y.-M. Chen and P.A. Whitson. (Spon. S. Fortney) NASA/Johnson Space Center and KRUG Life Sciences, Houston, TX 77058.

Clearance of ANP *in vivo* is rapid and nonguanylate cyclase receptors are thought to be responsible for its metabolism. In the present study, we investigated the cellular mechanism of ANP degradation by a bovine pulmonary artery-derived endothelial cell line, CPA 47. The kinetics showed rapid binding of ANP at 37°C (10 min), but a 76% decrease in specific binding after 60 min. The decrease in ANP binding was not due to downregulation of receptors since there was no change in ANP binding after preincubating cells with unlabeled ANP. Furthermore, there was little or no intracellular accumulation of ANP in the continuous presence of ANP or when cells were prebound with ANP and warmed to 37°C. These data suggest that ANP was not being internalized. However, a 95% loss in specific ANP binding was observed when ANP was preincubated with cells and then transferred to fresh cells for binding, indicating that ANP was being modified at the cell surface or by a soluble enzyme or both. HPLC analysis was used to determine the extent of ANP degradation and/or modification in the medium by CPA 47 cells. ANP was 90% degraded after 1h at 37°C or after 4h at 4°C. The conditioned medium from these cells also degraded between 40 and 60% of the ANP. These data suggest that these endothelial cell have both a soluble and cell surface-associated proteolytic activities. EDTA (2 mM) was found to inhibit the proteolytic activity in the medium by >90%. Cell surface activity was demonstrated by low temperature (50% degradation at 4°C in 1 h) or 0.2 M sucrose (28% degraded at 37°C in 20 min) which inhibits receptor-mediated internalization. These studies indicate that endothelial cells may play a crucial role in the *in vivo* clearance and metabolism of ANP.

49.15

PUTRESCINE RESTORES THE BASAL AND STIMULATED SECRETION OF ATRIAL NATRIURETIC PEPTIDE INHIBITED BY DIFLUOROMETHYLORNITHINE. Ulka R. Tipnis and Paul J. Boor. Univ. of Tx. Med. Br., Galveston, TX 77550

Atrial natriuretic peptide (ANP) is synthesized and secreted predominantly by atrial myocytes. Our previous studies (Tipnis, et al., J. Mol. Cell Cardiol., 21:743, 1989) demonstrated that Ornithine decarboxylase, the rate-limiting enzyme in the biosynthesis of polyamines, is immunolocalized in atrial secretory granules that store ANP, thereby suggesting a role for polyamines in ANP secretion. We have used difluoromethylornithine, an irreversible and covalent inhibitor of ODC activity and polyamine synthesis, to investigate the functional significance of polyamines in ANP secretion. Rats were given 2% DFMO in drinking water and received 200 mg/kg of DFMO (i.p.) in saline. Controls were given plain drinking water and saline (i.p.). Carotid artery was cannulated and ANP secretion was stimulated by arginine-vasopressin (AVP) (1 μg/animal, i.v.) and blood samples were removed at 0-30 min, centrifuged, extracted through SEPCOL1, lyophilized, and assayed by radioimmunoassay. Basal levels of plasma ANP in control were 89 pg/ml, while those in DFMO group averaged 25 pg/ml. Following AVP administration, plasma ANP levels in both groups increased to a peak at 5 min and returned to control level by 30 min. However, ANP levels at the time of peak secretion in control were two fold higher (532 pg/ml) than those in the DFMO group (269 pg/ml). Following the prior administration of putrescine (80 μM/kg), basal levels of ANP in control and DFMO group were 118 pg/ml and 67 pg/ml, respectively. However, putrescine restored the plasma ANP response to AVP-stimulation at 5 min, i.e. in control + putrescine and DFMO + putrescine groups ANP levels were 626 + 42 and 426 + 75 pg/ml respectively (not significantly different). These results demonstrate that putrescine is an intracellular modulator of ANP secretion.

49.16

BLUNT CIRCADIAN RHYTHMS OF BLOOD PRESSURE AND ATRIAL NATRIURETIC PEPTIDE IN CHRONIC RENAL FAILURE. Francesco Portaluppi,* Paolo Gilli,* Giorgio Trasforini,* Luciana Vergnani,* Maria Rosaria Ambrosio,* Roberta Rossi* and Ettore degli Uberti* (SPON: E. Roti). University of Ferrara, Ferrara, Italy I-44100

Hypertension due to chronic renal failure (CRF) is a condition in which the nocturnal drop of blood pressure (BP) can be lost or reversed. We investigated the circadian rhythm of atrial natriuretic peptide (ANP) and its relation with the BP pattern of 12 hypertensive patients in CRF matched by sex, age and mean 24-h BP levels with 12 patients affected by essential hypertension (EH). Noninvasive BP monitoring was performed with an oscillometric device and plasma ANP levels were measured by radioimmunoassay under carefully standardized conditions. In EH, plasma ANP concentration was highest at 4 AM (34±1 pmol/L) and lowest at 4 PM (16±1 pmol/L), while a nocturnal BP drop was evident. In CRF, no significant circadian change of ANP was detectable (22±3 and 20±4 pmol/L, respectively) and the nocturnal fall of BP was lost. These findings are further evidence in favor of a role for ANP in the alteration of BP homeostasis occurring during CRF. They suggest the existence of a causal relation between the circadian rhythms of ANP and BP.

49.17

DIFFERENTIAL REGULATION OF CARDIAC HORMONE GENES. Mona Nemer and Lina Dagnino, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA H2W 1R7

The heart synthesizes a family of peptide hormones which have potent natriuretic, diuretic and vasodilatory properties. These peptides which include ANF and isoANF/BNP contribute to cardiovascular homeostasis although their respective roles remain unclear. We hypothesized that differential expression of the genes encoding these two peptide hormones might reflect different physiological functions in health and disease. To this end, we developed a quantitative PCR protocol to measure both ANF and isoANF mRNA in cardiac and extracardiac tissues during normal development and in pathophysiological conditions. In addition, we examined the regulatory elements of the rat isoANF gene and compared them with those involved in ANF gene transcription using DNA mediated gene transfer into primary cardiocyte cultures. All our data so far are consistent with a differential temporal and spatial regulation of these two cardiac genes. Indeed, within the heart itself, ANF and isoANF genes are differentially expressed in atria and ventricles, and whereas the atria are the major site of ANF synthesis, ventricles are the main site of isoANF production since they account for over 90% of cardiac isoANF mRNA and peptides. In addition, there is a difference in the developmental regulation of the two natriuretic peptide genes and although they are both regulated by hypertrophy and increased blood pressure, the response to these stimuli is not coordinated temporally. Finally, and consistent with differential expression, regulatory promoter elements of the isoANF gene were mapped and found to be distinct from those of the ANF gene indicating the existence of more than one putative mechanism for regulation of cardiac genes.

50.1

SMOKE INHALATION INDUCES INDIRECT LUNG DAMAGE HM Loick*, LD Traber*, R Tokyay*, HA Linares*, DN Herndon, DL Traber. Univ. of Tex. Med. Br. & Shriners Brns Inst., Galveston, TX, 77550. The chemicals in smoke induce lung damage directly and by activation of granulocytes(PMN). We hypothesized that damage could be induced in areas of the lung which were not injured by the smoke. In 6 chronically instrumented sheep, the right lung and lower trachea was insufflated with smoke. Before and 30 min. after smoke the bronchial tissue blood flow (BTQ) was measured in the smoked and in the air insufflated lung with an endoscopic laser flow probe. All animals were studied for 24 hours. Lungs were harvested at autopsy for tissue PMN count (count/0.0645 mm^2), wet/dry ratio (W/D) and determination of tissue conjugated dienes (CD), products of lipid peroxidation. The lungs of healthy sheep served as controls. Data are mean ± SE and analyzed by t-test. BTQ of the bronchial wall was 36.5±2.4 and 35±2.9 ml/min/100g in the right and left lung, respectively. After smoke BTQ was increased only in the smoked lung (52 ml/min/100g). The tissue PMN count was significantly increased in the smoked lungs (11.5±0.9) compared to normal lungs (7.4±0.4). W/D and tissue CD were increased in both the smoked (W/D: 5.3±0.1, CD: 3.5±0.1) and air insufflated lungs (W/D: 4.9±0.2, CD: 2.5±0.2) compared to normal lungs (W/D: 4.3±0.1, CD: 1.9±0.1). Smoke can induce tissue damage indirectly. We believe that the underlying pathogenesis is due to a release of mediators over time from damaged areas into the interstitial space and down the airways to uninjured tissue.

50.2

PERIODICITY OF TRACHEAL CILIARY BEAT FREQUENCY IN VIVO. Tarun Chandra, Lid B. Wong, Irving F. Miller and Donovan B. Yeates. University of Illinois at Chicago and Veterans Administration, West Side, Chicago, Illinois, 60612

Cytosolic calcium oscillations may be associated with the maintenance of cellular functions. Any periodicity of ciliary beat frequency, CBF, may be dependent on calcium induced calcium release. 8 dogs were anesthetized with pentobarbitol (40 mg/kg). The cuff of the endotracheal tube was inflated in the distal trachea. CBF in the mid trachea was measured in real time at 5 s intervals using nonstationary heterodyne laser light scattered from the ciliated epithelium. 4mg/kg of hexamethonium and 4 mg/kg of indomethacin were administered i.v. to inhibit neural and cyclooxygenase pathways. To increase intracellular calcium through non-receptor mechanisms aerosolized A23187 (10^{-9} to 10^{-6} M) were delivered to the trachea. Following the administration of aerosolized nifedipine (2 mg/ml) the trachea was again challenged with A23187. The baseline CBF in each dog was observed to oscillate between 6 and 12 Hz with a period of 3.3 min. A23187 synchronized these oscillations as evident when the temporal nature of CBF was averaged over all dogs. The amplitude of CBF stimulation initiated by 10^{-6} M A23187 was inhibited by nifedipine. The period of the CBF was not changed by A23187 with or without pretreatment with nifedipine. These data indicate that the induced stimulation of CBF was dependent on voltage operated calcium channels but period of CBF was not.

