Conference Office

The Conference Office is located in the Longboat Room of the Hyatt Sarasota Hotel, 1 Sarasota Bay, 1000 Blvd of the Arts, Sarasota, FL 34236, telephone: 813-366-9000 (ask for Longboat Room).

On-site registration

The scientific registration fee includes the opening reception, banquet, entrance to scientific sessions, and receipt of the Program/Abstract Volume.

The guest registration fee includes the opening reception and banquet. Guest registrants may not attend scientific session.

Registration — Longboat Room:

Hours:

Wednesday, October 5	2:00 PM—8:00 PM
Thursday, October 6	7:30 AM—4:30 PM
Friday, October 7	8:00 AM—4:30 PM
Saturday, October 8	8:00 AM—4:30 PM

Fees:

APS Member	$235.00
Nonmember	$280.00
Retired Member	$ 75.00
Student	$ 75.00
Guest	$ 40.00

(Nonscientist family members of registrants)

Press

Press badges will be issued in the Conference Office only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public information, public affairs, etc.) may register as nonmembers in the registration area.

Publications

The Program/Abstract Volume (the August issue of *The Physiologist*) was mailed to all APS members and will be given to registrants on-site. Replacement copies may be purchased for $20.00 in the APS/Management Office.

Message Center

The message board will be located in the Longboat Room by the Registration Desk. Registrants should check for messages daily. Please suggest that callers who wish to reach you during the day leave a message with the Conference Office during registration hours.

Airline Reservations

Arrangements have been made with Delta Airlines to offer registrants special discounts. Reservations may be made by calling the airlines directly or by using your choice of travel agent. To take advantage of the Delta discounts, you must call 1-800-241-6760 and refer to file #V0012.

Car Rental

Alamo Car Rental has been appointed the official car rental company for the meeting. Special discounted rates have been extended to any participant. Reservations may be made by calling toll-free 1-800-732-3232. Be sure to identify yourself as an APS Meeting attendee and give the dates of the Meeting, I.D. #377555 and Plan Code GR to guarantee the special rate.

Airport Transportation

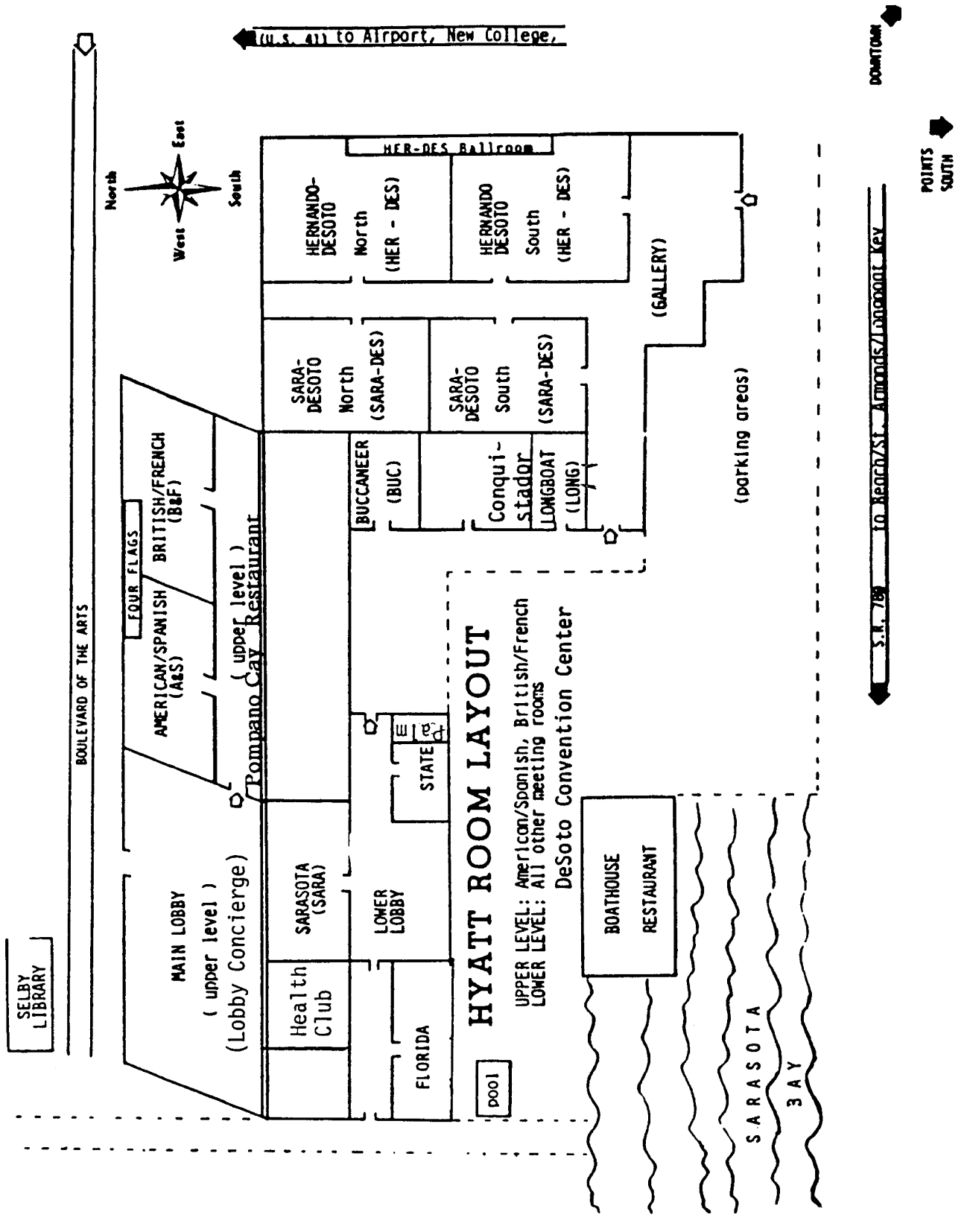
Travel time from the Sarasota/Bradenton Airport to the Hyatt Sarasota is approximately 10 minutes. The Hyatt Sarasota offers a complimentary shuttle service from the Sarasota/Bradenton Airport to the hotel. To take advantage of the airport transportation service: upon arrival, go to the baggage area and pick up the courtesy phone for the Sarasota Hyatt who will immediately send a shuttle.

Social Program

Opening Reception — The Opening reception will be held poolside (weather permitting) 6:00—8:00 PM on Wednesday, October 5.

APS Banquet and Lecture — All registrants are invited to attend the Saturday evening banquet on October 8. A cash bar reception is scheduled at 6:00 PM in the Gallery followed by dinner at 7:00 PM. The lecture entitled "Complex Mechanochemical Signal Transduction Involved in the Regulation of Development" will be presented by Dr. David McClay of Duke University. Tickets are required for admittance. **Each registrant will receive a coupon in the registration packet which MUST be exchanged for a dinner ticket before 10:00 AM on Friday, October 7.**

HOTEL FLOORPLAN



1994 APS Conference: October 5-8, 1994

Mechanotransduction and the Regulation of Growth and Differentiation

Hyatt Sarasota, Sarasota, Florida

Wednesday October 5, 1994	Thursday October 6, 1994	Friday October 7, 1994	Saturday October 8, 1994
Registration: 2:00–8:00 PM *Longboat Room*	Registration: 7:30 AM–4:30 PM *Longboat Room*	Registration: 8:00 AM–4:30 PM *Longboat Room*	Registration: 8:00 AM–4:30 PM *Longboat Room*
1.0 Evening Lecture 5:00–6:00 PM *Hernando Ballroom*: How Hearing Happens: Mechanoelectrical Transduction by Hair Cells of the Internal Ear Speaker: **A. James Hudspeth**	2.0 Symposium 8:30–11:30 AM *Hernando Ballroom*: Musculoskeletal Responses to Mechanical Stimuli Chair: **Herman Vandenburgh** Participants: **Radovan Zak, Kenneth Baldwin, Frank Booth, Elisabeth Berger, D.B. Jones**	8.0 Symposium 8:30–11:30 AM *Hernando Ballroom*: Cardiovascular Adaptations to Mechanical Stimuli I Chair: **Peter Davies** Participants: **Lowell Langille, Peter Davies, Robert Nerem, Michael Gimbrone, Bauer Sumpio**	12.0 Symposium 8:30–11:30 AM *Hernando Ballroom*: Mechanisms of Mechanochemical Signal Transduction Chair: **Peter Watson and Fred Sachs** Participants: **Herman Vandenburgh, Pete Watson, Jonathan Ashmore, Ching Kung, Owen Hamill**
Opening Reception: 6:00–8:00 PM *Gallery*	3.0 Symposium 7:00–10:00 PM *Hernando Ballroom:* Pulmonary Responses to Mechanical Stimuli Chair: **D. Eugene Rannels** Participants: **D. Eugene Rannels, Robert Mercer, Scott Randell, Robert Paine III, Leland G. Dobbs**	9.0 Symposium 7:00–10:00 PM *Hernando Ballroom:* Cardiovascular Adaptations to Mechanical Stimuli II Chair: **Howard Morgan** Participants: **Seigo Izumo, Yoshio Yazaki, Kenneth Chien, George Cooper, David Warshaw**	13.0 Symposium 2:00–5:00 PM *Hernando Ballroom* Regulation of Cell Shape and Function by the Extracellular Matrix Chair: **Martin A. Schwartz** Participants: **Donald Ingber, Keith Burridge, Thomas Parsons, Martin Schwartz, Zena Werb**
	Poster Viewing 3:30–5:00 PM *Sara Ballroom* 4.0 Pulmonary responses to mechanical stimuli 5.0 Musculoskeletal responses to mechanical stimuli 6.0 Cardiovascular adaptations to mechanical stimuli: peripheral vasculature 7.0 Cardiovascular adaptations to mechanical stimuli: myocardial cells	Poster Viewing 3:30–5:00 PM *Sara Ballroom* 10.0 Mechanisms of mechanochemical signal transduction 11.0 Regulation of cell shape and function by the extracellular matrix	14.0 Banquet Lecture 8:30–9:30 PM Complex Mechanochemical Signal Transduction Involved in the Regulation of Development Speaker: **David McClay**

Wednesday, October 5

Evening Lecture

1.0 How Hearing Happens: Mechanoelectrical Transduction by Hair Cells of the Internal Ear

5:00 PM — Hernando Ballroom

Speaker: **A. James Hudspeth**, Univ. of Texas Med. Sch. Dallas

Thursday, October 6

Symposium

2.0 Musculoskeletal Responses to Mechanical Stimuli

8:30 AM — Hernando Ballroom
Chair: **Herman Vandenburgh**

8:30 Skeletal muscle use and regulation of contractile protein gene expression. **Radovan Zak.** Univ. of Chicago.
9:00 Discussion.
9:05 Influence of hypokinesia on skeletal muscle biochemistry. **Kenneth Baldwin.** Univ. of California, Irvine.
9:35 Discussion.
9:40 Mechanisms of skeletal muscle atrophy during immobilization disuse. **Frank Booth**. Univ. of Texas Med. Sch., Houston.
10:10 Discusion.
10:15 In vitro studies into the regulation of growth in Calvaria-derived osteogenic cells. **Elisabeth Berger.** ACTA-Free Univ., Amsterdam, The Netherlands.
10:45 Discussion.
10:50 Biochemical signal transduction of mechanical strain in osteoblast-like cells. **D.B. Jones**. Univ. of Munster, Germany.
11:20 Discussion.

Symposium

3.0 Pulmonary Responses to Mechanical Stimuli

7:00 PM — Hernando Ballroom
Chair: **D. Eugene Rannels**

7:00 Physical signals in compensatory growth of the lung. **D. Eugene Rannels**. Penn. State Univ.
7:30 Discussion.
7:35 Distribution of strain across the alveolar region of the lung. **Robert Mercer**. Duke Univ.
8:05 Discussion.
8:10 Developmental regulation of airway epithelial cell morphology. **Scott Randell**. Duke Univ.
8:40 Discussion.
8:45 Linkage of shape and differentiation in alveolar epithelial cells. **Robert Paine III**. Univ. of Michigan.
9:15 Discussion.
9:20 Mechanical signals for surfactant secretion by pulmonary type II cells. **Leland G. Dobbs.** UCSF.
9:50 Discussion.

Posters

4.0 Pulmonary Responses to Mechanical Stimuli

Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.

Board #

1 4.1 Reduction in wall tension in isolated pulmonary vassels stimulates matrix metalloprotease activity. **C.A. Tozzi, S. Thakker-Varia, and D.J. Riley.** UMDNJ-R.W. Johnson Med. Sch. and VA Med. Ctr., Lyons, NJ.
2 4.2 Anitsense oligodeoxynucleotides targeting platelet-derived growth factor-β mRNA inhibit mechanical strain-enhanced fetal rat lung cell proliferation. **M. Liu, J. Liu, S. Buch, A.K. Tanswell, and M. Post.** Hosp. for Sick Children, Toronto.
3 4.3 Physical responses of the trachea to transection in the living rat. **M. Lorber.** Georgetown Univ.
4 4.4 Bronchomotor tone in the early fetal lung: is it involved in normal lung growth? **M. Sparrow and H.W. Mitchell.** Univ. of Western Australia, Nedlands.

Poster

5.0 Musculoskeletal Responses to Mechanical Stimuli

Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.
Board #

5 5.1 Muscle damage/repair and muscle conditioning mechanisms in dynamic cardiomyoplasty. **U. Carraro, C. Rizzi, A. Bruson, C. Catani, P. Mikus, A. Pierangeli, and G. Arpesella.** Padova Univ., Bologna Univ., and Chieti Univ., Italy.
6 5.2 Influence of compressive load on matrix synthesis in cartilage: possible signalling mechanisms. **J.P.G. Urban and A.C. Hall.** Oxford Univ., United Kingdom.
7 5.3 Transcriptional regulation in mechanically overloaded skeletal muscle of transgenic mice. **I. Rivera-Rivera, J.L. Wiedenman, L-Y. Gao, D. Vyas, L.T. Kwan, and R.W. Tsika.** Univ. of Illinois, Urbana.
8 5.4 Regulation of proliferation and platelet-derived growth factor expression in palmar fibromatosis (Dupuytren's disease) by mechanical strain. **B.A. Alman and D.A. Greel.** Tufts Univ.

9 5.5 Mechanical stress and expression of stress protein in growth and differentiation of skeletal muscle. **Y. Atomi.** Univ. of Tokyo, Japan.

Poster

6.0 Cardiovascular Adaptations to Mechanical Stimuli: Peripheral Vasculature

Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.

Board #

10 6.1 Calcium-permeable mechano-activated adapting channel in rat mesangial cells. **V. Chen, H. Guber, C. Shuman, and C.E. Palant.** Brooklyn VA Hosp. and SUNY Hlth. Sci. Ctr., Brooklyn.

11 6.2 Feed artery hemodynamics change with muscle length in hamster retractor muscle. **D.G. Welsh and S.S. Segal.** John B. Pierce Lab., Yale Univ.

12 6.3 Mechanisms of transduction of elevated vascular wall stress into increased production and accumulation of arterial elastin. **F.W. Keeley, L. Bartoszewicz, and P. Robson.** Hosp. for Sick Children, Toronto.

13 6.4 A model for subjecting vascular wall cells to simultaneous pulsatile fluid and mechanical anastomotic shear stress. **G.J. L'Italien, A. Brenbrahim, S. Dhara, C.J. Kwolek, B.B. Milinazzo, D.F. Warnock, J.P. Gertler, R.W. Orkin, and W.M. Abbott.** Mass. Gen. Hosp.

14 6.5 Structural changes and their dynamic stability of human endothelial cell after applying a shear stress. **J.K. Chang, J.H. Kim, J. Kim, D.C. Han, and B.G. Min.** Seoul Natl. Univ., Korea.

15 6.6 Three-dimensional changes of human endothelial cell morphology under fluid flow. **D.I. Shin, J.K. Chang, J.H. Kim, J. Kim, D.C. Han, and B.G. Min.** Seoul Natl. Univ., Korea.

16 6.7 Integrin expression by porcine coronary vascular smooth muscle cells. **J. Mogford, G.E. Davis, and G.A. Meininger.** Texas A&M Univ.

17 6.8 Mechanical strain enhances expression of the PDGF-A and EGR-1 genes in vascular smooth muscle cells. **G.J. Vives, E. Wilson, T. Collins, V. Sukhatme, and H.E. Ives.** UCSF, Beth Israel Hosp., and Harvard Univ.

18 6.9 Mechanical strain induces expression of smooth muscle myosin in vascular smooth muscle cells. **H.P. Reusch, H. Wagdy, and H.E. Ives.** UCSF.

19 6.10 Intercellular communication vs. receptor-mediated mechanisms in ATP and bradykinin-induced increases in endothelial intracellular calcium. **H.M. Honda, J.I. Goldhaber, L.L. Demer, and J.N. Weiss.** UCLA.

20 6.11 Mechanical strain induces monocyte chemotactic protein-1 gene expression mediated via protein kinase C pathway in human endothelial cells. **D.L. Wang, B.S. Wung, S. Usami, and Y.J. Shyy.** Academia Sinica, Taipei, Taiwan, ROC.

21 6.12 Flow-mediated NO release in endothelial cells: role of calcium and K^+ channels. **W.C. O'Neill, P.B. Perry, and D. Steinberg.** Emory Univ.

22 6.13 Distribution of shear stress on the surface of aligned and non-aligned endothelial monolayers. **K.A. Barbee, T. Mundel, R. Lal, and P.F. Davies.** Univ. of Chicago.

23 6.14 10047 Ca^{2+} entry in A7r5 vascular smooth muscle cells modulated by stretch. **V. Ruiz-Velasco, B.M. Mayer, and L.J. Hymel.** Tulane Univ.

24 6.15 Elevated stretch and shear stress produce distinctly different changes in capillary hydraulic conductivity. **D.A. Williams and V.H. Huxley.** Univ. of Missouri.

25 6.16 Pressure/agonist-dependent modulation of arterial calcium sensitivity. **M. Cipolla and G. Osol.** Univ. of Vermont.

26 6.17 Pressure-induced alterations in gene expression in the isolated rate mesenteric artery. **S.P. Allen, H.M. Liang, S.B. Parker, M.N. Tran, and R.L. Prewitt.** Eastern Virginia Med. Sch.

27 6.18 Cyclic mechanical strain differentially modulates procoagulant activity in endothelial cells derived from different vascular beds. **M. Silverman, V.G. Manolopoulos, and P.I. Lelkes.** Univ. of Wisconsin, Milwaukee.

28 6.19 Differential modulation of endothelial cell adhesion molecules by chemical and mechanical activation. **S. Zhang, H. Zhang, V.G. Manolopoulos, and P.I. Lelkes.** Univ. of Wisconsin, Milwaukee.

29 6.20 Elevated pressure alters morphology and extracellular matrix deposition of cultured endothelial cells. **M.M. Samet, D.M. Wankowski, and P.I. Lelkes.** Univ. of Wisconsin, Milwaukee.

30 6.21 Alteractions in endothelial integrins in response to hydrostatic pressure. **E.A. Schwartz, R. Bizios, and M.E. Gerritsen.** Rensselaer Polytech. Inst. and Miles Inc.

31 6.22 Ca^{2+} dependent and Gd^{3+} sensitive morphological changes of cultured endothelial cells by cyclic mechanical stretch. **K. Naruse, M. Inoue, T. Yamada, and M. Sokabe.** Nagoya Univ., Japan.

Posters

7.0 Cardiovascular Adaptations to Mechanical Stimuli: Myocardial Cells

Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.

Board #

32 7.1 Extracellular ATP modulates stretch-activated channels in chick heart cells. **A.M. Ruknudin, H. Hu, and F. Sachs.** SUNY, Buffalo.

33 7.2 Cardiac ventricular septal defects: analysis of effects on pressure-volume transduction, by allometric assay of hearts in infants/children. **R.P. Spencer.** Univ. of Connecticut.

34 7.3 Cardiac responses to chronic angiotensin II hypertension in sympathectomized dogs. **H. McDonald, Jr. and L.N. Cothran.** Howard Univ.

35 7.4 Mechanical strain stimulates extracellular matrix gene expression in adult rat cardiac fibroblasts. **A.A. Lee, F.J. Villarreal, A.D. McCulloch, W.H. Dillmann, and J.W. Covell.** UCSD.

36 7.5 Use of differential display technology in the identification of upregulated transcripts during acute right ventricular overload. **G.T. Schyler, A.S. Ridenour, D.R. Menick, J.D. Rozich, T.X.**

O'Brien, and G. Cooper IV. Med. Univ. of South Carolina, Gazes Cardiac Res. Inst., and VA Med. Ctr., Charleston, SC.

37 7.6 Neonatal rat cardiocytes subjected to in vitro load stress acutely upregulate the mitochondrial F_1-ATPase β-subunit. **T.X. O'Brien, D.R. Menick, M.S. Rackley, P.J. McDermott, and G. Cooper IV.** Med. Univ. of South Carolina, Gazes Cardiac Res. Inst., and VA Med. Ctr., Charleston, SC.

38 7.7 The rapid upregulation of nuclear and mitochondrial encoded subunits of the F_1 ATPase and cytochrome oxidase compleses in response to cardiac load. **D.R. Menick, T.X. O'Brien, D.E. McDermott, J.D. Rozich, G.T. Schuyler, P.J. McDermott, and G. Cooper IV.** Med. Univ. of South Carolina, Gazes Cardiac Res. Inst., and VA Med. Ctr., Charleston, SC.

39 7.8 Effects of pressure overload hypertrophy on passive stiffness and viscous damping in the isolated cardiocyte. **M.R. Zile, J.M. Buckley, K.E. Richardson, and G. Cooper IV.** Med. Univ. of South Carolina, VA Med. Ctr., and Gazes Cardiac Res. Inst., Charleston, SC.

40 7.9 Growth effects of electrically stimulated contraction on adult feline cardiocytes in primary culture. **P.J. McDermott, S. Kato, C.T. Ivester, G. Cooper IV, and M.R. Zile.** Med. Univ. of South Carolina, VA Med. Ctr., and Gazes Cardiac Res. Inst., Charleston, SC.

41 7.10 Autoregulation of tubulin mRNA expression and organization of the microtubule cytoskeleton in ischemic and dilated cardiomyopathy. **R.D. Bies.** Univ. of Colorado Hlth. Sci. Ctr., Denver.

42 7.11 Nitric oxide released from carotid sinus sensory nerves inhibits activity of baroreceptor A-fibers. **X. Su and M.W. Chapleau.** Univ. of Iowa and VA Med. Ctr., Iowa City.

43 7.12 Protein kinase C-epsilon translocates in response to both angiotensin II and swimming in the adult rat heart. **D.L. Geenen, P.M. Buttrick, A. Malhotra, and J. Scheuer.** Montefiore Med. Ctr. and Albert Einstein Col. of Med.

44 7.13 Hypotonic stretch increases the slow component of delayed rectifier potassium current in guinea pig ventricular myocytes. **J.G. Maylie and W.J. Groh.** Oregon Hlth. Sci. Univ.

45 7.14 Contractile activity modulates expression of the polymerase 1-associated ribosomal transcription factor, UBF, in cardiomyocyte cultures. **R.D. Hannan, J. Luyken, and L.I. Rothblum.** Weis Ctr. Res., Geisinger Clinic, Danville, PA.

Friday, October 7

Symposium

8.0 Cardiovascular Adaptations to Mechanical Stimuli I

8:30 AM — Hernando Ballroom

Chair: **Peter Davies**

8:30 Developmental adaptation of blood vessels to mechanical cues in the newborn mammal. **Lowell Langille.** Toronto Gen. Hosp.

9:00 Discussion.

9:05 Signal transduction of hemodynamic forces at the endothelium. **Peter F. Davies.** Univ. of Chicago.

9:35 Discussion.

9:40 Flow regulation of relaxation in vascular tissues. **Robert M. Nerem.** Georgia Inst. Tech.

10:10 Discussion.

10:15 Modulation of endothelial gene expression by mechanical forces. **Michael A. Gimbrone.** Brigham & Women's Hosp.

10:45 Discussion.

10:50 Stretch-induced morphological and biochemical changes in endothelial and smooth muscle cells. **Bauer E. Sumpio.** Yale Univ.

11:20 Discussion.