50.3

CHARACTERIZATION OF LEUKOTRIENE C_4 RECEPTORS IN BULLFROG LUNG. M.J. Chiono*, J.J. Andazola*, O. Torres,*, and C.A. Herman. Department of Biology, New Mexico State University, Las Cruces, NM 88003.

LTC_4 has potent contractile effects on isolated bullfrog lung strips. Specific binding sites for [^{3}H] leukotriene C_4, ([^{3}H]-LTC_4), were characterized in membrane preparations from American bullfrog (*Rana catesbeiana*) lungs. Binding assays were done at 23°C for 30 min with serine borate (10 mM) to prevent metabolism of LTC_4 to LTD_4. Specific binding reached steady state within 10 min and was reversible. Scatchard analysis predicted a single class of binding sites with a K_d of 48.1 nM and a B_{max} of 54.7 pmol/mg protein. LTC_4 was the most potent inhibitor of [^{3}H]-LTC_4 specific binding with a K_i of 33 nM. LTD_4 and LTE_4 had K_i values of 3350 nM and 5979 nM, respectively. The K_i values for the mammalian LTD_4 antagonists LY171883, L-649,923 and ICI 198,615 were 144900 nM, 117750 nM and 117750 nM, respectively. Glutathione and hematin had K_i values of 57000 nM and 29000 nM, respectively. In addition, no activity of glutathione S-transferase was detected in the membrane preparation suggesting that the binding site is not the enzyme. Guanosine 5'triphosphate and guanyl-5'-yl-imidodiphosphate did not affect specific binding of [^{3}H]-LTC_4 indicating that a G_i protein is not involved in the transduction mechanism. The data suggest that LTC_4 exerts its action by binding to receptors in bullfrog lung.

50.4

CHARACTERIZATION OF LEUKOTRIENE B_4 BINDING SITES IN BULLFROG LUNG MEMBRANES. J.J. Andazola,*, J.A. Underwood,*, M.J. Chiono,* and C.A. Herman. Department of Biology, New Mexico State University, Las Cruces, NM 88003.

Leukotriene B_4 (LTB_4) binding assays were carried out using lung membrane preparations from the American bullfrog (*Rana catesbeiana*). Lung tissue was homogenized with a polytron. The homogenate was centrifuged at 1000 x g and the supernatant centrifuged at 30000 x g. The final pellet was resuspended, frozen in liquid nitrogen, and was stored at -80°C until use. Incubations were carried out at 23°C for 30 min in 20 mM Tris-HCl (pH 7.5) containing 10 mM $MgCl_2$. Competition studies demonstrated LTB_4 and 20-OH LTB_4 were the most potent competitors of [^{3}H] leukotriene B_4, ([^{3}H]-LTB_4) specific binding, with K_i values of 6.96 nM and 195.00 nM, respectively. LTC_4, LTD_4 and LTE_4 failed to inhibit the binding of [^{3}H]-LTB_4 with concentrations from 10^{-9} - 10^{-5} M. When 10^{-4}M GTPγS was added to the LTB_4 binding assay, a K_i of 24.6 nM was obtained, suggesting the LTB_4 receptor is associated with a guanine nucleotide regulatory protein. Scatchard analysis indicated a single class of binding sites with a K_d of 4.3 nM and a B_{max} of 1744.6 fmol/mg protein. This K_d is similar to the K_d of 3.85 nM reported for guinea pig lung macrophages. These data suggest LTB_4 binds to specific receptors in bullfrog lung membrane preparations although the location of binding remains to be determined.

50.5

AXON REFLEX IN RESINIFERATOXIN (RTX)-INDUCED BRONCHOCONSTRICTION (RIB) OF GUINEA PIGS. H.-Q. Zhang*, and Y.-L. Lai. University of Kentucky College of Pharmacy, Lexington, KY 40536

To study the role of axon reflex in RIB in vivo, 24 guinea pig weighing 284 ± 8 g were randomly divided into four groups: *Gp 1*, control (n=6); *Gp 2*, chlorisondamine (n=6); *Gp 3*, tetrodotoxin (n=6); and *Gp 4*, tachykinin depletion (n=6). Each animal was anesthetized with sodium pentobarbital, cannulated with a tracheal cannula and venous catheter, paralyzed with gallamine triethiodide, and artificially ventilated. All animals were treated with atropine (0.2 mg/kg) and phenoxybenzamine (0.5 mg/kg). RTX (2 µg/kg),which is an ultrapotent capsaicin analog and causes release of tachykinins, was intravenously injected to induce bronchoconstriction. Immediately upon the RTX injection each control animal exhibited decreases in vital capacity, maximal expiratory flow and respiratory compliance, as well as increases in functional residual capacity and residual volume, indicating severe bronchoconstriction. The airway spasm then decreased gradually toward the baseline values. The animals in *Gp 3* indicated a partial abolishment of the RIB, while in *Gp 4* the abolishment was complete. *Gp 2* did not display a significant change from the control group. Since it is known that tetrodotoxin blocks nerve conduction, the data suggest that the axon reflex plays a significant role in RIB, which is apparently mediated via tachykinins. Supported by HL40369.

50.6

OXYGEN RADICALS IN EXSANGUINATION-INDUCED BRONCHOCONSTRITION (EIB) IN GUINEA PIGS. K.-R. Zhou*, and Y.-L. Lai, University of Kentucky College of Pharmacy, Lexington, KY 40536

To investigate the role of oxygen radicals in EIB, 33 guinea pigs weighing 348 ± 10 g were divided into five groups: *Gp 1*, control (n=7); *Gp 2*, chronic dimethylthiourea (DMTU, n=8); *Gp 3*, acute DMTU (n=7); *Gp 4*, superoxide dismutase (SOD, n=6); and *Gp 5*, catalase (n=5). DMTU, SOD, and catalase were used to scavenge hydroxyl radical, superoxide anion, and hydrogen peroxide, respectively. In *Gp 2*, DMTU was administered for 3 days (the daily doses were 250, 125, and 125 mg/kg, ip) before the study. In addition, 750 mg/kg of DMTU was infused via a venous catheter into each animal for 30 min before the baseline study. This latter acute DMTU treatment was also given to *Gp 3* animals. In *Gp 4* and *Gp 5*, SOD (120,000 U/animal) and catalase (400,000 U/animal) solutions were respectively infused for 15 min before the baseline study. All animals were anesthetized, sternotomized, and artificially ventilated. At 5 min intervals until 30 min after exsanguination, decreases in the maximal expiratory flow and dynamic compliance were used as indicators of EIB. Exsanguination in the control group caused a gradual increase in EIB that was significantly ameliorated by chronic DMTU, but was not significantly altered by other treatments. These results suggest that EIB is modulated by oxygen radicals, which may be involved in the biosynthesis and/or axonal transport of tachykinins. Supported by HL 40369.

50.7

SARCOMERE LENGTH RANGE OF IN SITU RAT DIAPHRAGM. D.C. Poole and O. Mathieu-Costello. Dept. of Medicine, U.C.S.D., La Jolla, CA. 92093-0623.

Maximum transdiaphragmatic pressure decreases sharply (~70%) with increasing lung volume from functional residual capacity (FRC) to total lung capacity (TLC) (Braun et al. J. Appl. Physiol. 53: 405-412, 1982). One putative cause of this is that as lung volume increases, the capacity for tension development of the diaphragm is reduced. Thus, as sarcomere length becomes progressively shorter on the descending limb of the sarcomere length-active tension curve, tension development becomes increasingly impaired. However, to our knowledge, sarcomere length in diaphragm has never been related to lung volume and/ or airway pressures in situ. Eleven rats were perfusion-fixed with glutaraldehyde at different airway pressures, from -20 cmH_2O (corresponding to residual volume, RV) to +25 cmH_2O (TLC) and during sub- or supramaximal stimulation. Sarcomere and filament lengths in costal diaphragm were determined using microscopic techniques. At FRC, sarcomere length was 2.66±0.07 μm. From RV to TLC, sarcomere length ranged from 2.98±0.05 to 2.21±0.04 μm, respectively. Direct supramaximal stimulation of the diaphragm reduced this further to 2.12±0.04 μm. Considering that peak active tension (l_o) is developed at a sarcomere length of ~2.70 μm and using the $\%l_o$ to active -tension relationship of McCully and Faulkner (J. Appl. Physiol. 54: 1681-1686, 1983), the ~17% reduction in sarcomere length from FRC to TLC suggests that only a modest portion of the reduced transdiaphragmatic pressure at high lung volumes can be attributed to decreased sarcomere length per se. It is likely, therefore, that other factors e.g. gross geometrical changes are important determinants of transdiaphragmatic pressure generation at high lung volumes.

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50.8

V_1-VASOPRESSINERGIC PULMONARY VASODILATION IN ISOLATED RAT LUNGS. T.C. Resta*, R.D. Russ and B.R. Walker. Dept. of Physiology, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Controversy exists concerning the specific receptor subtype(s) involved in vasopressinergic pulmonary vasodilation. Therefore, experiments were performed on isolated, salt-perfused rat lungs to investigate the putative roles of V_1- and V_2-receptors in this response. Lungs were removed from male Sprague-Dawley rats, suspended in a humidified chamber at 38°C and perfused with a physiological saline solution at 30 $ml \cdot min^{-1} \cdot kg$ body $weight^{-1}$. Pulmonary arterial pressure was measured as an index of pulmonary vascular tone. Control pulmonary arterial pressure was approximately 8.5 mmHg and did not differ between groups. After time for stabilization, the pulmonary vasculature was preconstricted with the synthetic thromboxane analog U-46619. Administration of a 50 ng bolus of arginine vasopressin (AVP) to preconstricted lungs elicited a 65% reversal of pulmonary vasoconstriction. Similar vasodilatory responses were observed upon administration of either 50 ng of the selective V_1-receptor agonist [Phe^2, Ile^3, Orn^8]-AVP, or 50 μg of oxytocin. Vasodilatory responses to AVP, V_1-agonist, and oxytocin were attenuated by 93% or greater ($p<0.05$) following pretreatment with 10 μg of the V_1-receptor antagonist $d(CH_2)_5$ Tyr(Me)-AVP. However, pretreatment with 20 μg of the V_2-receptor antagonist [$d(CH_2)_5$, D-Ile^2, Ile^4]-AVP did not affect the AVP-mediated vasodilatory response. Furthermore, administration of the V_2-receptor agonist dDAVP (50 ng) to preconstricted lungs elicited no significant vasodilation. We conclude that AVP induces vasodilation of preconstricted pulmonary vasculature in the isolated, salt-perfused rat lung via stimulation of V_1-vasopressinergic receptors. (Supported by NHLBI Grant HL-42778.)