Symposium

9.0 Cardiovascular Adaptations to Mechanical Stimuli II

7:00 PM — Hernando Ballroom

Chair: **Howard E. Morgan**

7:00 Stretch-induced anabolism in perfused rat and neonatal pig hearts. **Seigo Izumo.** Harvard Med. Sch.

7:30 Discussion.

7:35 Molecular mechanisms of load-induced cardiomyocyte hypertrophy. **Yoshio Yazaki.** Univ. of Tokyo, Japan.

8:05 Discussion.

8:10 Adrenergic induction of proto-oncogene expression in cardiomyocyte hypertrophy. **Kenneth R. Chien.** UCSD.

8:40 Discussion.

8:45 Mechanical stretch as a signal for cardiocyte growth. **George Cooper.** VA Hosp., Charleston, SC.

9:15 Discussion.

9:20 Contractile and biochemical properties of isolated vascular smooth muscle cells. **David Warshaw.** Univ. of Vermont.

9:50 Discussion.

Poster

10.0 Mechanisms of Mechanochemical Signal Transduction

Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.

Board #

1 10.1 Mechanotransduction across the urokinase receptor. **N. Wang, E. Planus, J.J. Fredberg, and G. Barlovatz-Meimon.** Harvard Sch. Publ. Hlth. and Univ. of Paris XII, Creteil, France.

2 10.2 Mechanical regulation of intracellular Ca^{2+} in endothelial cells. **J.F. Minore and W.C. O'Neill.** Emory Univ.

3 10.3 Volume dependent phosphorylation of myosin light chain in aortic endothelial cells. **J.D. Klein and W.C. O'Neill.** Emory Univ.

4 10.4 Mechanical load alters expression of the inositol 1,4,5-triphosphate receptor mRNA expression in human smooth muscle cells. **J. Yamamoto, E. Chikamatsu, T. Fischer, T. Iwasaki, T. Lawrence, M. Tsuzaki, and A.J. Banes.** Univ. of North Carolina.

5 10.5 Mechanical load upregulates connexin-43 expression in MC-373 osteoblasts. **A. Keen, P. Hu, W.T. Lawrence, and A.J. Banes.** Univ. of North Carolina.

6 10.6 Cyclin D1 expression in hSMC is stimulated by cyclic mechanical load in dose-dependent manner. **E. Chikamatsu, Y. Nimura, J. Yamamoto, P. Hu, T. Fischer, T. Lawrence, M. Tsuzaki, T. Brown, and A.J. Banes.** Univ. of North Carolina.

7 10.7 Characterizing whole-cell mechanosensitive currents in chick heart. **H. Hu and F. Sachs.** SUNY, Buffalo.

8 10.8 Role of G-proteins in mechanical signal transduction. **S.R.P. Gudi, N-Q. Li, and J.A. Frangos.** Penn. State Univ., Univ. Park.

9 10.9 Mechanical strain is sensed by vascular smooth muscle cells through an interaction with extracellular matrix proteins. **E. Wilson, K. Sudhir, and H.E. Ives.** UCSF.

10 10.10 Mechanical strain increases mRNA for platelet-derived growth factor-β chain in vascular smooth muscle cells. **Y-H. Ma and H.E. Ives.** UCSF.

11 10.11 Activation of cyclic-AMP signaling inhibits volume-sensitive osmolyte efflux in cardiac myocytes. **J.J. Smith, E.S. Moore, D. Leiser, and M. Lieberman.** Duke Univ.

12 10.12 Cytoskeletal rearrangement activates the swelling-induced chloride current in cardiac myocytes. **J. Zhang, R.L. Rasmusson, and M. Lieberman.** Duke Univ.

13 10.13 Load-accelerated protein synthesis of stretched adult cardiocytes depends upon stretch--activated calcium influx. **R.L. Kent and G. Cooper IV.** VA Med. Ctr., Gazes Cardiac Res. Inst., and Med. Univ. South Carolina, Charelston, SC.

14 10.14 Ligand-induced Ca^{2+} uptake via the acetylcholine receptor is inhibited reversibly by pluronic F-68. **E.J. Massaro, L. Aloi, Y.C. Izu, J. Niggel, and F. Sachs.** Duke Univ., US EPA-Res. Triangle Pk., NC, and SUNY, Buffalo.

15 10.15 Percolation as a possible model for biological signaling. **G. Forgacs.** Clarkson Univ.

16 10.16 Mechanical stimulation of neurites of nodose baroreceptor neurons in culture induces a whole cell current. **J.T. Cunningham, L. Fankhauser, R.E. Wachtel, and F.M. Abboud.** VA Med. Ctr., Iowa City and Univ. of Iowa.

17 10.17 Chronic, intermittent mechanical strain induces whole cell conductance increases via modulation of mechanosensitive channels in osteoblast-like cells. **R.L. Duncan and K.A. Hruska.** Jewish Hospital of St. Louis.

18 10.18 Mechanosensing in arabidopsis: towards a genetic analysis based on mechano-inducible luminescent phenotypes. **G.A. Karlin-Neumann and R.W. Davis.** Stanford Univ.

19 10.19 Isolation of a mechanosensitive subpopulation of vagal sensory neurons in vitro. **G. Hajduczok.** SUNY, Buffalo.

20 10.20 Osmotic activation of mechanosensitive channels in vesicles isolated from xenopus oocytes. **H. Sackin and W. Schutt.** Cornell Univ. Med. Col.

21 10.21 Stretch stimulates proliferation of renal epithelial cells. **G.A. Tanner, P.F. McQuillan, M.R. Maxwell, J.K. Keck, and J.A. McAteer.** Indiana Univ.

22 10.22 Ca^{2+} dependent mechanosensitivity in cardiac fibroblasts. **P. Kohl, A. Kamkin, I. Kiseleva, and M.J. Lab.** Univ. of Oxford, UK.

23 10.23 Mechanosensitive activation of Ca^{2+} transport pathways in HT29 human colon cancer cells. **L.J. Hymel, Y. Qin, and T. Ertl.** Tulane Univ.

24 10.24 Osmotic stimulation of axonal elongation is mediated by an increase in tension sensitivity of growth. **S.R. Heidemann, P. Lamoureux, and C. Lin.** Michigan State Univ.

25 10.25 Effects of fluid flow on osteocytes. **J. Klein-Nulend, N.E. Ajubi, A. van der Plas, C.M. Semeins, P.J. Nijweide, and E.H. Burger.** ACTA--Free Univ., Amsterdam, and Univ. of Leiden, The Netherlands.

26 10.26 Focal contacts and lamellar protrusion are increased after mechanical wounding by agents that inhibit phosphoinositide breakdown. **G.E. Moeller, L.E. Hinman, and P.J. Sammak.** Univ of Minnesota.

27 10.27 Effect of abdominal vibroacoustic stimulation on acceleration levels at the fetal head in sheep. **R.M. Abrams, A.J.M. Peters, and K.J. Gerhardt.** Univ. of Florida.

Posters

11.0 Regulation of Cell Shape and Function by the Extracellular Matrix

Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.

Board #

28 11.1 Dynamics of mechanical stress-regulated modulation of cell shape, microfilaments and macromolecular biosynthesis in fibroblasts. **H. Doong, R.C. Lee, and S.C. Tripathi.** Univ. of Chicago.

29 11.2 Human Culture-derived macrophages contain a K^+ channel that modulates the cytoskeleton and is activated by membrane stretch and cytokines. **S.N. Breit, M.R. Bootcov, T.J. Campbell, P.W. Franch, D.K. Martin.** St. Vincent's Hosp., and Univ. of New South Wales, Sydney, Australia.

30 11.3 A study of parameters affecting fibroblast morphology in response to an applied mechanical force. **R.A. Grymes and C. Sawyer.** Bionetics Corp. and NASA-Ames Res. Ctr.

31 11.4 Predictions of a model for cell/intercellular matrix interactions in shortening smooth muscle. **R.A. Meiss.** Indiana Univ.

32 11.5 Modulation by reversible inhibition of $\beta 1$ integrin by antisense oligonucleotides of myoblast attachment to EMC and proliferation. **M.L. Massimino, E. Rapizzi, M. Cantini, M. Sandri, A. Bruson, C. Catani, L. Dalla Libera, and U. Carraro.** Univ. of Padua, Italy.

Saturday, October 8

Symposium

12.0 Mechanisms of Mechanochemical Signal Transduction

8:30 AM — Hernando Ballroom
Chair: **Peter Watson and Fred Sachs**

8:30 Stretch-induced activation of phospholipase activity in rat skeletal muscle myotubes in vitro. **Herman Vandenburgh.** Brown Univ. and Miriam Hosp., Providence, RI.
9:00 Discussion.
9:05 Structural diversity and molecular requirements for mechanochemical transduction in the adenylyl cyclase family. **Peter A. Watson.** Geisinger Clin., Danville, PA.
9:35 Discussion.
9:40 Stretch-sensitive channels in normal and MDX mouse skeletal muscle. **Jonathan Ashmore.** Univ. of Bristol, UK.
10:10 Discussion.
10:15 Biochemical properties of stretch-activated ion channels. **Ching Kung.** Univ. of Wisconsin.
10:45 Discussion.
10:50 Effects of stretch-activated ion channel activation on intracelllular calcium. **Owen Hamill.** Univ. of Texas Med. Branch, Galveston.
11:20 Discussion.

Symposium

13.0 Regulation of Cell Shape and Function by the Extracellular Matrix

2:00 PM — Hernando Ballroom
Chair: **Martin A. Schwartz**

2:00 Control of cell growth and gene expression by cell shape. **Donald Ingber.** Children's Hosp., Boston.
2:30 Discussion.
2:35 Regulation of intracellular tyrosine phosphorylation by integrins. **Keith Burridge.** Univ. of North Carolina.
3:05 Discussion.
3:10 Regulation of immediate-early gene expression by extracellular matrix. **Thomas Parsons.** Univ. of Virginia.
3:40 Discussion.
3:45 Regulation of Na^+/H^+ exchange and inositol lipid metabolism by integrins. **Martin A. Schwartz.** Scripps Res. Inst., La Jolla, CA.
4:15 Discussion.
4:20 Regulation of protease gene expression by cell shape, the cytoskeleton and integrins. **Zena Werb.** UCSF.
4:50 Discussion.

Banquet Lecture

14.0 Complex Mechanochemical Signal Transduction Involved in the Regulation of Development

8:30 PM — Hernando Ballroom

Speaker: **David McClay**, Duke Univ.

4.1

REDUCTION IN WALL TENSION IN ISOLATED PULMONARY VESSELS STIMULATES MATRIX METALLOPROTEASE ACTIVITY. C.A. Tozzi, S. Thakker-Varia, and D.J. Riley. UMDNJ-Robert Wood Johnson Medical School, New Brunswick NJ 08903; Lyons VA Medical Center, Lyons NJ 07939.

Remodeling of hypertensive pulmonary arteries (PAs) regresses after blood pressure is reduced. We hypothesized that abrupt reduction in wall tension in isolated segments of hypertensive PAs stimulates expression of matrix metalloproteases. We have demonstrated that release of mechanical tension in isolated (PA) segments stimulates proteolysis (Clin. Res. 1992:40;327A; Exp. Biol. A775, 1993.). Segments of PAs from rats exposed to 10% O_2 for 10 d in an organ bath and subjected to a tangential load equivalent to 50 mmHg for 2 h then released or maintained for 6 h. Metalloprotease activity was measured in tissue homogenates by degradation of [^{14}C]-transferrin substrate, gelatinolytic activity by degradation of [^{3}H]-gelatin, and collagenolytic activity by degradation of [^{3}H]-collagen. Protein levels of collagenase and stromelysin were assessed by Western blot analysis. Release of tension stimulated proteolysis (1.7±.5 in release vs 0.8±0.06 x10^2 dpm/protein in maintained for [^{14}C]-transferrin $p<0.05$, $n=4$) and gelatinolysis (2.6±0.3 in release vs 1.9±0.2 x 10^3 dpm/protein in maintained for [^{3}H]-gelatin, $p<0.05$, $n=4$). Collagenolyitc activity was not stimulated by release of tension. Western blot analysis demonstrated the presence of a doublet for collagenase at 57 and 54 kd as well as a 52 kd band for stromelysin. We conclude that an abrupt reduction in wall tension stimulates the expression of stromelysin and gelatinase. Hemodynamic forces may modulate vascular remodelling by releasing factors that regulate proteolytic activity within the vessel wall and initiate breakdown of excess matrix proteins.

4.2

ANTISENSE OLIGODEOXYNUCLEOTIDES TARGETING PLATELET-DERIVED GROWTH FACTOR-B mRNA INHIBIT MECHANICAL STRAIN-ENHANCED FETAL RAT LUNG CELL PROLIFERATION. Mingyao Liu,* Jason Liu,* Shilpa Buch,* A. Keith Tanswell* & Martin Post.* MRC Group in Lung Development, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

We have previously shown that an intermittent strain regimen, which simulates fetal breathing movements, enhances DNA synthesis and cell division of fetal rat lung cells maintained in organotypic culture. In the present study we report that mechanical strain increased platelet-derived growth factor (PDGF) B-chain and PDGF ß-receptor (PDGF-ßR) mRNAs within 5 min after the onset of strain. PDGF-BB and PDGF-ßR proteins increased after 24 h of intermittent strain. The strain-induced stimulatory effect on fetal lung cell proliferation was blocked by tyrphostin 9 (1 µM), a PDGF receptor associated tyrosine kinase inhibitor, antisense PDGF-B oligodeoxynucleotides (15 µM), and PDGF-BB neutralizing antibodies (10 µg/ml). These results suggest that physical forces play an important role in regulating PDGF-B and PDGF-ßR expression, which then in turn may mediate strain-induced cell proliferation. (Supported by MRC of Canada).

4.3

PHYSICAL RESPONSES OF THE TRACHEA TO TRANSECTION IN THE LIVING RAT. Mortimer Lorber. Georgetown University School of Medicine, Washington, DC 20007

The flexibility of the rat trachea is well known. This study reveals another mechanical characteristic. It is tensed even when the neck is flexed. In eight female rats (body wt. 187±6 g) the cervical trachea was exposed and the thyroid removed. Beginning rostrally, four dots of dye were placed on alternate tracheal cartilages. With a dividers and micrometer the three inter-dot lengths were measured. Width was similarly measured. Thickness was determined by a calipers. Transection of the fibroelastic membrane caused the rostral interval of 1.76±0.07 mm to decrease by 15% ($P < 0.01$) and the caudal one of 1.72±0.24 mm to decrease by 10% ($P < 0.05$). In contrast, at the transection site between dots 2 and 3 the gape was 4.23±0.97 mm, a 249% increase over the baseline interval of 1.70±0.20 mm ($P < 0.001$). Mural retraction alone would increase the distance between dots 2 and 3 only by about 25% (15% + 10%). As the gape was ten times that, the segments had also been acted upon by a more powerful force which in each rat visibly distracted the caudal tracheal segment toward the chest - the negative intrathoracic pressure. As no change in its 3.00±0.11 mm width occurred, the trachea had to thicken while it shortened. Thickness was 2.71±0.06 mm rostrally and 2.55±0.08 mm caudally. It could only be measured following excision. It is concluded that both intrinsic retractive and extrinsic distractive forces tense the trachea in situ. If no tension existed the transected edges would have lain adjacent.

4.4

BRONCHOMOTOR TONE IN THE EARLY FOETAL LUNG: IS IT INVOLVED IN NORMAL LUNG GROWTH? M. Sparrow and H.W. Mitchell Dept Physiol, Univ. Western Australia, Nedlands 6009, Australia

Force acting across the lung tissue of the developing foetus is considered to be the stimulus for normal lung growth. We hypothesised that bronchomotor tone in the lower airways of the early foetus may serve this role. We sought, and then characterised, narrowing of the bronchial tree and the movement of the lung liquid in the lungs of first trimester foetal pigs. The entire bronchial tree of 8-30g foetal pigs (30-45 days gestation) was freed of parenchyma and vasculature. Transmitted light was used to sharply image the airway wall and lumen. Narrowing was video-recorded in real time and quantitated using NIH Image 1.54 software. Airway narrowing was strongly developed throughout the bronchial tree to within 75µm of the epithelial buds indicating rapid onset of airway function after differentiation from the mesenchyme. Small airways from 25-300µm lumen diameter narrowed by 80% in response to a variety of stimuli. Spontaneous narrowing was seen throughout the bronchial tree in most lungs at 37°C. Contractions varied from localised movements of the wall to strong peristaltic-like waves moving over ~2mm of airway. Both spontaneous and activated narrowing produced a flow of the lung fluid along the lumen which carried with it particulate cell debris. We suggest that bronchomotor tone maintains the lung liquid at a positive pressure in the airways. Spontaneous narrowing is activated in response to small pressure fluctuations in localised regions of the airways and serves to move lung liquid along the airways preventing the accumulation of cellular debris in the lumen of the rapidly growing airways and maintaining an even positive pressure in the lumen of the bronchial tree. This may be a stimulus contributing to the growth of the lung tissue.

MUSCULOSKELETAL RESPONSES TO MECHANICAL STIMULI

5.1

MUSCLE DAMAGE/REPAIR AND MUSCLE CONDITIONING MECHANISMS IN DYNAMIC CARDIOMYOPLASTY.

U. Carraro[1], C. Rizzi[1*], A. Bruson[1*], C. Catani[1*], P. Mikus[2*], A. Pierangeli[2*], G. Arpesella[3*] CNR Unit Muscle Biol&Physiopath, Dept Exp Biomed Sci, Padova University[1]; Catt Cuore e Grossi Vasi, Bologna University[2]; Catt Cardiochirurgia, Chieti University[3], I-35131 Italy.

Motor units of skeletal muscles differ in their ability to withstand fatigue at different sustainable power outputs. At the myofiber level this property is correlated with both a high oxidative potential and a distinct set of contractile proteins. Several independent parameters can be evaluated to type skeletal myofibers: light and electron morphology; contractile properties; enzymatic activities; histochemical and immuno-histochemical staining; structural characteristics of contractile proteins by their gel electrophoretic behaviours, peptide mapping, and aminoacid sequence at protein or gene level. Ease, reliability, sensitivity and discriminating power make SDS PAGE-immunoblotting of: 1) myosin heavy chains and myoglobin (markers of myofiber transformation/regeneration), and of extracellular matrix proteins (markers of cumulative damage), the first choice in experimental and clinical applications, because only a-few-milligram biopsy is needed to perform molecular typing and histopathology. Combining sessions of non-invasive measurements of dynamic contractile properties of LD with myoglobin and CPK serum leakage follow-up and needle muscle biopsies a discontinuous pattern of bradycardic-like electrostimulation can be semi-continuously monitored, correlating power output to muscle transformation-trophism-damage.

5.2

INFLUENCE OF COMPRESSIVE LOAD ON MATRIX SYNTHESIS IN CARTILAGE: POSSIBLE SIGNALLING MECHANISMS. Jill P.G. Urban and Andrew.C. Hall
Physiology Laboratory, Oxford University, Oxford, England, U.K.

Articular cartilage and intervertebral disc routinely experience high compressive loads during normal activity. When these cartilages are loaded, the cells and matrix deform, the hydrostatic pressure rises and fluid moves under the resulting pressure gradient. Extracellular hydrostatic pressures can rise to nearly 200 atmospheres articular cartilage is loaded. The extent of fluid expression can also be significant; the disc loses and regains around 20% of its interstitial fluid during a diurnal loading cycle, leading to proportional changes in extracellular osmolality. We have found that both hydrostatic pressure and changes in osmotic pressure can affect metabolism of chondrocytes *in vitro*, whether the chondrocytes are isolated or *in situ* in the matrix. Short exposures (secs) of hydrostatic pressure within the physiological range, stimulate matrix synthesis rates by 30-100%. In contrast, increases in osmolality, induced either by loading the matrix or by changing the osmolality directly, lead to a similar, dose-dependent fall in synthesis. Both changes in osmolality and hydrostatic pressure affect ion and amino-acid transport across the chondrocyte plasma membrane, while changes in osmolality also alter cell volume. Both these effects will alter intracellular composition, and this change in composition may be responsible in part for the effect of hydrostatic and osmotic pressures on chondrocyte metabolism.

5.3

TRANSCRIPTIONAL REGULATION IN MECHANICALLY OVERLOADED SKELETAL MUSCLE OF TRANSGENIC MICE. Ilia Rivera-Rivera*, Jennifer L. Wiedenman*, LiYing Gao*, Dharmesh Vyas*, Lola T. Kwan* and Richard W. Tsika, Dept of Physiology, Univ. of Illinois, Urbana, IL 61801

Mechanically overloaded adult skeletal muscle undergoes hypertrophic growth and selective changes in the transcription (repression/induction) of both metabolic and contractile protein genes. To better understand transcriptional regulatory mechanisms which occur in response to work overload we have begun to delineate cis-acting elements in the promoters of the mouse muscle creatine kinase (MCK), and the mouse and human β-myosin heavy chain (β-MHC) genes in the mechanically overloaded plantaris muscle of transgenic mice. Northern blot analysis revealed a 3.5 fold repression of endogenous MCK specific transcripts after 2 days of overload whereas endogenous β-MHC specific transcripts where not significantly induced until 3 weeks post-overload. Analysis of MCK and β-MHC transgene expression levels were measured as chloramphenicol acetyltransferase activity (CAT assays). Our previous work has identified an upstream fragment (-3300 to -1256) which strongly represses (5.6 fold) MCK expression in response to 2 days of work overload. Preliminary analysis of new transgenic lines suggests this element resides upstream of nucleotide -2200. Multiple independent transgenic lines which harbor mouse β-MHC transgenes (-5600 and -600 bp) and human β-MHC transgenes (-1285 and -600) are induced (4 -12 fold) after 6 to 8 weeks of work overload. These data provide the first evidence that both the MCK and β-MHC genes are transcriptionally regulated in response to a mechanical overload in adult skeletal muscle, and that the regulation of these two genes occurs over very different time courses.

5.4

REGULATION OF PROLIFERATION AND PLATELET-DERIVED GROWTH FACTOR EXPRESSION IN PALMAR FIBROMATOSIS (DUPUYTREN'S DISEASE) BY MECHANICAL STRAIN. Benjamin A. Alman* and Debra A. Greel*. Tufts University School of Medicine, Boston, MA 02111.

Palmar fibromatosis causes contracture of specific palmar fascial bands. Primary cell cultures were derived from involved palmar fibromatosis from 8 patients, uninvolved palmar fascia (Skoog's fibers) from 4 of these patients, and normal palmar fascia from 4 additional patients. Cultures were synchronously grown on collagen coated silastic membranes subjected to cyclic strain (25% strain at 1 Hz) or without strain. Proliferation assay showed an increase in proliferation in all cultures subjected to stretch. The increase was highest in palmar fibromatosis and lowest in normal fascia. Stretch increased Platelet-derived Growth factor (PDGF) A and B chain expression in palmar fibromatosis and in Skoog's fibers. The level of expression was higher in fibromatosis cultures. Normal fascia did not express PDGF. PDGF neutralizing antibody decreased the proliferative response to stretch in the fibromatosis cultures. The observed anatomic location of palmar fibromatosis can be explained based on the cells' response to mechanical strain (fascial bands subjected to repetitive stretch), which may be mediated by PDGF.

5.5

MECHANICAL STRESS AND EXPRESSION OF STRESS PROTEIN IN GROWTH AND DEFFERENTIATION OF SKELETAL MUSCLE. **Yoriko Atomi University of Tokyo, Tokyo, Japan 153**

Skeletal muscle is a tissue which differentiates and functionates in response to mechanical stress. Passive stretch/muscle length greatly influences muscle weight. 22-kDa protein which specifically decreases in slow muscle atrophy induced by hindlimb suspension and increase with passive stretch is identified to be αB-crystallin. Recent data show αB-crystallin is one of small heat shock protein (sHSPs) and associates with cytoskeletal proteins and Z-band of myofibrils. α-Crystallin is a shapelon of vimentin in vitro study. We analyzed the expression (localization) of αB-crystallin by polyclonal antibody produced by C-terminal peptide of it and other stress proteins such as HSP27 and HSP70 by monoclonal antibodies (Sigma Chemical) in embryonic chiken cultured cells, rat L6 and mouse C2C12 cells and rat skeletal and heart muscles . αB-crystallin expresses in myoblast and whole myotube, but shows striations in matured myotubes, which autonomically begins to contract. 20% DMSO enhances this striated localization, while heat schock at 43 C for 30min doesn't do it. Passive stretch of chicken myotube on silicone dishes also enhances marked striated localization of αB-crystallin in chicken and L6 myotubes. sIISP27 (I-band) and HSP70(in nuclear) expresses at fusion of myoblast to myotube in L6 cells, but they doesn't not always express in other duration. In adult rat skeletal muscle, αB-crystallin shows fiber-type related expressions in cross-section, and striated localization in longitudinal sections. In our recent study, αB-crystallin is a Ca_{2+} binding protein. Taken together, the role of stress protein, αB-crystallin seems a Ca_{2+} dependent chaperone for cytoskeletal protein networks in muscle.