50.9

CHRONIC HYPOXIA DOES NOT ATTENUATE VASOPRESSINERGIC PULMONARY VASODILATION. R.D. Russ and B.R. Walker. Dept. of Physiology, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

We have previously demonstrated that arginine vasopressin (AVP) dilates preconstricted pulmonary vasculature via the release of endothelium-derived relaxing factor (EDRF). However, recent evidence suggests that EDRF release in response to other agents may be supressed in lungs from animals chronically exposed to hypoxia. Therefore, we investigated the putative role of EDRF in vasopressinergic vasodilation in lungs from rats acclimated at P_B = 380 mmHg for 4 weeks. Lungs from control and chronically hypoxic rats were suspended in a humidified chamber (38°C), and perfused at 30 $ml \cdot min^{-1} \cdot kg\ BW^{-1}$ with a physiological saline solution. Pulmonary artery pressure was measured as an index of pulmonary vascular tone. Following a 20 minute equilibration period, the pulmonary vasculature was preconstricted with the synthetic thromboxane analogue U-46619. At the height of vasoconstriction, a 50 ng bolus of AVP was administered and the resulting vasodilatory response observed. Administration of AVP to chronically hypoxic and control lungs resulted in a 73±2% and 68±3% decrease in pulmonary artery pressure, respectively. Separate groups of lungs were pretreated with the EDRF synthesis inhibitor N^{ω}-Nitro-L-Arginine (N-ARG; 18.45mM). N-ARG pretreatment attenuated the vasodilatory response to AVP by 81% and 82% in chronically hypoxic and control lungs, respectively. Addition of 10 $\mu g \cdot ml^{-1}$ meclofenamate to inhibit prostaglandin biosynthesis did not affect responses in any of the groups tested. We conclude that AVP causes dilation of the pulmonary vasculature via the release of EDRF in lungs from both control and chronically hypoxic rats. (Supported by HL-42778)

50.10

DOES PGE_2 RELEASE FETAL NON-PITUITARY IMMUNOREACTIVE ACTH FROM A PULMONARY SOURCE? Timothy A. Cudd, Colin Sumners, M. Ian Phillips, and Charles E. Wood. Department of Physiology, University of Florida, Gainesville, FL 32610

The late term sheep placenta secretes large quantities of prostaglandin E_2 (PGE_2) into the fetal circulation. Prostaglandins are involved in mediating developmental changes in the fetus including changes in blood pressure and blood flow in many organs including the lungs. It has been hypothesized that PGE_2 stimulates the late term rise in fetal plasma cortisol and the initiation of parturition. We have recently reported increases in plasma IR-ACTH from a non-pituitary source in late gestational sheep fetuses infused with PGE_2. We hypothesize that the fetal lung is the source of the increased late gestational plasma ACTH. Pulmonary tissue from late gestational fetal sheep was examined for the presence of immunoreactive ACTH using radioimmunoassay (RIA) and immunocytochemical methods (n=6). Fetal pulmonary tissue contained 21.1±4.1(SEM) ng ACTH immunoreactivity per gram tissue wet weight measured by RIA. We calculate that late gestational fetal lungs contain about 2.7 μg of IR-ACTH, an amount comparible to that found in late gestational fetal pituitary. ACTH immunoreactivity was also detected by immunocytochemical techniques in cells lining the alveolar space though it was not possible to identify the specific cell type. The presence of pulmonary immunoreactive ACTH supports the hypothesis that pulmonary ACTH may be released into the fetal circulation in response to late gestational increases in plasma PGE_2 concentrations. Support: NIH grant HD24250, Established Investigator Award, American Heart Assoc.(CEW); Post Doc. Fellowship, American Heart Assoc., Florida Affiliate (TAC).

50.11

PULMONARY VASODILATOR RESPONSES TO ACETYLCHOLINE AND ISOPROTERENOL ARE ABOLISHED IN CONSCIOUS DOGS FOLLOWING LEFT LUNG AUTOTRANSPLANTATION. K. Nishiwaki*, D.P. Nyhan*, P. Rock*, W.P. Peterson*, P.M. Desai*, C.G. Pribble*, B.X. Tong*, and P.A. Murray. The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Acetylcholine (Ach) and isoproterenol (Iso) cause vascular relaxation via increases in cytosolic concentrations of cyclic GMP and cyclic AMP, respectively. We investigated the effects of intravenously administered Ach (0.05 μg/kg/min) and Iso (0.1 μg/kg/min) on the left pulmonary vascular pressure-flow ($LP\dot{Q}$) relationship of conscious dogs following left lung autotransplantation (LLA). LLA was achieved by serial anastomoses of the left pulmonary artery, left pulmonary veins and left mainstem bronchus. $LP\dot{Q}$ plots were constructed by continuously measuring the pulmonary vascular pressure gradient (PAP-LAP:mmHg) at multiple levels of left pulmonary $\dot{Q}$ (ml/min/kg) by gradually (~ 1 min.) inflating an occluder implanted around the right pulmonary artery. $LP\dot{Q}$ plots were generated in 6 dogs 2 - 3 weeks post-LLA before and during Ach and Iso. Compared to identically-instrumented normal conscious dogs, PAP-LAP was approximately doubled at each value of $\dot{Q}$ following LLA. For example, at $\dot{Q}$=80, PAP-LAP was increased ($p < 0.01$) from 19 ± 2 to 39 ± 5. However, despite this marked pulmonary vasoconstriction following LLA, neither Ach nor Iso had any significant effect on PAP-LAP at any value of $\dot{Q}$. At $\dot{Q}$=80, PAP-LAP was 39 ± 5 before and 40 ± 6 during Ach, and PAP-LAP was 38 ± 6 before and 37 ± 8 during Iso. Thus, LLA results in chronic pulmonary vasoconstriction, as well as a total loss of pulmonary vasodilator responsivity to Ach and Iso in conscious dogs. Supported by NIH 40361.

50.12

HYPOXIC PULMONARY VASOCONSTRICTION IS INCREASED IN COPPER DEFICIENT RATS. Corrie B. Allen* and Jack T. Saari. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202

The mechanism by which alveolar hypoxia produces vasoconstriction within the pulmonary vasculature remains poorly defined. Dietary deficiency of copper results in changes in a number of enzymes (cytochrome oxidase, superoxide dismutase, glutathione peroxidase, lysyl oxidase) which may be involved in this response. Weanling male rats were fed semipurified diets containing adequate (>5 ppm, n=16) or deficient (<1 ppm, n=16) copper for 5-8 weeks. Isolated perfused lung preparations were used to assess vasoconstrictor responses to 0.1 μg angiotensin II (AII) and hypoxia (3% O_2). Responses to AII and hypoxia were repeated five times. While there were no significant differences in baseline perfusion pressures between copper-deficient and copper-adequate lungs ($p=0.65$), the vasoconstriction in response to both acute AII and hypoxic ventilation was greater in the copper-deficient lungs in the last four of the five experimental bouts (Bonferroni contrasts, $p<0.05$). These findings suggest that individuals exposed to acute alveolar hypoxia (i.e. aircraft pilots, visitors to high terrestrial altitudes) may experience exaggerated pulmonary vascular responses to the alveolar hypoxia if they are copper-deficient. Further study is required to define amounts of copper consumption which prevent this exaggerated response.

50.13

WARM BUTANOL VAPOR THERAPY IN ASPIRATIONAL LUNG EDEMA. W. H. Waugh, East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Ethyl alcohol vapor (e.g. 20%) as defoaming agent in lung edema (Luisada Clin Pharm Therap 5:628,1964) appears disreputable, partly because foam bubbles of alveolar origin in lung edema are destroyed only partly & very slowly by air saturated with ethanol vapor (Pattle J Path Bacti 72:1956). As antifoam agent, I tested 7% 1-butanol (But) at 39°C, in a lung edema model in pentobarb-anesthetized rabbits breathing spontan thru a 10-ml tracheostomy mask. Air humidified by H_2O or 7% But at 39°C was started 30 min after instilled 1.1 ml/kg of soln [1.2 molal sorbitol (19 g%)-140 mmolal NaCl-10 mmolal HCl, pH 2.0].

H_2O Vapor Rx	PaO_2^x	$PaCO_2^x$	PapH	$PaHCO_3^-$	MAP^x
Before (n=7)	68±1.7*	40±1.4*	7.36±0.03	22±1.4*	96±3.1*
Edema, 30"	35±1.8*	34±1.8*a	7.39±0.04	20±1.3*	87±3.1*
Rx, 15"	36±1.4	32±1.8	7.40±0.04	20±1.6	85±2.2
Rx, 60"	38±2.2	31±1.5a	7.41±0.03	19±1.1	83±3.0
But Vapor Rx	[x = mm Hg; *, a, & b = mean diff P < 0.05]				
Before (n=6)	71±3.2*	38±1.1*	7.40±0.02	23±0.8*	91±1.5*
Edema, 30"	35±2.6*a	33±1.6*	7.43±0.02a	21±0.9*a	82±2.4*
Rx, 15"	40±2.5ab	33±1.2a	7.39±0.01	19±0.7ab	80±1.4a
Rx, 60"	45±1.7b	30±1.2a	7.34±0.03a	16±1.0b	63±1.3a

After 60 min Rx, edema proteins (mg/ml) were 2.6±0.2 & 2.6±0.2; lung/body wt ratios (g/kg) were 9.0±0.5 & 9.5±0.3, resp. Thus, warm But vapor appears effective for hypoxia in pulmonary edema associated with high permeability alveolar damage, without serious butyl alcohol-induced acidosis or CNS depression unless vapor Rx is protracted.