CARDIOVASCULAR ADAPTATIONS TO MECHANICAL STIMULI: PERIPHERAL VASCULATURE

6.1

CALCIUM (Ca) -PERMEABLE MECHANO-ACTIVATED (MAC) ADAPTING CHANNEL IN RAT MESANGIAL CELLS (RMC's). Victor Chen*, Helena Guber*, Clyde Shuman*, Carlos E. Palant* Nephrology and Endocrinology Sections, Brooklyn VA Hospital and SUNY Health Science Ctr. at Brooklyn, NY 11209

Chelation of extracellular Ca prevents the increase in kinase activity that precedes protein synthesis in RMC's stimulated by mechanical stretching. This has led authors to propose a Ca-permeable MAC in RMC's. To explore this, RMC's in their 3rd to 20th culture passage were examined with patch-clamp techniques. In cell-attached patches with 100 mM $BaCl_2$ pipets, single channel inward currents with a conductance g = 21 pS (r^2 = 0.89, n = 16 cells) and a reversal potential E_{rev} = -70 mV (with respect to V_{pipet}) were elicited. Intracellular Cl activity and membrane potential measurements gave an estimated E_{rev} for Cl = -13 mV (with respect to V_{pipet}). In control patches, channels exhibited a cumulative open probability (N*p) of 0.01-0.1 and a mean open time of approx. 100 ms. Channel kinetics were independent of holding voltages and were insensitive to Nitrendipine (10μ M, n=6 cells). In 8 cells, a flickery block occurred upon addition of $CoCl_2$ (5 mM) to patch pipets, resulting in a reduction of g of 62%, with respect to control) Stepped suction (-5 to -25 mm Hg) applied to the recording pipet resulted in bursts of channel openings which showed quick adaptation. Hyperpolarizing holding voltages decreased the rate of adaptation to suction stimuli. An increase in N*p was also observed upon reduction of extracellular osmolarity with the response adapting in similar fashion. In conclusion, RMC's possess two types of MAC's, a weakly adapting Ca impermeable, monovalent cation- MAC (Kidney Int. 43:535, 1993) and a strongly adapting Ca-permeable MAC that responds phasically. This phasic response is presumably necessary so as not to override mechanisms that maintain a low cytosolic Ca. The Ca-permeable MAC may be involved in stretch-induced contraction of RMC's and in stretch-induced stimulation of growth promoting signals.

6.2

FEED ARTERY HEMODYNAMICS CHANGE WITH MUSCLE LENGTH IN HAMSTER RETRACTOR MUSCLE. Donald G. Welsh and Steven S. Segal. John B. Pierce Laboratory, Yale University School of Medicine, New Haven CT 06519.

We tested the hypothesis that changing muscle length (Lm) would alter hemodynamics and vasomotor activity of feed arteries (FAs) which supply the muscle microcirculation. Male hamsters (n=13, 100±4g; mean±SE) were anaesthetized (pentobarbital) and the retractor muscle prepared for intravital microscopy. FA diameter, red blood cell velocity and transmural pressure (servo-null) were monitored at 85% to 130% of Lm in vivo. Increasing Lm under control conditions reduced FA diameter (from 73±4 to 59±4 μm; $p<0.05$) and blood flow (from 73±14 to 40±8 nl/s: $p<0.05$). Neither wall shear rate (WSR; 1700±180 s^{-1}) nor pressure (68±5% of systemic arterial) changed with Lm. Topical sodium nitroprusside (SNP; 10 μM) was used to eliminate active responses and reveal the direct effects of Lm on FA perfusion. At Lm = 85%, SNP dilated FAs (to 96±4 μm; $p<0.05$), increased flow (to 209±24 nl/s; $p<0.05$) & WSR (to 2350±150 s^{-1}; $p<0.05$), and reduced pressure (to 59±3%; $p<0.05$). Increasing Lm during SNP reduced WSR ($p<0.05$) and increased pressure ($p<0.05$); diameter and flow were unchanged. These findings indicate that feed arteries external to the muscle respond actively to changes in Lm, reducing blood flow while maintaining WSR and microvascular perfusion pressure.

Supported by the American Heart Association (GIA and EI).

6.3

MECHANISMS OF TRANSDUCTION OF ELEVATED VASCULAR WALL STRESS INTO INCREASED PRODUCTION AND ACCUMULATION OF ARTERIAL ELASTIN. Fred W. Keeley*, L. Bartoszewicz*, and P. Robson*. Div. of Cardiovasc. Research, Hospital for Sick Children, Toronto, Canada M5G 1X8

Elevated vascular wall stress appears to be a driving force for the accumulation of arterial connective tissue proteins in both normal development and hypertension. Increased elastin production in response to elevated wall stress in *in vitro* aortic organ culture models is a rapid and graded response which does not require an intact vascular endothelium. Depletion of extracellular calcium, or blockade of calcium channels with verapamil inhibits secretion and assembly of elastin but not synthesis of the protein. Gadolinium-sensitive, stretch-activated calcium channels are not involved in the stress-induced response. Stress-induced elastin production is also not dependent on release of calcium from sarcoplasmic reticulum stores, although flooding of the cytoplasm with calcium through the action of ryanodine, thapsigargin or caffeine selectively blocks the stress-induced response. Inhibition of phospholipase C and protein kinase C also selectively inhibit the response to increased wall stress. Stress-induced elastin production is correlated with a rapid increase in tyrosine phosphorylation of several cellular proteins, and both increased phosphorylation and increased elastin production are inhibited by tyrphostin-25 but not by genistein. Stress-induced increases in aortic elastin production are not correlated with increased steady state levels of mRNA for elastin, suggesting a mechanism involving increased translational efficiency.

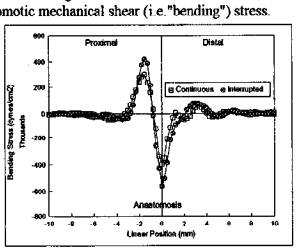
6.4

A Model For Subjecting Vascular Wall Cells To Simultaneous Pulsatile Fluid and Mechanical Anastomotic Shear Stress. *GJ L'Italien, A Benbrahim, S Dhara, CJ Kwolek, BB Milinazzo, DF Warnock, JP Gertler, RW Orkin, WM Abbott. Vascular Surgery Department, Massachusetts General Hospital, Boston, MA. 02114.*

We have previously described an *in vitro* system, the Vascular Simulating Device (VSD), capable of subjecting cells grown inside silicone rubber tubes to arterial levels of pressure, pulsatile strain, and fluid shear (J Vasc Surg, in press). Here we describe a modification to the system, (VSD-ANAST), which can expose cells to the dynamic mechanical and fluid shear conditions present in the end-to-end vascular anastomosis.

Fourteen silicone rubber tubes 6.5±0.1 mm in diameter and 80 mm in length were transected and anastomosed with polypropylene suture using either the interrupted (n=7) or continuous (n=7) technique. Tubes were subjected to arterial levels of pressure and flow and scanned with a helium-neon laser micrometer in the peri-anastomotic region. Measurements of diameter, wall strain and thickness were used to compute the anastomotic mechanical shear (i.e."bending") stress.

There was no significant difference in the stress pattern for either suturing technique (Figure). The peak bending stress which occurred 1.8 mm proximal to the anastomosis (430±70 dyne/cm^2 x 10^6) was significantly greater than at the distal site (148±16 dyne/cm^2 x 10^6,p<.001), which was seen 3.0 mm distal to the anastomosis. The observed levels of mechanical shear stress were similar to values reported by others for host artery-graft anastomoses (J. Biomech 20:795-803, 1987). In addition, wall strain at the anastomosis was reduced by 40±4%, as we have seen *in vivo*.



VSD-ANAST reproduces the mechanical conditions found at the artery-graft anastomotic junction. Furthermore, we have demonstrated that tube cell cultures subjected to these conditions can be fixed in situ and examined microscopically, thus permitting cell characterizations (morphology, proliferation, immunostaining, and in situ hybridization) correlating biologic cell behavior with anastomotic biomechanical conditions.

6.5

STRUCTURAL CHANGES AND THEIR DYNAMIC STABILITY OF HUMAN ENDOTHELIAL CELL AFTER APPLYING A SHEAR STRESS. Jun K. Chang*, Jin H. Kim*, Jongwon Kim*, Dong C. Han*, Byoung G. Min*. Seoul National University, Seoul, 110-744, Korea.

Structural changes in the pattern of actin cytoskeleton (CSK) and the dynamic stability of its organization were analyzed quantitatively and analytically using cultured human umbilical vein endothelial cell (HUVEC) monolayer after a shear flow exposure. Morphological parameters of the cell and of the microfilaments (MF) were measured on fluorescent micrographs of the cells stained with FITC-conjugated phalloidin. The computer model of CSK was developed with morphological parameters of actin CSK based on tensegrity architecture. HUVECs on fibronectin-coated polyurethane were elongated and aligned to the direction of the flow after the shear flow exposure (10 dyne/cm^2, 0-24 hours exposure). After 6 hours, the stress fibers were formed in the proximal (relative to flow direction) cell regions. This change in MF preceded cell elongation and orientation. After 24 hours, HUVECs were elongated and oriented to the direction of flow, and MF bundles were localized along the long axis of the cell. Throughout the computer simulations, cytoskeletal alignment to the direction of stress was the most efficient way to increase the tension of the actin CSK with maintaining the dynamic stability of CSK to shear stress. These results indicate that the cytoskeletal redistribution is the early cellular response to the onset of shear stress and that this pattern of redistribution to the direction of external mechanical stress shows the maximized structural integrity and stability of CSK.

6.6

THREE-DIMENSIONAL CHANGES OF HUMAN ENDOTHELIAL CELL MORPHOLOGY UNDER FLUID FLOW. Dong I. Shin*, Jun K. Chang*, Jin H. Kim*, Jongwon Kim*, Dong C. Han*, Byoung G. Min*, Seoul National University, Seoul, 110-744, Korea.

A parallel-plate, channel flow chamber and image processing system were developed to investigate the three-dimensional changes of cultured human umbilical vein endothelial cell (HUVEC) morphology on the polymeric surfaces. The change of cell shape were examined by a CCD camera attached to the microscope and connected to the image analysis system and a video recorder. The fine adjust nut of microscope was directly connected to the step motor which synchronized with the electrical shutter of CCD. Contours of cell on specific height level were collected and reconstructed to analyze the morphologic parameters of HUVEC. HUVECs cultured on fibronectin-coated polyurethane were aligned to the direction of flow under the fluid flows (10 dyne/cm^2). Three-dimensional morphology was recorded automatically for every ten minutes after flow starts up to twelve hours. We observed i) alignment of the long axis of cell to the direction of flow, ii) movement of nucleus to the distal (relative to flow direction) cell region, iii) height decrease along the long axis of cell, and iv) distal directionality of cell process of HUVEC after the shear flow exposure. These results indicate that height decrease along the long axis of cell can reduce up to 40 % of shear stress acting on the plasma membrane and that these three-dimensional morphologic changes are necessary for adherence under fluid flow.

6.7

INTEGRIN EXPRESSION BY PORCINE CORONARY VASCULAR SMOOTH MUSCLE CELLS. Jon Mogford*, George E. Davis* and Gerald A. Meininger. Department of Medical Physiology, Texas A&M University Health Science Center, College Station, TX 77843.