50.14

HETEROTOPIC LUNG TRANSPLANT:AN AUXILLIARY OXYGENATOR. Bernard E. Pennock, Robert R. Lazzara*, Anthony Furnary*, Dennis Trumble*, Peter D. Kaplan* and James A. Magovern* Medical College of PA at Allegheny General Hospital, Pittsburgh, PA 15212

Left lungs from donor dogs were transplanted into the retroperitoneal spaces of three recipient dogs. The donor pulmonary artery and left atrial cuff were anastomosed to the external iliac artery and vein. The transplanted lung was ventilated with 100% 02. With ventilation to the native lung removed, the following mean values were obtained:

	Transplant lung		Systemic	
	Pulm art	Pulm vein	Arterial	Venous
Pressure (mmHg)	29	24	86	5.3
Blood flow (L/min)	1.1		4.9	
p02	372	514	372	79
pC02	47	40	47	52

A mathematical model of this circulatory and gas exchange system was developed. The experimental data and model show that the transplanted lung can provide supplemental gas exchange, or under defined conditions, can provide total support. The heterotopic transplant could be useful in the treatment of life threatening reversible pulmonary failure.

50.15

EFFECTS OF POSITIVE END-EXPIRATORY PRESSURE (PEEP) ON NON-STEADY STATE CO_2 KINETICS IN THE DOG. B Mazumdar*, SA Isserles*, AV Montano*, and PH Breen*. (SPON: A Leff). Dept. of Anesthesia & Critical Care, University of Chicago, Chicago, IL 60637

Six chloralose-urethane anesthetized dogs underwent median thoracotomy (open pleural spaces) and constant O_2 ventilation. Measurements included VCO_2/br, pulmonary VCO_2 per breath by computer integration of exhaled flow and PCO_2; Q_T, cardiac output; $P_{ET}CO_2$, P_aCO_2 & P_vCO_2, end-tidal, arterial & mixed venous CO_2; and V_D/V_T, physiologic dead space fraction. The table shows mean (SD) values at Baseline (B) and 1, 2, 5, 15, and 25 min after application of 10 cmH_2O PEEP. (*denotes significant ($p<.05$) difference from 'B')

	B	1	2	5	15	25
VCO_2/br (ml)	9.9 (3.6)	*5.1 (2.6)	*6.5 (2.7)	*7.1 (3.0)	*7.2 (3.0)	*7.4 (3.0)
P_aCO_2 (Torr)	41 (5)	*36 (6)	39 (3)	43 (4)	44 (5)	45 (6)
Q_T(L/min)	2.9 (.5)	*2.3 (.5)	*2.2 (.6)	*2.0 (.5)	*2.0 (.6)	*2.0 (.6)
P_vCO_2 (Torr)	52 (5)	51 (5)	53 (2)	56 (3)	59 (4)	*62 (8)
$P_{ET}CO_2$ (Torr)	34 (2)	31 (5)	34 (3)	38 (3)	39 (3)	*40 (4)
V_D/V_T (%)	66 (8)	*76 (8)	*76 (8)	*76 (7)	*74 (7)	*74 (7)

At 1 min after PEEP, VCO_2/br decreased. The increase in functional residual capacity of 793± 206 ml diluted alveolar gas and reduced P_aCO_2. Q_T decreased to reduce CO_2 delivery to the lung. Then, P_vCO_2 steadily increased to restore CO_2 delivery to the lung. The value of increased $P_{ET}CO_2$ (at constant minute ventilation) might otherwise suggest increasing pulmonary CO_2 elimination. However, VCO_2/br remained significantly lower 25 min after 'B' because increased V_D/V_T during PEEP reduced alveolar ventilation. Thus, PEEP reduced CO_2 elimination from the lung that was not reflected by $P_{ET}CO2$. (Supported by grants from NIH (HL-42637) and Foundation for Anesth. Educ. & Research).

50.16

MALIGNANT HYPERTHERMIA: A CANINE MODEL OF CO_2 KINETICS. PH Breen*, AV Montano*, and SA Isserles*. (SPON: A Leff). Dept of Anesthesia & Critical Care, University of Chicago, Chicago, IL, 60637

An increase in end-tidal PCO_2 ($P_{ET}CO_2$) is an early monitor of malignant hyperthermia (MH). To investigate the time course of $P_{ET}CO_2$ during MH, we have used a canine model to simulate the pharmacokinetics of the CO_2 load that occurs in MH. CO_2 is added to venous blood in a bubble oxygenator. After Animal Care Committee approval, in 6 anesthetized (chloralose-urethane) and heparinized open-chest dogs (24± 4 kg), we inserted cannuli into the superior and inferior vena cavae to drain venous return into a bubble oxygenator. A calibrated roller pump returned blood to the right atrial appendage at constant cardiac output (Q_T) (1.6± .3 L/min). $P_{ET}CO_2$ was measured by side-stream infra-red capnography and VCO_2 was measured by integrating exhaled flow and tidal PCO_2. After Baseline measurements, CO_2 was added to carrier flows through the bubble oxygenator (FCO_2 = 13± 3%). Measurements were repeated up to 25 min. By 25 min after CO_2 input to the system, (as if muscle hypermetabolism was occurring), the mixed venous PCO_2 (P_vCO_2) increased significantly from 47± 6 to 76± 13 mmHg ($p<.05$, mean± SD), resulting in parallel increases ($p<.05$) in arterial PCO_2 (P_aCO_2, 35± 4 to 48± 7 mmHg), $P_{ET}CO_2$ (28± 6 to 38± 6 mmHg), and VCO_2 (143± 57 to 204± 57 ml/min). We have modelled CO_2 kinetics during MH, independent of effects from changing Q_T. The increase in $P_{ET}CO_2$ of 10 mmHg (36%) occurred in the face of a 29 mmHg (62%) increase in P_vCO_2, reflecting storage of CO_2 in peripheral tissues. VCO_2 increased more (43%) than $P_{ET}CO_2$, since it also measured changes in exhaled CO_2 volume. CO_2 stores approached equilibrium within about 25 min. We suggest that these data help understand CO_2 kinetics during MH. (Supported by NHLBI grant HL-42637 & Foundation for Anesthesia Education & Research).

CONTROL OF BREATHING

51.1

TOPOGRAPHIC, MORPHOLOGIC AND GROWTH CHARACTERISTICS OF DISSOCIATED CELLS SENSITIVE TO CO_2 AND LOW pH ISOLATED FROM THE RESPIRATORY AREAS OF THE UPPER MEDULLA. S.C. Fitzgerald, M.A. Willis, Chang Yu, and H. Rigatto. Lab.Dev.Neurobiol.,NICHD, NIH, Bethesda, MD and Dept. Pediat., Univ. Manitoba, Canada.

Dissociated cells from the areas of the nucleus ambiguus and the nucleus tractus solitarius obtained by punch technique or block dissection [Brownstein and Palkovits, New York, Elsevier, 1987] from coronal slices [1mm thickness] at the level of the obex were cultured from fetal rats [Sprague-Dawley] at 18 to 21 days gestation. Vitrogen coated 35 mm tissue culture dishes were plated with cortical or medulla derived non-neuronal cells split from flask, at 0.15 million cells per dish. After the non-neuronal cells reached confluency and were treated with FUDR, neuronal cells were plated at a concentration ranging from 0.5 to one million cells per dish. Neurons grew extremely well on medulla and cortical background, but were sparse on vitrogen dishes without any background. Medulla was the background of choice. Tissue treated with papain instead of trypsin yielded cleaner preparations, and the cell yield from the tissue was greater. This method produced a neuronal population of heterogenous morphology including small and large bipolar, pyramidal, and multipolar cells. Neurons sensitive to CO_2 and/or low pH [n=68] did not appear to have any definitive morphologic characteristics, but most were multipolar. These neurons stained well with antibodies to neuro-specific enolase [NSE] and fragment C tetanus toxin. They did not seem to stain for choline acetyltransferase [ChAT]. However, other cells in these preparations were immunoreactive for ChAT. Use of alpha-1 or 2 probes for adrenergic receptors did not appear to target these cells. These findings suggest that neurons responsible for the central regulation of respiration can be cultured. These cultures may serve as a system for neurotransmitter, electrophysiological, and other morphological studies of cells from these regions of the brain.

51.2

CELLS INHERENTLY SENSITIVE TO CARBON DIOXIDE (CO_2) OBSERVED IN DISSOCIATED CULTURES OF THE UPPER MEDULLA IN THE FETAL RAT. H. Rigatto*, S.C. Fitzgerald, M.A. Willis and C. Yu. Lab. of Dev. Neurobiology, NICHD, NIH, Bethesda, Md. and the Dept. of Pediatrics, Univ. of Manitoba, Canada.

Dissociated fetal rat (Sprague-Dawley) cells in culture (n=87) obtained from the nucleus ambiguus and adjacencies (Na area, n=57) and the tractus nucleus solitarius and adjacencies (NTS area, n=30) were used for electrophysiologic patch recording. Cells with a regular firing pattern resembling pacemaker activity were more frequent in the NA area than in the NTS (25% vs 10%; $p<0.05$); those firing irregularly (29% vs 43%; p=0.11) and the silent cells (46% vs 47%; p=0.8) were similar in both areas. With small pulses (<100 ms) of media equilibrated with high CO_2 (PCO_2 190±20 torr; pH 6.704±.012), applied adjacent to the cell, firing frequency increased from 129±11 to 345±14 spikes/min (p=0.001) and amplitude decreased from 45±4 to 38±3 mV (p=0.002) in the pacemaker cells. Irregularly beating or silent cells usually required pulses greater than 200 ms and were insensitive or inhibited by CO_2. Input membrane resistance appeared not to change and membrane potential decreased by 4 to 10 mV during CO_2. Synaptic blocking with tetrodotoxin or high magnesium media did not prevent the response to CO_2. Media with HCl of similar low pH but normal PCO_2 stimulated and media with high oxygen (>600 torr) inhibited activity at rest. The findings suggest a) some cells, primarily in the NA area, even in culture, express a phenotype inherently designed to respond to CO_2 and/or low pH; and 2) these characteristics would allow them to be responsible for central respiratory chemoreceptor activity. We speculate that these cells may also be involved in the generation of the respiratory rhythm.