The purpose of this study is to characterize the integrin expression of porcine coronary vascular smooth muscle cells (VSMC). Cultured cells from the left anterior descending and circumflex arteries were grown and surface labeled with biotin. Detergent extracts from these cells were passed over five sepharose affinity columns containing RGD peptide, the 120 kDa cell-binding fragment of fibronectin, collagen type I, a 25 amino acid peptide derived from the CS1 alternatively spliced region of fibronectin, and Matrigel (as a laminin source). Electrophoresis and blotting of collagen type I and Matrigel eluted fractions revealed major bands migrating similar to $\alpha_1\beta_1$. Distinct β_1 integrins were observed eluting from the 120 kDa fibronectin and RGD columns while no integrins were detected from the CS1 column. Bands of lesser intensity consistent with the $\alpha_v\beta_3$ integrin were also seen eluting from the RGD column. VSMC's were found to attach to substrates coated with collagen type I, collagen type IV and laminin in a magnesium but not calcium-dependent manner. Cell attachment to fibronectin and an RGD-containing recombinant protein (Pronectin F) occurred in the presence of either divalent cation, although calcium supported less adhesion than magnesium on Pronectin substrates. The above work represents a preliminary characterization of integrins expressed by coronary VSMC's that most likely mediate interactions with the extracellular matrix. (Supported by NIH HL33324 to GAM and AHA, Texas Affiliate #92G-025 to GED.)

6.8

MECHANICAL STRAIN ENHANCES EXPRESSION OF THE PDGF-A AND EGR-1 GENES IN VSM CELLS

Glenn J. Vives*, Emily Wilson*, Tucker Collins*, Vikas Sukhatme* and Harlan E. Ives*. Div. of Nephrology University of California, San Francisco and Dept. of Pathology, and Beth Israel Hosp. Harvard Univ., Boston, MA

We have previously shown that cyclic mechanical strain (1 Hz) induces cell growth and DNA synthesis in cultured vascular smooth muscle cells through the autocrine expression of PDGF A and B chains. Using deletion analysis of PDGF-A chain promoter-CAT constructs and transient transfection, we found a 92 base pair (bp) region proximal to the transcription start site that confers responsiveness to mechanical strain. This 92 bp minimal promoter contains consensus sequences for 3 SP1 binding sites and 2 egr-1/Wilms tumor suppressor sites. We therefore have analyzed the effect of cyclic mechanical strain on the expression of the early growth response gene, egr-1. Steady state levels of egr-1 mRNA were determined using Northern blot analysis. The mRNA levels increased 4.2 fold within 15 minutes of exposure to mechanical strain; peak expression was 5.7 fold over basal at 30 min. Expression returned to below basal levels within 1 hour. These studies suggest that the strain-induced expression of PDGF-A chain gene may be mediated by enhanced expression of egr-1.

6.9

Mechanical Strain Induces Expression of Smooth Muscle Myosin in Vascular Smooth Muscle Cells H.P. Reusch, H. Waggy and H.E. Ives, University of California, San Francisco.

The distribution of myosin subtypes was examined in neonatal rat vascular smooth muscle cells (VSM) in response to cyclic mechanical strain. Cells were grown on silicone elastomer plates which were subjected to strain by cyclic (1 Hz) application of a vacuum. Myosin subtypes were identified by Western blots. In response to strain, smooth muscle myosin, using semiquantitative chemiluminescense and densitometric scanning, increased over time, achieving a maximum of 3-fold after 36h. Over the same time period, non-muscle myosin decreased by 50%. We have previously shown that VSM cells exposed to strain secrete both A- and B-chains of PDGF. To asses the role of this secreted PDGF in altering myosin subtypes, experiments were performed with exogenously added PDGF or neutralizing PDGF-Ab (3µg/ml). Smooth muscle myosin was decreased by approx. 50% and non-muscle myosin increased by approx. 60% in response to PDGF, opposite to the effect of strain. When cells exposed to strain were incubated with neutralizing Ab to PDGF-AB , the strain-induced increase in smooth muscle myosin was further enhanced to 4-fold and non-muscle myosin was further reduced to 0.5-fold compared to cells without antibodies. Thus, unlike PDGF which causes dedifferentiation of VSM cells, mechanical strain alters myosin subtype distribution towards that seen in differentiated VSM cells.

6.10

INTERCELLULAR COMMUNICATION vs. RECEPTOR-MEDIATED MECHANISMS IN ATP AND BRADYKININ-INDUCED INCREASES IN ENDOTHELIAL INTRACELLULAR CALCIUM

Henry M. Honda*, Joshua I. Goldhaber*, Linda L. Demer and James N. Weiss*. UCLA School of Medicine, Los Angeles, CA

ATP, bradykinin (Bk), and acetylcholine (Ach) lead to vasodilation by the release of nitric oxide which is mediated by increases in endothelial intracellular calcium (Ca) levels. To determine the role of receptor-mediated increases in EC $[Ca]_i$ vs. intercellular communication in response to agonists, we examined the effects of vasoactive agents on Fura-2 AM loaded bovine aortic endothelial cells (BAEC) *in vitro*. In order to distinguish the effects of receptor-mediated increases in EC Ca_i from propagation of a Ca response from adjacent cells, we examined EC upstream to the point of delivery of agonist under flow conditions. Application of 3 µM Ach (n=2), 10 nM Bk (n=5) or 10 µM ATP (n=9) led to the expected receptor-mediated increases in EC $[Ca]_i$. Fura-2 ratios increased from 0.70±0.22 (mean ± s.d.) at baseline to 1.39±0.18 for ACh, 1.46±0.19 for Bk and 1.41±0.38 for ATP. However there was no retrograde propagation of the signal with any of these agents at room temperature or at 37°C. In contrast, a mechanical stimulus led to propagation of a Ca wave 2-4 cells away from the stimulated cell. Conclusion: Receptor-mediated increases in Ca play the dominant role in the response of BAEC to vasoactive agents in contrast to the intercellular propagation of a Ca wave seen with a mechanical stimulus.

6.11

MECHANICAL STRAIN INDUCES MONOCYTE CHEMOTACTIC PROTEIN-1 GENE EXPRESSION MEDIATED VIA PROTEIN KINASE C PATHWAY IN HUMAN ENDOTHELIAL CELLS

D. L. Wang, B. S. Wung, S. Usami and Y.J. Shyy
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Monocyte chemotactic protein-1 (MCP-1), a potent monocyte chemoattractant secreted by endothelial cells (EC), plays a key role in the early events of atherogenesis. Since vascular ECs are constantly subjected to mechanical stresses, the effect of cyclical strain on expression of the MCP-1 gene in human umbilical vein ECs was examined. ECs grown on a flexible membrane base were deformed by negative pressure (16 kPa at 60 cycles/min). ECs subjected to strain for 12 hours showed increased monocyte adhesion by about 1.8-fold as compared to unstrained control cells. By the Boyden chambers technique, cultured media from strained cells showed more than twice the monocyte chemotactic activity of medium from control cells. Pretreatment of the collected media with polyclonal anti-MCP-1 antibody suppressed this MCP-1 activity. Northern blot analysis demonstrated that MCP-1 mRNA levels in cells subjected to cyclical strain for 1, 5 or 24 hours were double those in control cells. The induced MCP-1 level returned to its control basal level 3 hours after strain had been released. Pretreatment of ECs with a protein kinase C (PKC) inhibitor, calphostin C, abolished the strain-induced MCP-1 gene expression. Pretreatment of ECs with cAMP- or cGMP- dependent protein kinase inhibitors (KT5720 or KT5823) only partially inhibited the strain-induced MCP-1 gene expression. ECs treated with EGTA showed strongly inhibited MCP-1 gene expression. The intracellular calcium chelator BAPTA/AM caused only a minor decrease in MCP-1 mRNA levels. Both strained and unstrained ECs treated with the Ca^{2+} channel blocker, verapamil, showed a strong reduction in MCP-1 mRNA levels. Nuclear proteins isolated from strain-treated ECs retarded the migration of AP-1 oligonucleotide in a mobility shifting assay. These results indicate that mechanical strain can stimulate the secretion of MCP-1 from ECs by increasing MCP-1 gene expression, and that this strain-induced gene expression is predominantly mediated via the protein kinase C pathway and requires extracellular Ca^{2+}. This strain-induced MCP-1 expression from ECs might contribute to the trapping of monocytes in the subendothelial space during atherogenesis. (supported by National Science Council, Taiwan, ROC)

6.12

FLOW-MEDIATED NO RELEASE IN ENDOTHELIAL CELLS: ROLE OF CALCIUM AND K^+ CHANNELS. W. Charles O'Neill, Pamela B. Perry and Daniel Steinberg, Renal Division, Emory University, Atlanta, GA. 30322

The mechanism by which flow stimulates NO release from endothelium is unknown, but may involve K^+ channel activation leading to hyperpolarization and a subsequent rise in $[Ca_i^{2+}]$. We measured NO release (as nitrite) and K^+ channel activity (as $^{86}Rb^+$ release) at 1 min intervals in bovine aortic endothelial cells on collagen-coated microcarrier beads perfused in 0.47 cm diameter columns. Graded increases in flow from 0.25 ml/min progressively and reversibly increased NO release up to 507 ± 61(SE)% (n=11) at 2.0 ml/min. By contrast, stimulation by A23187 (Ca^{+2} ionophore) was only 137 ± 22% (n=3). In cells loaded with the Ca^{+2} chelator (BAPTA), which completely blocked agonist-induced Ca^{2+} increases, neither basal NO production nor the stimulation by flow (772 ± 201%, n=4) was inhibited, but the stimulation by A23187 was completely blocked. Stimulation by flow, although reduced, was still apparent in BAPTA-loaded cells in the absence of extracellular Ca^{+2}. The K^+ channel blockers tetraethylammonium (5 mM) or Ba^{+2} (1 mM), but not Gd^{+3} (20 µM), a blocker of stretch-activated channels, inhibited $^{86}Rb^+$ efflux under low flow and high flow. $^{86}Rb^+$ efflux transiently increased with increased flow and transiently decreased with decreased flow, suggestive of an unstirred layer/washout effect, rather than channel activation. This was confirmed by showing a similar pattern for dissociation of bound [^{3}H]ouabain (a flow-independent process), but channel activation is not absolutely ruled out. When Na^+ in the medium was replaced with K^+ to prevent hyperpolarization via K^+ channels, NO production was reduced but still increased with flow. We conclude that the mechanism by which flow increases NO production in endothelial cells does not involve activation of K^+ channels or an increase in intracellular $[Ca^{2+}]$.

6.13

DISTRIBUTION OF SHEAR STRESS ON THE SURFACE OF ALIGNED AND NON-ALIGNED ENDOTHELIAL MONOLAYERS.

Kenneth A. Barbee*, Trevor Mundel*, Ratneshwar Lal*, and Peter F. Davies.* University of Chicago, Chicago, Illinois 60637

The stresses acting on the endothelial surface due to flow were determined on a subcellular scale. Atomic Force Microscopy was used to measure the surface topography of confluent endothelial monolayers cultured under no-flow conditions or exposed to steady shear stress (12 dyn/cm^2, 24 hrs.). Flows over these surface geometries were simulated using computational fluid dynamics. From the simulated velocity fields, shear stresses acting on the cell surface were calculated. The flow perturbation due to the undulating surface produced cell-scale variations in shear stress causing significant gradients in shear stress. The reorganization of the endothelial surface in response to prolonged exposure to steady flow resulted in significant reductions in the peak shear stresses and shear stress gradients. From the relationship between surface geometry and the resulting shear stress distribution, we have defined a hydrodynamically meaningful shape factor which characterizes the three-dimensional morphological response of endothelial cells to flow. This analysis provides the exact stress loading conditions on a scale that is relevant to the study of physical mechanisms of mechanotransduction.

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6.14

Ca^{2+} ENTRY IN A7r5 VASCULAR SMOOTH MUSCLE CELLS MODULATED BY STRETCH. V. Ruiz-Velasco, B.M. Mayer* and L.J. Hymel
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To better understand the cellular mechanisms involved in the myogenic response in vascular smooth muscle cells (VSMC), we compared $^{45}Ca^{2+}$ entry in A7r5 VSMC under stretch (Flexercell Strain Unit, Flexcell Int. Corp) and static conditions, and under depolarizing and depolarizing/stretch conditions. Stretching the cells at 2.5mM $[K^+]$ caused a significant increase in Ca^{2+}influx rates (from 0.14 to 0.76 nmoles $Ca^{2+}/10^6$ cells/min). This increase was similar in magnitude to that observed under depolarizing and depolarizing/stretch conditions (0.44 and 0.63 nmoles $Ca^{2+}/10^6$ cells/min, respectively). Addition of 1µM isradipine caused a significant block (>88%) of the Ca^{2+} uptake under all conditions. A significant reduction in Ca^{2+} influx rate occurred under Na^+-free conditions when the cells were stretched, but no significant effect of Na^+ removal was observed under static conditions (0.007 vs 0.07 nmoles $Ca^{2+}/10^6$ cells/min, respectively). Stretching 7-day cells (nearly confluent) at 2.5mM K^+ caused the opposite effect as that observed in 10-day post-confluent cultures (described above). The Ca^{2+} influx rate was significantly reduced when the cells were stretched (from 0.18 to 0.08 nmoles $Ca^{2+}/10^6$ cells/min) but the 70mM K^+-induced Ca^{2+} entry was unaffected (0.29 and 0.54 nmoles $Ca^{2+}/10^6$ cells/min for stretched and unstretched, respectively). Removal of extracellular Na^+ had no effect on Ca^{2+} uptake under stretched or static conditions in 7-day cultures. Our results suggest that stretch of aortic VSMC enhances Ca^{2+} uptake through the same pathway involved in voltage-dependent, dihydropyridine-sensitive Ca^{2+} entry, and its effect is dependent upon extracellular Na^+. The modulation of Ca^{2+} entry in A7r5 VSMC by stretch may involve alteration of cell membrane potential by stretch-activated channels. (Supported by a grants from the American Heart Assn., LA Affiliate Inc., and the Louisiana Board of Regents.)

6.15

ELEVATED STRETCH AND SHEAR STRESS PRODUCE DISTINCTLY DIFFERENT CHANGES IN CAPILLARY HYDRAULIC CONDUCTIVITY (L_p). Donna A. Williams and Virginia H. Huxley. Dept. of Physiol., U. Missouri, Columbia, MO 65212

In vivo, capillaries are exposed continuously to physical forces including shear stress and transmural pressure, which produces stretch or strain. We have demonstrated previously that *in situ* shear stress and L_p correlate positively. In this study we distinguish between responses of capillary L_p to stretch relative to shear stress hypothesizing that L_p would increase with elevated shear stress. Mesenteries of pithed frogs (*Rana pipiens*; *n*=21) were exteriorized and superfused with frog Ringer's solution (14-16°C). Pipettes filled with 10 mg·ml^{-1} bovine serum albumin in Ringer's were used to cannulate single capillaries. Volume flux (J_V) was estimated using the modified Landis technique at pressures ranging from 20 to 40 cm H_2O. In 2 sets of vessels, basal L_p (L_p^C) was assessed. Next, pressure was raised (10 min) to 40 cm H_2O plus partial block (L_p^{STR}) to elevate stretch or no block (L_p^{SS}) to increase shear. With elevated stretch, L_p^{STR}/L_p^C increased 4-fold (*n*=11; *P*=0.007) and remained elevated for 8-10 min. Altered shear stress produced modest changes (L_p^{SS}/L_p^C = 1.3±0.3, *n*=7) with an initial spike (2.1 ± 0.8) and decline indicating that L_p responses to stretch relative to shear stress differ. These data imply mechanistic specificity for altering filtration as capillaries adapt to physical forces. Supported by HL34872. DAW is an AHA-MO Affiliate Postdoctoral Fellow.

6.16

PRESSURE/AGONIST-DEPENDENT MODULATION OF ARTERIAL CALCIUM SENSITIVITY. Marilyn Cipolla and George Osol. Univ. Vt. Coll. Med., Burlington, VT 05405

The purpose of this study was to investigate the roles of transmural pressure (TMP) and extracellular Ca^{++} in modulating receptor-mediated vs. receptor-independent vasoconstriction. Uterine arteries (ϕ=100-250μm) from adult Sprague-Dawley rats (n=15) were isolated, cannulated and studied *in vitro*, under pressurized conditions, while continuously measuring lumen diameter. Addition of KCl to the superfusate induced progressive constriction that was markedly potentiated by increasing TMP from 50→100mmHg: EC_{50}=31±3 mM vs. 21±3 mM, respectively ($p<0.05$). Conversely, sensitivity to the α_1-adrenergic agonist phenylephrine (PE) was unaffected: EC_{50} @50mmHg=0.43±0.09 μM; @100mmHg=0.40±0.07 μM ($p>0.05$). In the second part of the study, vessels were given maximal concentrations of either PE (10 μM) or KCl (124 mM) @ 50 mmHg, and exposed to 0.01 to 1.6 mM Ca^{++}. Maximal constrictions were of comparable magnitude, however, vessels were 10x more sensitive to Ca^{++} in PE vs. KCl: EC_{50}=0.03±0.01 mM vs. 0.33±0.14 mM; $p<0.05$. These data support the concept that mechanotransduction by the arterial wall involves a synergism between transmembrane ionic fluxes and the activation of second messenger systems that can profoundly alter vascular smooth muscle Ca^{++} sensitivity. [AHA/901261+921470].

6.17

PRESSURE-INDUCED ALTERATIONS IN GENE EXPRESSION IN THE ISOLATED RAT MESENTERIC ARTERY. Steve P. Allen*, Hong M. Liang*, Sheri B. Parker*, Minh N. Tran*, and Russell L. Prewitt. Eastern Virginia Medical School, Norfolk, VA. 23501

Hypertrophy of the vascular wall occurs in hypertension. To determine the capability of pressure alone to contribute to this response, two small mesenteric arteries (250 μm) were dissected from male Wistar rats and mounted on pipettes in a dual vessel chamber. Each artery was then superfused at 1ml/min with Dulbecco's modified Eagles medium/Ham's nutrient mixture F12, supplemented with penicillin (100U/ml), streptomycin (100μg/ml) and nystatin (100U/ml). The pressure (with no flow) in each artery was increased gradually to physiological values (90 mmHg) and the ability to constrict was tested with 60 mM KCl in the presence of 1μM phentolamine. After a washout period, the pressure in one artery was increased to 140 mmHg. At set times each pair of vessels was fixed overnight in 10% formalin and embedded in paraffin for sectioning. Sense and antisense ^{35}S-labeled riboprobes for c-*fos* and c-myc mRNA and 18S rRNA were transcribed from the respective cDNAs and used for *in situ* hybridization and quantitation by Phosphorimager. The hypertensive vessels showed an induction of c-*fos* at 30 minutes, an almost 5-fold increase in c-myc at 3 hours and a doubling of 18S rRNA at 6 hours, compared with controls. These results therefore show that an increase in arterial pressure alone is sufficient to induce the early response genes, and also increase 18S rRNA, and is thus likely to result in structural changes in these vessels. (This work was supported by a Grant-in-Aid from the American Heart Association.)

6.18

CYCLIC MECHANICAL STRAIN DIFFERENTIALLY MODULATES PROCOAGULANT ACTIVITY IN ENDOTHELIAL CELLS DERIVED FROM DIFFERENT VASCULAR BEDS. Matthew Silverman, Vangelis G. Manolopoulos, and Peter I. Lelkes, Univ. Wisc. Med. School, Lab Cell Biol., Milwaukee, WI 53201.

The apical cell surface of quiescent endothelial cells (ECs) *in situ* and also in culture exhibits very low procoagulant activity, as assessed by the expression of tissue factor (TF). EC activation with cytyokines results in a significant upregulation of TF. In this study we compared the effects of cytokine activation, cyclic mechanical strain, and a combination of both, on TF expression in human ECs derived from umbilical vein (HUVEC), aorta (HAEC), and dermal microvessels (HMVEC). In line with previous reports, activation of these three EC types with cytokines, such as lipopolysaccharide (LPS) or TNF-α, resulted in qualitatively similar, albeit quantitatively diverse elevation of TF expression. Exposure to cyclic strain for 5 hours did not induce TF expression in both types of large vessel-derived EC, while it slightly elevated TF exprerssion in HMVEC. Prolonged exposure to cyclic strain for up to 8 days resulted in a transient 4 to 5 fold elevation of TF, which peaked within 24 hours and then returned to baseline levels. Importantly, cyclic strain synergistically augmented cytokine induced TF expression in HMVEC, but not in the other EC types. Our data support the notion that the differential activation of EC procoagulant activity in response to mechanical activation reflects EC heterogenity in terms of the perception and transduction of mechanochemical signals.

6.19

DIFFERENTIAL MODULATION OF ENDOTHELIAL CELL ADHESION MOLECULES BY CHEMICAL AND MECHANICAL ACTIVATION Shaosong Zhang, Huiying Zhang, Vangelis G. Manolopoulos, and Peter I. Lelkes, Univ. Wisc. Med. School, Lab Cell. Biology, Milwaukee, WI 53201.

Endothelial cells (EC) communicate with each other and with other blood-borne cells via cell surface adhesion molecules, such as PECAM-1, VCAM-1 and ICAM-1. EC activation by cytokines results in upregulation of VCAM-1 and ICAM-1. Recent data suggest that VCAM-1 is down-regulated by flow-induced shear stress. By contrast, the mechanism of PECAM-1 regulation is unknown. Using enzyme linked immunoasays, we confirmed that ICAM-1 and VCAM-1 expression was upregulated in lipopolysaccharide (LPS)-treated cultured EC, derived from human umbillical vein (HUVEC) and dermal microvessels (HMVEC). On the other hand, upon exposure to cyclic mechanical strain for 24 hours, VCAM-1 and ICAM-1 were upregulated (approx by 30%) in HUVEC, but down-regulated (by ca., 25%) in HMVEC. The expression of PECAM-1 was not affected by cyclic mechamical strain in either EC type. However, as also confirmed by Western blotting, the expression of PECAM-1 was differentially modulated by LPS: In HUVEC and HMVEC, 24 hour exposure to LPS caused a dose-dependent downregulation, whereas PECAM-1 expression was upregulated in LPS-stimulated bovine aortic endothelial cells. Our data support the notion that the differential responses of EC to mechanical activation reflect EC heterogenity in terms of the perception and transduction of mechanochemical signals.

6.20

ELEVATED PRESSURE ALTERS MORPHOLOGY AND EXTRACELLULAR MATRIX DEPOSITION OF CULTURED ENDOTHELIAL CELLS Mark M. Samet, Dawn M. Wankowski and Peter I. Lelkes, Univ. Wisc. Med. School, Lab. of Cell Biology, Milwaukee, WI 53201.

Elevated blood pressure as can cause profound changes in morphology, proliferation and extracellular matrix production of endothelial cells. We hypothesized that we can mimic *in vitro* the effects of blood pressure on the endothelium by culturing endothelial cells under their normal or pathophysiological pressure regimens. Using a novel, pressurized cell culture system, we exposed for 60 hours confluent monolayers of pig pulmonary artery endothelial cells to a static pressure of 165 mm Hg. Under atmospheric pressure, the endothelial cell monolayer maintained a growth-arrested, cobblestone-like appearance. By contrast, elevated, static pressure increased cell density by 30%, reduced cell surface area by 25% and induced cell sprouting. The sprouting cells were of endothelial origin as assessed by their continued staining for specific EC markers. Elevated pressure also affected extracellular matrix production. By comparison to non-pressurized controls, we observed, by indirect immunofluorescence, a substantial down-regulation in laminin and fibronectin deposition into the matrix of the cells. By contrast, collagen IV expression was apparently not affected. We propose that these changes manifest endothelial cell participation in the remodeling of blood vessels during their exposure to pathophysiological pressures.