51.3

RESPIRATORY RESPONSES TO CO_2 IN THE BRAINSTEM PREPARATION OF *RANA CATESBEIANA*. E.G. Smith, R.J. Galante*, S. Lahiri, A.I. Pack. Center for Sleep & Respiratory Neurobiology and Department of Physiology, University of Penna., Phila., PA

In mammalian systems, increases in CO_2 produce an increase in ventilation. This is thought to be mediated, at least in part, by cells in the ventral medullary surface (the central chemoreceptors). Since the cellular basis for this response is unknown, we wondered whether the recently described *in vitro* brainstem preparation of the larval form of *Rana catesbeiana* showed chemosensitivity and, therefore, might be a preparation to study cellular mechanisms of CO_2 sensing. Brainstems were removed and secured in a recording chamber and superfused with standard modified Krebs solution. Discharges of the facial motor nucleus were recorded extracellularly to monitor respiration. Altering the pCO_2 (pH) in the superfusate changed the frequency of the rhythm related to lung ventilation, but not to gill ventilation. At different pH's we recorded the following average frequencies/hour of lung ventilation: (n=7) 119±21.0 (pH 7.2); 81±18.6 (pH 7.4); 14.3±2.0 (pH 7.8). The frequency of gill ventilation/ minute (n=7) was 38.7±3.5 (pH 7.2); 40.3±2.8 (pH 7.4); 42.5±3.6 (pH 7.8). Recently we have shown that there are putative pacemaker cells for lung, but not for gill ventilation in this preparation. With removal of Cl^- (replaced with gluconate) lung ventilation, but not gill ventilation, persists. We questioned whether the activity of the pacemaker system is altered by CO_2 (pH). We found that CO_2 in the absence of Cl^-, in a different series of studies, still changed the frequency of lung ventilation (n=7) 56.6±9.5 (pH 7.2); 43.7±7.0 (pH 7.9). In conclusion, the brainstem of this preparation (800 μm thick) has chemosensitive elements, and these elements are connected to the putative pacemaker cells for lung ventilation (Supported by HL 39775, T32HL 07027-16).

51.4

INHIBITION OF THE ROSTRAL MEDULLARY RAPHE NUCLEUS (RMRN) UPON RESPIRATORY-MODULATED FACIAL DISCHARGE IN THE CAT. J.-C. Hwang, L.-C. Hu* and S.-E. Wang*. Dept. of Biology, National Taiwan Normal University, Taipei, Taiwan 11718, R.O.C.

To examine respiratory-modulated facial response to RMRN activation, activities of both the facial (FN) and the phrenic nerves were monitored in decerebrated, vagotomized, paralyzed and ventilated cats. Electrical stimulation (12.5 to 100 uA, 80 Hz and 0.5 ms pulse duration) and glutamate injection was performed to activate the RMRN. The animal was maintained at normocapnia and hypercapnia in hyperoxia. The results showed that respiratory-modulated activity of the FN was reduced while non-respiratory was excited in response to the RMRN activation. The reduction of FN activity with RMRN activation was due to the decrease of discharge rate of inspiratory facial motoneurons. At high concentration of CO_2, diminishes in discharge rate of facial motoneurons and whole FN activity with RMRN activation were attenuated. Moreover, some silent facial motoneurons were recruited when the RMRN was activated. These results suggest that the RMRN may exert an inhibition upon respiratory-modulated facial activity whereas excitation on non-reapiratory facial discharge (Supported by NSC grant #80-0211-B-003-05).

51.5

HYPOGLOSSAL (HG) AND PHRENIC (PR) NERVE RESPONSES TO CAROTID BARORECEPTOR STIMULATION. MJ WASICKO, C UKABAM, SL KNUTH, JC LEITER. DARTMOUTH MEDICAL SCHOOL, HANOVER, NH.

Elevated arterial BP leads to a preferential inhibition of the activity of HG compared to PR. To determine the role of the carotid baroreceptors (BARO) in this reflex, we studied 4 decerebrate, vagotomized, paralyzed, mechanically ventilated cats. The left carotid sinus was stimulated by inflating a 3F balloon catheter, inserted in the lingual artery and positioned at the carotid sinus; the right carotid sinus was denervated. BP was maintained constant. Steady-state BARO stimulations were 30 sec in duration; 5-7 stimulations were performed in each cat before and after denervation of the left carotid sinus. The following results were obtained: BARO stimulation inhibited peak inspiratory PR and HG activity to 79.7±4.2 and 56.7±4.6 % control, respectively. Barodenervation abolished this response. With BARO intact, HG returned immediately to control levels upon cessation of the steady-state BARO stimulation. After cyclic (1/sec) BARO stimulation, however, HG exhibited sustained inhibition (> 5 breaths post-BARO stimulation). We conclude: 1) elevated BP leads to preferential inhibition of HG by direct stimulation of carotid BARO; 2) brainstem centers involved in this reflex are sensitive to the dynamics of the BARO afferent information. These data have implications for obstructive sleep apnea: increases in BP that occur in the post-apneic period may lead to increased pharyngeal collapsibility through BARO mediated inhibition of upper airway dilator muscle activity. Support: HL01998, HL07449.

51.6

Analysis of pharyngeal resistance and genioglossal EMG activity using a model orifice flow. J.C. Leiter. Dartmouth Med. Sch., Hanover, N.H. 03756.

I have applied a model of orifice flow to pharyngeal pressure, flow and genioglossal electromyographic (EMG_{ge}) measurements made in humans during inspiration. The results suggest that airflow is turbulent in the pharynx for flow rates between 0.3 and 1.5 L/sec. Furthermore, the area of the pharynx appears to increase as flow increases, but the actual change in pharyngeal diameter necessary to fit the pressure-flow data is quite small (0.11 to 0.87 cm, depending on the assumptions in the model). By analyzing pressure and flow as a function of EMG_{ge}, I have also estimated the effect of a given level of EMG_{ge} activity on orifice area. The flow related increase in orifice area can be attributed, in part, to the activation of the genioglossus muscle. However, other flow-related factors also contribute to pharyngeal dilation as airflow increases. Different airway shapes (circular and elliptical) and orientations (major axis anteroposterior and lateral) were incorporated into the model calculations, and genioglossal muscle shortening increases pharyngeal area and reduces pharyngeal resistance more effectively when the pharynx is elliptical with the long axis of the ellipse oriented laterally. The results of this analysis suggest that the genioglossus may operate at a significant disadvantage in patients with Obstructive Sleep Apnea in whom the pharyngeal lumen is small and oriented with the longer axis anteroposteriorly. Support: HL01998.

51.7

DIFFERENTIAL EFFECTS OF EXTRACELLULAR ATP ON CAROTID CHEMOSENSORY RESPONSES TO HYPOXIA AND NICOTINE. D. Spergel (SPON: S. Lahiri). Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6085.

It is unclear which neurotransmitter(s) mediates the carotid chemosensory response to hypoxia. ATP exists in glomus cell dense-core granules from which catecholamines are secreted during hypoxic or nicotinic stimulation. The hypothesis that ATP is also released and acts as a neurotransmitter in the carotid body was tested using carotid chemosensory discharges in vitro. Exogenous ATP, ATP analogues and ATP hydrolysis products were used. Their effects on chemosensory discharge and responses to ischemic hypoxia evoked by perfusate stop-flow and to a pulse of 4 nmole nicotine were measured. The cat carotid body was perfused and superfused with modified Tyrode solution (PCO_2<1 Torr, pH 7.4, 36°C) equilibrated at PO_2=150 Torr and PO_2<40 Torr, respectively. Pulses of ATP showed dose-dependent stimulation of chemosensory discharge (ED_{50}=60 nmole). The responses to ATP analogues, α,β-methylene ATP and ATP-γ-S, were larger and slightly longer lasting. The responses to similar doses of ADP, AMP and adenosine were smaller than those to ATP. Constant infusion of 1.65 mM ATP desensitized the responses to ATP and nicotine after the initial stimulation but did not desensitize the response to stop-flow. The results suggest a) that carotid body cells have ATP receptors, b) that extracellular ATP suppresses the excitatory response to nicotine but is not involved in O_2 chemotransduction, and c) that the desensitized nicotine receptors are not a prerequisite for O_2 chemotransduction. (Supported by NIH grants 2-T32-HL-07027-16 and HL-43413-02.)

51.8

MENTHOL INDUCED HYPOPNEA IS GREATLY DIMINISHED BY TRIGEMINAL NERVE BLOCKADE. G.E. Woodson, F. Sant'Ambrogio, J.W. Anderson, G. Sant'Ambrogio. UTMB, Galveston, Texas, 77550

Stimulation of upper airway cold receptors, by L-menthol, reduces ventilation. Most of the receptors responsible for this reflex are in the nose. To determine whether this response is due to trigeminal or olfactory afferents, the effect of l-menthol was studied before and after trigeminal block by intraorbital injection of 2% lidocaine (0.1 ml). Anesthesia was induced in 9 guinea pigs (urethane, 1g/kg), tracheotomy was performed, and the upper airway was isolated to permit passage of air (30 ml/s) in the expiratory direction. Airflow, tidal volume, and laryngeal temperature were recorded in control conditions and during passage of warm air (37°C, saturated) with addition of l-menthol (390 ng/ml). Warm air + l-menthol reduced ventilation to 27 ± 8% of control ($P<0.01$) before trigeminal block and to 90 ± 8% of control ($P>0.05$) after block. The ventilatory depression was mostly due to prolongation of T_E, 1,152 ± 262% ($P<0.01$) before and 142 ± 17% ($P<0.01$) after block. The effects of trigeminal blockade are significant ($P<0.01$). One hour after lidocaine injection there was only a partial recovery. Intraorbital injection of equal volumes of saline did not modify respiration. We suggest that the response to menthol is mostly due to activation of trigeminal cold receptors. However, a role for olfactory receptors cannot be ruled out. (NIH Grant HL-20122 and DC-00009)

51.9

MENTHOL IN THE UPPER AIRWAY DEPRESSES VENTILATION IN NEWBORN DOGS. Franca B. Sant'Ambrogio, James W. Anderson and Giuseppe Sant'Ambrogio. Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550-2774.

Upper airway cooling decreases ventilation, particularly in newborn animals in which superior laryngeal nerve (SLN) afferents mediate the response. Since laryngeal cooling activates cold receptors and inhibits respiratory modulated mechanoreceptors it is difficult to determine which type of receptor is responsible. To this purpose we used *l*-menthol, a specific stimulant of cold receptors in the absence of any temperature change. Experiments were carried out in 6 anesthetized puppies (5-14 days old) breathing through a tracheostomy, with the upper airway functionally isolated. Warm (37°C), saturated air was delivered through the upper airway without and with addition of *l*-menthol (390 ng/ml) for 10 s in the expiratory direction. Airflow, tidal volume and esophageal pressure were recorded. The test trials with *l*-menthol markedly depressed ventilation ($\dot{V}_E$, 45±14% of control, $P<0.05$), mostly due to a prolongation of T_E (402±110% of control, $P<0.05$). SLN section reduced considerably the response to *l*-menthol ($\dot{V}_E$, 82±8%; T_E, 118±14%); the residual effect was abolished by nasal anesthesia. In one puppy we recorded from the SLN the activity of 2 cold receptors and ascertained the prompt stimulatory effect of *l*-menthol. This results support a preponderant role of cold receptors in the response to upper airway cooling. [Supported by NIH Grant HL-20122]

EXERCISE/HYPOXIA

52.1

TRAINING AND POST-EXERCISE HYPOTENSION WITH HYPERTENSIVE RATS (SHR). Martin D. Devine, Lisa A. Sebastian, Kim A. Monnin, and Charles M. Tipton*. Dept. of Exercise and Sport Sciences, School of Health Related Professions, University of Arizona, Tucson, AZ 85721.

Previous studies with nontrained (NT) hypertensive rats have shown that 25 minutes or more of moderate exercise (60-70% $\dot{V}O_2$ max) will be associated with a significant reduction in the post-exercise mean blood pressure (MBP) during a 60-minute recovery period when compared to their pre-exercise value. To gain insight on the responsible mechanisms as well as to learn whether endurance training (12-16 weeks at 40-70% $\dot{V}O_2$ max) would facilitate the process, we anesthetized 18 male trained rats and placed a sterile thermal probe and cannulae into the aortic arch, carotid artery, and jugular vein in order to measure MBP and cardiac output ($\dot{Q}$, thermal dilution) and to calculate cardiac index (CI), total peripheral vascular resistance (TPR) and the total peripheral vascular resistance index (TPRI). After 2-7 days of recovery, the rats performed an exercise test (60-70% $\dot{V}O_2$ max) and received either sterile saline or naloxone (1 mg·kg^{-1}) infusions. As with the NT rats, moderate acute exercise was associated with a significant reduction in post-exercise MBP ($F = .001$) in rats receiving saline. However, the reductions were not significantly lower or more prolonged when compared to NT groups. As expected, naloxone prevented the post-exercise fall in MBP. Analysis of variance or co-variance results did not indicate whether the reduction was due to changes in either $\dot{Q}$, CI, TPR, or TPRI. From these collective findings we have concluded that acute exercise by hypertensive rats will significantly lower post-exercise MBP; a history of chronic exercise does not facilitate the process; an opioid receptor(s) inhibitor will prevent the reduction, and it remains uncertain how the mechanism is altering measures of cardiac output and total peripheral vascular resistance.

(Supported in part by Grant HL-33782-05).

52.2

EFFECTS OF HIGH AMBIENT HEAT AND COOLING ON HEART RATE DRIFT AND WORK ENDURANCE. A.Sucec,* D.Trone,* R.Pozos,* R. Hesslink,* C. Bischoff,* E.LaBranch* (Spon: M. Buono) Naval Health Research Center, San Diego, Ca 92186

High heat loads have been shown to increase heart rate (HR) and reduce the duration of work over the course of steady state exercise to exhaustion. The purpose of this investigation was to ascertain the effectiveness of a portable individual ice cooling system (CS) at high heat load (dry bulb 49°C, 20%RH) (HH+CS) as compared to no cooling control (HH) at dry bulb 49°C, 20 %RH. Both slopes of HR response and work duration (WD) in minutes were assessed. Eight, unacclimatized male Marines, with means for; age=21 yrs, height=180cm and weight=78.3kg, walked at 4.8 km/hr and a two percent grade in chemical defense gear with a load of 34kg over nude weight on two different occasions. The results indicate that the cooling system reduced HR slope ($p<.05$) from 1.15 (HH) to 0.82 (HH+CS). Likewise, WD was improved from 24.3 (HH) to 32 min (HH+CS) ($p<.05$). These finding support the use of light weight cooling systems for increasing WD and reducing heart rate drift in high heat conditions.

52.3

ROLE OF PLASMA VOLUME IN CARDIOPULMONARY FUNCTION DURING EXERCISE IN THE EQUINE ATHLETE. H.H. Erickson, M.K. Hopper*, R.L. Pieschl*, N.G. Pelletier*, and C.P. Coyne*. College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506

Plasma volume increases with training and decreases in horses given furosemide to prevent exercise-induced pulmonary hemorrhage. Six horses were evaluated on a high-speed treadmill on the level at mild, moderate, and intense exercise (6-14 m/sec). Under control conditions, right atrial pressure (RAP) increased from 6 mm Hg at rest to 20, 28, and 40 mm Hg during exercise. Pulmonary artery pressure (PAP) increased from 30 mm Hg at rest to 49, 55, and 80 mm Hg during exercise. Systemic arterial pressure (SAP) increased from 115 mm Hg at rest to 151, 165, and 186 mm Hg during exercise. Cardiac output (CO) increased from 33 l/min at rest to 205, 261, and 293 l/min during exercise. Heart rate increased from 39 b/min at rest to 167, 183, and 203 b/min during exercise. Plasma volume (PV) increased from 28.9 to 35.3 liters with dextran infusion (DI). Following DI, RAP increased from 10 mm Hg at rest to 25, 31, and 45 mm Hg during exercise. CO increased from 54 l/min at rest to 242, 287, and 339 l/min during exercise. PV decreased from 28.9 to 26.5 liters with furosemide (1 mg/kg) administered three hours before exercise. FUR resulted in a significant reduction in RAP to 12, 17, and 31 mm Hg during exercise. There was a significant reduction in PAP from 80 mm Hg to 58 mm Hg at the highest level of exercise. CO, SV, and SAP were also reduced with furosemide. The results indicate that changes in PV can influence RAP and subsequently SV, CO, and vascular pressure responses to exercise in the equine athlete. (Supported by the American Quarter Horse Association)

52.4

EFFECTS OF HIGH INTENSITY INTERVAL TRAINING ON CREATINE DEPLETED RAT DIAPHRAGM M. Burch*, G.Howell*, H. Park*, H.L. Sweeney*, R.P. Farrar. (SPON: J Ivy). Dept. of Kinesiology, Univ. of Texas, Austin, TX, 78712 and Dept. of Physiology, Univ of Penn

Prolonged (4hr/day) endurance training has been demonstrated to induce a shift in type of diaphragm muscle of rats to a greater percentage of IIB fiber (Green H.J., et al. 1989). This study was designed to evaluate the interaction of an increased functional demand imposed upon the diaphragm by high intensity interval training, and the loss of temporal and spatial buffering of changes in the phosphorylation potential induced by creatine depletion. Fifty male Fischer 344 rats were divided into two primary groups. One was fed a control diet, the other a diet containing 3% w/w ß-GPA. The groups were further subdivided into a sedentary and a high intensity trained group. The animals were treated for 12-15 weeks. The diaphragm was removed and frozen for histochemistry, with a portion being utilized for citrate synthase activity. Surprisingly the interaction of ß-GPA and interval training did not alter the activity of the diaphragm muscle as measured by citrate synthase activity. The results from the sedentary control group were 41% Type I , 24% Type IIA, and 34% Type IIB. No significant changes were seen in the sedentary ß-GPA group. The interval trained group showed a shift of an increase to 40% in Type IIB fibers, a non-significant increase to 27% Type IIA, with a drop in Type I to 31%. The interval trained ß-GPA group showed an attenuation and/or reversal of this shift, maintaining a more equal fiber type distribution of 34% Type I, 35% Type IIA, and 31% Type IIB. Therefore, it appears the depletion of creatine phosphate in the cell induced a signal in the animals that were interval trained to maintain a higher percentage of IIA fibers.

52.5

THE EFFECT OF CREATINE DEPLETION AND ENDURANCE TRAINING UPON WORK CAPACITY AND CARDIAC FUNCTION. R.P Farrar*, D.Bowles*, H. Park*, H.L.Sweeney*, and J.W.Starnes*. (SPON: J.Wilmore) Dept. of Kinesiology, Univ. of Texas, Austin, TX. 78712 and Dept of Physiology, Univ. of Penn.

F344 male rats were placed in one of two main groups, creatine depleted or control diet, and then each group further subdivided into sedentary, endurance or interval trained groups. The treatments were evaluated in terms of *in vitro* heart function, myosin isozyme composition, citrate synthase, changes in *in vivo* work capacity (VO_2) and run-time to exhaustion. Creatine depletion, achieved through feedings of 3% ß-guanidinopropionic acid(ßGPA) in the diet, had no effect upon VO_2max when compared to their contol-diet matched groups. Creatine depletion doubled the run-to-exhaustion time in the sedentary group, reduced it significantly in the endurance group, and had no effect on the interval group, relative to the control-diet matched groups. ßGPA feeding did not alter the citrate synthase activity nor myosin isozyme patterns of the heart when compared to their control-diet matched groups. Significantly lower cardiac outputs were observed in all ßGPA treated groups when the hearts were evaluated against 130mm Hg afterload and paced at 420bpm in the working heart model. The interval trained ßGPA hearts had significantly higher cardiac outputs than all other ßGPA groups. Creatine depleted hearts appear unable to meet the demands of high heart rate and high afterload *in vitro*. The interaction of creatine depletion and training resulted in cardiac hypertrophy, a normal training response in VO_2max, but only interval training attenuated the decline in cardiac outputs against a high heart rate and high afterload.

52.6

THE INTERACTION OF CHRONIC ß-GUANIDINOPROPIONIC ACID (ßGPA) FEEDING AND INTERVAL TRAINING UPON SKELETAL MUSCLE FUNCTION H. Park*, G.Howell, H.L.Sweeney* R.P.Farrar* (SPON: J. Ivy). Dept. of Kinesiology, Univ. of Texas, Austin, TX, 78712 and Dept. of Physiology, Univ of Penn.

Depletion of creatine by the feedings of ßGPA in the diet has been utilized to determine whether changes in the spatial and temporal buffering of the phosphorylation potential can induce changes in aerobic capacity of the muscle as well as expression of myosin isozymes. In order to exacerbate changes in the phoshorylation potential high intensity interval training, repeated sprints of 60m./min, was imposed upon F344 male rats that were placed on a diet of 3% ßGPA. Following 12-15 weeks of this protocol the plantaris muscle was evaluated for changes in contractile, histochemical and biochemical properties. The interval training did not alter contractile function, but did increase citrate synthase activity by 50%. ßGPA feedings alone did not alter contraction time, half relaxation time or tetanic force, but the fatigue index was increased by 200%, while citrate synthase increased by only 25%. The interaction of the two increased contraction time and half relaxation time by 50%, increased fatigue index by 450%, decreased tetanic force by 60 %, but did not change specific tension. Histochemical analysis demonstrated that ßGPA feedings alone did not induce fiber type transformation, but that interval training caused a significant ($p \leq 0.05$) increase in type I fibers at the expense of type IIB fibers. The interaction of interval training and creatine depletion induced significant fiber type shifts with type IIB decreasing by 50%, type IIA increasing by 35%, and type I increasing by 100%. Interval training imposed upon creatine depleted muscles significantly increases the signals for fiber type transformation.

52.7

MODULATION OF HYPOXIC TOLERANCE BY NALOXONE AND MORPHINE IN MICE. Kimberly P. Mayfield and Louis G. D'Alecy*. The University of Michigan, Ann Arbor, MI 48109

Our laboratory has demonstrated that a non-lethal, hypoxic pretreatment increases hypoxic survival time (HST) in mice. To determine if endogenous opioids alter HST we administered naloxone (1 mg/kg i.p.) at minus 5 min, morphine (10 or 20 mg/kg i.p.) at minus 30 min, or saline (0.3 ml i.p.) at corresponding times prior to the hypoxic pretreatment. Sixty percent of the mice received the pretreatment consisting of three hypoxic exposures (4.5% oxygen balance nitrogen for 1.5, 2, and 2.5 min) separated by 5 minutes of room air. The remaining mice did not receive a pretreatment but instead were maintained on room air for this duration. All mice were tested for hypoxic survival by first exposing them to 20 sec of 8.5% oxygen balance nitrogen followed by exposure to 4.5% oxygen balance nitrogen. The HST was recorded as the time from the onset of the 4.5% oxygen to the cessation of spontaneous ventilation. Control mice (non-pretreated, saline injection) had a pooled mean HST of 147±9 sec (n=46). The pretreatment significantly increased HST ($p<0.001$, Mann Whitney U Test). Saline injected, pretreated mice had HST's of 434±61 sec (n=20, injected at minus 5 min) and 437±35 sec (n=49, injected at minus 30 min). Naloxone significantly ($p<0.01$) blunted the pretreatment effect producing a mean HST of 215±28 sec (n=17) which was not significantly different ($p=0.238$) from non-pretreated, saline-injected mice. Mice given 10 mg/kg morphine had an augmented pretreatment effect with a mean HST of 550±51 sec compared to pretreated, saline-injected mice. However, 20 mg/kg morphine dampened the pretreatment effect decreasing the mean HST to 353±31 sec which is a shorter survival time than the saline controls. These preliminary results suggest that endogenous opioids may be involved in the protective adaptation to hypoxia induced by prior exposure to non-lethal hypoxia.

52.8

HYPOCAPNIA, HIGH ALTITUDE, AND EGGSHELL CONDUCTANCE TO WATER VAPOR (GH_2O). S.C. Hempleman, F.L. Powell, T.P. Adamson* and R.E. Burger*. U.C. San Diego, La Jolla, CA 92093-0623 and U.C. Davis, Davis, CA 95616.

We hypothesized that hypocapnia associated with high altitude hypoxia in hens is responsible for the reduced GH_2O in avian eggs. If true, correcting hypocapnia (but not hypoxia) at altitude should return GH_2O to normal values. Seven hens native to 1200m were exposed to high altitude (3800m), and then to high altitude with 28 torr $PICO_2$ to relieve hypocapnia (3800m+CO_2). Eggshell GH_2O was measured gravimetrically, shell thickness was measured micrometrically ($\bar{x}$±sem):

	1200m	3800m	3800m+CO_2
Number of Eggs	102	82	118
GH_2O (mg/d/torr)	13.9±.2	12.6±.2	11.1±.2
Thickness (mm)	.297±.003	.287±.003	.305±.003
Pore Area (mm^2)	1.97±.03	1.72±.03	1.61±.03

Relieving hypocapnia at altitude reduced both GH_2O and pore area of the eggshell ($p<.05$), thus we must reject our initial hypothesis. Other altitude stimuli (eg. hypoxia or hypobaria) may cause the reductions in eggshell GH_2O and shell pore area we observed at 3800m. Hypocapnia does appear to cause thinning of eggshells at altitude ($p<.05$). Supported by NIH grants HL17731 and HL02071.

52.9

OXYGEN CONSUMPTION OF THE AXOLOTL, *Ambystoma mexicanum*, AS A FUNCTION OF VENTILATORY MODE DURING A PROGRESSIVE HYPOXIC CHALLENGE. C. F. Zwemer* H. D. Prange* and J. M. Stager. Physiology Section, Medical Sciences Program, Indiana University, Bloomington, IN 47405.

Are different metabolic strategies employed when bimodal breathers are forced to ventilate exclusively through the aquatic mode? To address this question, we measured the oxygen consumption ($\dot{V}O_2$) in $ml \cdot g^{-1} \cdot hr^{-1}$ of 11 adult axolotls during progressive aquatic hypoxia (PO_2 from 150 to 0 mmHg). Aquatic and aerial $\dot{V}O_2$ was measured during 30 min exposure to different aquatic PO_2's. The relationship between $\dot{V}O_2$ and PO_2 was best represented by a quadratic regression where $\dot{V}O_2 = 0.10 - 1.04 \cdot 10^{-3} \cdot PO_2 + 3.70 \cdot 10^{-6} \cdot PO_2^2$ (r=.83) for axolotls with access to air. For those without access to air, $\dot{V}O_2 = -3.40 \cdot 10^{-3} + 2.93 \cdot 10^{-4} \cdot PO_2 - 7.43 \cdot 10^{-7} \cdot PO_2^2$, (r=.91). These regressions differ significantly ($p<.0001$) and diverge significantly, ($p<.0001$) below a PO_2 of about 90 mm Hg. These results demonstrate that, when challenged by aquatic hypoxia, VO_2 is a function of the availability of access to air and that metabolism may be depressed when axolotls are forced to breathe solely in water. (Funded in part by Indiana University Doctoral Student Grant-in-Aid)

52.10

HYPOXIC ENHANCEMENT OF VASOPRESSIN RESPONSE TO HEMORRHAGE. M. R. Eichinger * and J. R. Claybaugh. Tripler Army Med. Ctr., Hon., HI. 96859

Cardiovascular and hormonal responses to hemorrhage with hypoxia were studied in conscious goats. Following control, goats continued in normoxia (NH) or were exposed to hypoxia (HH, FiO2=10%) for 120 min. After 60 min of normoxia or hypoxia, a hemorrhage was initiated (0.05 ml/kg/min for 30 min). Mean arterial blood pressure (MABP) and heart rate (HR) were monitored throughout; arginine vasopressin (AVP), atrial natriuretic factor (ANF), and plasma renin activity (PRA) were assayed. MABP was maintained through 20 min of hemorrhage in the NH group, and then decreased by 30 min of blood loss. In contrast, MABP was reduced by 20 min of hemorrhage in the HH group and remained decreased. AVP responses mirrored MABP changes in the NH group with a significant increase simultaneous to MABP decrease. However, AVP was significantly elevated 10 min prior to a decrease in MABP in the HH group. Further, lowest MABP values were not different between the groups, yet AVP was two-fold higher in the HH group. PRA increased in parallel in both groups up to 20 min; at 30 min NH group was higher than HH group and remained higher post-hemorrhage. Hypoxia significantly attenuated the ANF reduction in response to hemorrhage. Thus, hemorrhage during hypoxia results in an earlier development of hypotension and enhances the AVP response to MABP reduction.

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52.11

HYPOXIA MODULATES SYMPATHETIC VASOCONSTRICTION
LiQian Chen and A.P. Shepherd, Dept. of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284.

In a previous study, we noted that mesenteric venous PO2 and O2 consumption fell during the reductions in intestinal blood flow caused by sympathetic stimulation and that alpha-2 receptor antagonists enhanced autoregulatory escape (the partial recovery that flood flow undergoes in spite of sustained sympathetic stimulation). In addition, other studies indicated that decreased PO2 selectively inhibits the responsiveness of postjunctional alpha-2 receptors to UK-14,304. Therefore, we investigated the role of hypoxia in the modulation of sympathetic vasoconstriction by 1) infusing hypoxic blood intra-arterially and determining the effects of hypoxia on sympathetic vasoconstriction, 2) ascertaining the effects of hypoxia on the release rate of endogenous NE during sympathetic stimulation, and 3) determining whether hypoxia exerts effects in vivo on postjunctional responses to the selective alpha-1 and alpha-2 agonists, phenylephrine and clonidine. Our findings were that 1) infusing hypoxic blood to lower arterial PO2 from 125 to 52 mmHg attenuated the initial vasoconstrictor response after 30 sec of stimulation, 2) hypoxia caused blood flow to return further toward control in spite of continued stimulation, 3) hypoxia impaired neither NE uptake nor NE release at either 30 sec or 6 min of stimulation in the presence of cocaine, and 4) hypoxia inhibited the intestinal vasoconstrictor effects of selective alpha-2, but not alpha-1, agonists. The results support the hypothesis that hypoxia or an agent released during hypoxia inhibits sympathetic vasoconstriction by selectively antagonizing alpha-2-mediated postjunctional responses to neuronally released norepinephrine (supported by PO1 HL-36080).

BODY FLUID REGULATION

53.1

SELECTIVE INHIBITION OF ALDOSTERONE SYNTHESIS BY MDL 19,347 (18-ACETYLENIC DEOXYCORTICOSTERONE) IN SODIUM-DEPLETED MONKEYS. P.R. Kastner, R.A. Bohnke*, C.L. Wright*[1], and J.O. Johnston[1]. Marion Merrell Dow Research Institute, Indianapolis, IN 46268 and [1]Cincinnati, OH 45215

Sequential oxidation of deoxycorticosterone (DOC) at C11 and C18 by P450 monooxygenase(s) constitutes the final steps of aldosterone biosynthesis. Selective inhibition of C18 hydroxylation (corticosterone methyl oxidase) of corticosterone would limit aldosterone formation and potentially decrease sodium retention and potassium excretion associated with hyperaldosterone states. The purpose of this study was to evaluate MDL 19,347, an enzyme-activated substrate analog of DOC, as an aldosterone synthesis inhibitor. Sodium-depleted rhesus monkeys with plasma aldosterone levels 20-fold higher than normal were utilized in this study. Preincubation of adrenal mitochondria with 1 μM of MDL 19,347 for 20 min prior to a 2 hr incubation with 1 μM of [^{3}H]-DOC resulted in 80-100% inhibition of the formation of [^{3}H]18-hydroxycorticosterone and [^{3}H]-aldosterone without inhibition of 11β-hydroxylation of DOC. Intravenous MDL 19,347, 3 mg/kg (n=4), resulted in a 50% decrease in plasma aldosterone levels at 6 hr after dosing. These data indicate that MDL 19,347 decreases plasma aldosterone by selectively inhibiting C18 hydroxylation and are consistent with the presence of two distinct adrenal P450 hydroxylases, 11β/18-hydroxylase and corticosterone C18 methyl oxidase, in the biosynthesis of aldosterone. MDL 19,347 provides a novel approach for treatment of conditions associated with hyperaldosteronism.

53.2

INTERACTIONS OF HUMAN RECOMBINANT RELAXIN (hRlx) WITH FLUID BALANCE AND HEMODYNAMICS IN CONSCIOUS FEMALE RATS. D.G. Ward and M.J. Cronin. Dept. Endocrine Res., Genentech, Inc., South San Francisco, CA 94080.

Relaxin is a hormone of pregnancy that was reported to effect blood pressure in anesthetized and hypertensive rats (Life Sci 37; 1351, 1985: J Neuroendo 2; 53, 1990), but not in pregnant normotensive and hypertensive rats (J Obstet Gynecol 161; 618, 1989: Ward, Baertschi & Cronin, Am J Physiol 1991, in press). Nonpregnant Sprague Dawley (SD) or Spontaneously Hypertensive rats (SHR) were prepared either with osmotic minipumps and arterial cannulae or with minipumps alone. Blood volumes were determined after 5 d, and water intake, urine volume, food intake, mean arterial pressure (MAP) and heart rate (HR) were obtained daily. Bioactivity of hRlx was confirmed with increased uterine weights and circulating hRlx was determined by an ELISA. Relative to controls, infusion of hRlx at either 1 or 10 ng/min for 1 wk did not change blood volume or MAP in SD or SHR. Small but significant, dose-related increases in HR occured in SD and SHR, but increases in water intake and urine volume occured only in SD. In SD with minipumps alone, dose-related increases in water intake and urine volume were confirmed. In a separate study of SHR infused with 2 ng/min hRlx for 2 wk without measurements of blood volume, MAP did not change while there were small and significant increases in water intake, urine volume and HR. In summary, hRlx had a modest but consistent influence on fluid balance and heart rate with no effect on blood volume or MAP in conscious SD and SHR.

53.3

HYPOTHALAMIC INFUSION OF ACTIVIN-A INCREASES WATER CONSUMPTION AND URINE VOLUME IN THE FEMALE RAT. Ralph H. Schwall*, Lyn B. Jakeman*, and C. Anthony Altar*. (SPON: Michael J. Cronin) Genentech, Inc., South San Francisco, CA 94080

Activin-A was first purified from ovarian follicular fluid, but activin A (βA) immunoactivity has subsequently been found in other tissues, such as the nucleus tractus solitarius and its projections into the hypothalamus (Sawchenko et al., 1988 Nature 334, 615-617). To understand activin's role within the CNS, we have monitored body weight, food and water consumption, fecal mass and urine volume during infusion of activin-A into the paraventricular nucleus of the hypothalamus. Adult female Sprague-Dawley rats (~200 g) were placed in metabolic cages and 2 days of baseline data were collected. Bilateral intracranial catheters were then implanted, under ketamine-xylazine anesthesia, with the ends 0.8 mm dorsal to the paraventricular nucleus. Vehicle (phosphate-buffered saline) or recombinant activin-A (0.9 μg/d per side) was infused continuously via osmotic minipump. Daily water consumption and urine volume increased in activin-treated animals beginning on day 3 and reaching a maximum on day 5 [WATER: Vehicle = 23.5 ± 2.1 ml (Mean ± SEM, n=6); Activin = 62.2 ± 9.0 ml, P<0.001; URINE: Vehicle =11.3 ± 1.3 ml; Activin = 45.8 ± 8.4 ml; P<0.001]. This increase was maintained through day 8, when the study was terminated. The same dose of cytochrome C or recombinant inhibin-A, or a lower dose of activin-A (0.009 μg/d) had no effect on water consumption or urine volume. In addition, water consumption and urine volume in vehicle-infused animals were not different from values in sham-operated controls. Weight gain, food consumption and fecal mass transiently decreased on the day following surgery in all groups, but rapidly returned to presurgical levels. None of the tested proteins had any effect on these parameters. Our results suggest that activin-A may play a physiological role in the central regulation of fluid balance.

53.4

DOPAMINE SPECIFICALLY INHIBITS PHOSPHATE TRANSPORT IN THE RAT RENAL BRUSH BORDER MEMBRANE. R.P. Glahn*, M.J. Onsgard*, T.J. Berndt, T.P. Dousa, and F.G. Knox. Mayo Clinic & Foundation, Rochester, MN 55905

Although previous experiments have shown that dopamine (DA) is phosphaturic, it is not known whether this effect is independent of the effects of DA on Na^+ transport. The present study was designed to determine the effects of DA administered in vivo on the Na^+-dependent uptake of inorganic phosphate (Pi), proline, glucose, and sulfate by renal brush border membrane vesicles (BBMV). Paired studies were performed in acutely TPTX male Sprague Dawley rats. The rats were fed a low phosphate diet (LPD, .07% Pi) for 2 days to enhance basal rate of Na^+-P_i transport. After surgical preparation and equilibration, DA (350 μg/kg bolus plus 35 μg/kg/min infusion), parathyroid hormone (PTH; 33 U/kg bolus plus 1 U/kg/min infusion), or 0.9% NaCl was infused for 90 minutes at a rate of 3 ml/hr. The kidneys were then harvested and BBMV were prepared. DA decreased Pi transport by 22.4 ± 4.1% (P=.01), but had no effect on proline, glucose, or sulfate uptake. By comparison, PTH inhibited Pi transport by 45.2 ± 3.9% (P=.01). Thus, the effect of DA is approximately half of the maximal inhibition of P_i transport by PTH. We conclude that DA specifically inhibits proximal tubular P_i uptake.

53.5

INDOMETHACIN ABOLISHES THE RESTORATION OF THE PHOSPHATURIC RESPONSE TO PTH BY CHRONIC RENAL DENERVATION (DNX) IN PHOSPHATE (P_i) DEPRIVED RATS.

T. J. Berndt, A. A. Khraibi, C. Calcagno*, and F. G. Knox. Mayo Medical School, Rochester, MN 55905

Previous studies demonstrated that DNX restored the phosphaturic response to PTH in P_i-deprived rats. The present studies were performed to evaluate the role of prostaglandin (PG) synthesis in the restoration of the phosphaturic response to PTH by DNX. Unilateral renal DNX or sham surgery was performed 1 week prior to the acute experiment. Four groups of rats were fed low P_i diet (0.07%) for 2 days. All rats were TPTX. Indomethacin (3 mg/kg) or vehicle was administered 1 hr prior to the control clearance. After a 2 hr equilibration period, a control clearance was taken and PTH was administered (33 U/kg+1 U/kg/min). Forty-five minutes later an experimental clearance was taken.

Fractional Excretion of P_i

	Vehicle		Indomethacin	
	C	PTH	C	PTH
DNX+PTH	6±3	30±5* (n=12)	0.2±.1	4±2$^{\$}$ (n=5)
Sham+PTH	0.6±.3	10±4* (n=9)	2±1	10±5 (n=5)

*$p<.05$ paired t, control vs PTH; $^{\$}p<.05$ group t, indomethacin compared to vehicle treatment.

Indomethacin abolished the restoration of the phosphaturic effect of PTH by chronic DNX in P_i-deprived rats. These results suggest that PGs play a role in the interaction of the renal nerves with P_i transport.

53.6

INCREASED CO_2 STIMULATES INOSITOL TRIPHOSPHATE (IP_3) IN ISOLATED CELLS OF TOAD URINARY BLADDER.

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The urinary bladder of Bufo marinus excretes H^+ and this excretion is increased by increased CO_2 (respiratory acidosis), metabolic acidosis (MA) and various hormonal agents. It was recently reported by this laboratory that MA and insulin stimulates formation of inositol phosphates (IP) in this tissue (Fed. Proc. 5(4), A763, 1991). The purpose of this experiment was to determine if CO_2 stimulates IP formation in isolated cells of toad bladder. Cells from 15 paired hemibladders were isolated from normal toads by treating bladder sacs with collagenase (100 U/ml). The cells from each hemibladder were suspended in 2 ml of Ringer's solution containing ^{3}H-myoinositol (10 μCi) and then incubated for 2 hrs at 25°C. The cells were washed, suspended in Ringer's containing LiCl (10 mM) and the experimental cells were challenged with 5% CO_2 for 20 min. Cells from the paired hemibladders (control) were maintained in room air for 20 min. Cells were then homogenized and the IP fractions quantitated by column chromatography and liquid scintillation counting. The results were expressed as DPM (μM PO_4)$^{-1}$(min)$^{-1}$. The IP_3 in control cells was 126 and in 5% CO_2 cells 163 (mean difference 37±13.2, $P<0.05$). We conclude that increased CO_2 stimulates IP_3 in toad bladder and IP_3 may be a second messenger in mediating the response to ↑ CO_2.

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