6.21

ALTERATIONS IN ENDOTHELIAL INTEGRINS IN RESPONSE TO HYDROSTATIC PRESSURE. Eric A. Schwartz, Rena Bizios and Mary E. Gerritsen. Rensselaer Polytechnic Institute, Troy NY 12201, and Miles Inc. West Haven CT, 06516

Vascular endothelial cells display morphological alterations, i.e. elongation and cytoskeletal rearrangement, as well as increased cell proliferation, in response to sustained hydrostatic pressure (J Cell Physiol 157:603-614). In the present study we have evaluated the effects of hydrostatic pressure on the expression of integrins in human umbilical vein endothelial (HUVEc) cells. HUVEc on rigid substrates were exposed to sustained hydrostatic pressure for 4 days. Integrin expression was examined by fluorescence microscopy. HUVEc monolayers exposed to 5 cm H_2O for 4 days sloughed off very easily from the coverslips during the process of fixation or staining, compared to similarly manipulated monolayers exposed to 0.3 cm H_2O (control) for the same time period. Immunohistochemical analysis demonstrated that control HUVEc stained intensely for the integrin subunits $\alpha2$,$\alpha3$ and $\alpha5$, while cells exposed to hydrostatic pressure demonstrated a dramatic reduction in the expression of these integrin subunits. The reduction in the expression of integrins following exposure to hydrostatic pressure may contribute to the unstable monolayer structure and furthermore, demonstrates that exposure of HUVEc to hydrostatic pressure can influence cell adhesion to substrates.

6.22

CA^{2+} DEPENDENT AND GD^{3+} SENSITIVE MORPHOLOGICAL CHAGNES OF CUL TURED ENDOTHELIAL CELLS BY CYCLIC MECHANICAL STRETCH. Keij Naruse, Masumi Inoue, Takako Yamada and Masahiro Sokabe. Dept Physiol, Nagoya Uni Sch Med, Nagoya 466, Japan

Recently we have reported that applying a mechanical stretch pulse to cultured endothe lial cells induced a gadolinium (Gd^{3+}) sensitive Ca^{2+} transient that might be mediated b Ca^{2+} permeable stretch activated (SA) channels on cell membranes (Naruse & Sokabe, An J Physiol, 1993). In addition we have found that, upon an application of cyclic unidirec tional stretches to the cells, they became elongated and oriented perpendicularly to th stretch direction. The present study was designed to know the relationship between th stretch induced -Ca^{2+} transient and -morphological changes. Also we made a preliminar experiment on the cytoskeleton involvement in the stretch induced morphological changes

Human umbilical vein endothelial cells (HUVECs) cultured on an elastic silicon mem brane coated with fibronectin were subjected to unidirectional cyclic (1Hz) stretches. Fitee minutes after the onset of stretches, significant changes in the cell shape became could b observed; elongation and alignment of their longitude perpendicular to the stretch direction These morphological changes completed in several hours. When extracellular Ca^{2+} concen tration was reduced to less than 1μM, the morphological response was completely sup pressed, while 10 μM Ca^{2+} was enough to induce the shape change. Gd^{3+}, a potent blocke of SA channels, also completely blocked the morphological responses, suggesting that Ca^{2} transients mediated by a SA channel activation is essential. Associated with the shap change, an increase in a F-actin content and an alignment of stress fibers along the longi tude of the cells were observed. As the total content of actin was constant during stretches polymerization of G-actin might proceed with the morphological change.

To know the details of the course of the morphological change, the cells were precondi tioned by a three hrs cyclic stretches to elongate and align them in the same direction, the applied cyclic stretches perpendicularly to the direction. The cells began to shorten thei longitude and to elongate their shor axis. The short/long axis ratio turned to almost one i 30 min. and became elongated perpendicularly to the preconditioned original axis in a hour , suggesting that a depolymerization of F actins in an early phase followed by polymelization of G-actins occurred. Depletion of external Ca^{2+} or Gd^{3+} application com pletely inhibited the latter phase but not the early phase, suggesting that the stretch induce Ca^{2+} transient is essential to the polymelization process as well as the elongation and align ment of the cells.

CARDIOVASCULAR ADAPTATIONS TO MECHANICAL STIMULI: MYOCARDIAL CELLS

7.1

EXTRACELLULAR ATP MODULATES STRETCH-ACTIVATED CHANNELS IN CHICK HEART CELLS. Abdul M. Ruknudin, Hai Hu and Fred Sachs. Dept. of Biophysical Sciences, SUNY, Buffalo, NY 14214

Adenosine 5'-triphosphate (ATP) is released during hypoxia and ischemia in heart. We tested the effects of extracellular ATP on stretch-activated channels (SACs) of chick cardiomyocytes. Using cell-attached patches, the sensitivity of the SACs to stretch was measured by their NPO in a range of suction applied by hydrostatic pressure. We found that extracellular ATP increased sensitivity of the SACs to stretch in these cells. The pressure sensitivity curves shifted towards left showing higher sensitivity with increasing concentrations of Mg-ATP on the extracellular side of the cell. Using pipette diffusion technique, we measured the NPO in the same patch before and during exposure of SACs to 100 μM ATP. The higher the concentration, the more rapid was the onset of action consistent with a dose dependent mechanism. The activation of SACs were observed for a non-selective cation (50 pS) channel and for K-selective (100 pS) channel. The concentrations of ATP tested were between 1 and 100 μM and higher the concentration, the quicker was the activation. Cells treated prior with 4,4'-diisocyanatostilbene-2,2'-disulfonic acid (DIDS) or co-exposure of DIDS and ATP did not seem to affect the effects of ATP on SACs. Extracellular Ca^{2+} was not required for the ATP effect on SACs. Both ADP and AMP at 100 μM had similar effects on SACs but with reduced potency. ATPγS at 100 μM was able to enhance the activity of SACs; but required prolonged exposure indicating that phosphorylation of the SACs may be responsible for increase in sensitivity to stretch and for activation of SACs by these adenosine phosphate compounds.

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7.2

CARDIAC VENTRICULAR SEPTAL DEFECTS- ANALYSIS OF EFFECTS ON PRESSURE-VOLUME TRANSDUCTION, BY ALLOMETRIC ASSAY OF HEARTS IN INFANTS/CHILDREN. Richard P. Spencer. Univ. Connecticut Health Center, Farmington, CT 06030.

Cardiac ventricular septal defects (usually shunting of blood from left to right sides) produces pressure and volume changes in the developing hearts of infants and children. These effects are manifested by an increase in the weight of the heart walls. Prior assays have principally been by using heart/body weight ratios, which have not delineated the altered growth pattern as compared with normal children. We therefore analyzed results of Smolinsky et al (Isr. J. Med. Sci. 29:769, 1993), on 77 children, by employing 3 age divisions (0 to 1 year with 40 cases, 1^{+} to 9.3 years with 20 cases, and over 10 years of age with 17 cases). The allometric equation was analyzed in logarithmic form as: log H = K + p.log B, where H is heart weight and B is body weight (g). Correlation coefficients for the 3 groups were 0.76, 0.75 and 0.72 (all highly significant). Using data for normal groups, it was demonstrated that both the slope and intercept terms of the equation differed between affected children and normals. In addition, coefficients changed between age groups, being most different in the first year of life and then apparently showing adaptation to the pressure-volume effect. Allometric analysis of heart/body weights may also permit assay of effects of interventions.

7.3

CARDIAC RESPONSES TO CHRONIC ANGIOTENSIN II HYPERTENSION IN SYMPATHECTOMIZED DOGS. Harrison McDonald, Jr. * and L.N. Cothran. Howard University, College of Medicine, Washington, DC 20059

Previously, we demonstrated in conscious instrumented dogs (CID), that myocardial contractility is increased in association with chronic angiotensin II hypertension ($CA_{II}H$). To assess the role of the sympathetic nervous system in this regard, experiments were conducted in 5 dogs with ventricular and aortic pressure cells and catheters to measure left ventricular pressure (LVP) and aortic pressure (AP) 2 wks before sympathectomy (SNX) and 4 wks during $CA_{II}H$ and SNX. The 1st derivative of the LVP (dP/dt) was obtained by electronic differentiation and used as an index of contractility. 2-D echocardiograms (2DE) were recorded three times weekly throughout the study to access changes in left ventricular mass (LVM). During the control period, infusion of AII at a rate of 50 ng/kg/min was characterized by a 59% increase in AP ($p<0.01$) and a 14% decrease in maximal dP/dt ($p<0.01$) with no change in heart rate (HR). For the four wks following SNX with 6-hydroxydopamine and implantation of Alzet mini pumps to deliver the same dose of AII, the responses to AII were characterized by a 43% increase in AP ($p<0.01$) in association with a 34% increase in dP/dt ($p<0.05$) and a 18% reduction in HR ($p<0.05$). Selective blockade of V_1 and β_1 receptors did not alter this response. α_1 blockade during $CA_{II}H$ caused only a small but significant decrease in AP (10%, $p<0.05$). Selective blockade of the AII type 1 receptors (AT_1) with Losartan, restored both the AP and the dP/dt to control levels and also caused an additional 18% reduction in the heart rate ($p<0.01$). After 4 wks of $CA_{II}H$, the dogs were humanely killed, the hearts were removed and the actual LVM was compared to the LVM estimated for wk 4. Comparison of the actual LVM to the estimated control LVM revealed a 15% increase in LVM ($p<0.01$) occurred during the 4 weeks of $CA_{II}H$. These results indicate that in CID with SNX $CA_{II}H$ is supported primarily (90%) by the direct vasoconstrictor action of AII. Myocardial contractility is increased by the direct action of AII on cardiac AT_1 receptors. Losartan restores both of these variables to control levels while further reducing HR. LVM is markedly increased in this model of $CA_{II}H$.

7.4

MECHANICAL STRAIN STIMULATES EXTRACELLULAR MATRIX GENE EXPRESSION IN ADULT RAT CARDIAC FIBROBLASTS. A.A. Lee*, F.J. Villarreal*, A.D. McCulloch, W.H. Dillmann*, and J.W. Covell. Depts. of AMES-Bioengineering and Medicine, UC San Diego, La Jolla CA 92093

The cardiac extracellular matrix (ECM), predominantly composed of fibrillar collagens, may undergo significant remodeling during cardiac hypertrophy. We have previously shown that pressure-overload in the adult rat heart increases mRNA levels for fibronectin, collagen types I and III, and transforming growth factor-β_1. In this study, we examined the effects of mechanical stretch on cultured adult rat cardiac fibroblasts. Cells were plated onto a collagen-coated silicone membrane which was stretched 10% along the length of a rectangular culture chamber. Cell deformation was quantified by two-dimensional (2D) homogeneous finite strain analysis. The 2D mechanical strain components, E_{11}, E_{12}, and E_{22}, were determined by measurements of the displacements of latex microspheres attached to the cell surface (axis 1 in stretch direction; axis 2, perpendicular). Cell strain components were uniform for most of the culture system except for cells located near the clamped edges of the substrate. In the center of the culture chamber, E_{11} = 0.11 and E_{22} = -0.05, with negligible shear (E_{12}). Near a clamped edge, E_{11} = 0.12, E_{12} = -0.08, and E_{22} = -0.01. Total RNA was extracted from adult rat cardiac fibroblasts which were unstretched or stretched at 10% for 24 h. Levels of mRNA for collagens I and III increased 2- and 3-fold, respectively, in stretched cells compared with control. Fibronectin mRNA levels increased 5-fold under stretch. Conclusions: (1) In a uniaxial stretch apparatus, mechanical strains are 2D, mostly uniform, and include both tensile and compressive strain components; (2) the application of these 2D strains increased ECM mRNA levels in adult rat cardiac fibroblasts. These findings suggest that mechanical stimulation may play an important role in the process of ECM remodeling in the heart. Supported by NIH and NSF.

7.5

Use of Differential Display Technology in the Identification of Upregulated Transcripts During Acute Right Ventricular Overload.

Gregg T. Schuyler, Allen S. Ridenour, Donald R. Menick, John D. Rozich, Terrence X. O'Brien, George Cooper IV, Medical University of South Carolina, Gazes Cardiac Research Institute, and VA Medical Center, Charleston, SC.

Acute pressure overload has been shown to induce transient changes in gene expression of several proto-oncogenes. With time, this increase in load will result in cardiac hypertrophy. Acute right ventricular pressure overload (RVPO) was induced in a feline model by inflating a balloon in the pulmonary outflow tract. This techniques allows the LV to remain hemodynamically unaffected and serve as a same animal control. Northern analysis of *c-fos* mRNA levels with this model have reproducibly shown a significant increase in the RV and undetectable levels in the LV. A differential display technique was optimized for the detection of rare messages in cardiac tissue in response to acute RVPO. Total RNA extracted from the RV and LV free wall of a 1 hr. RVPO cat was reverse transcribed, amplified by PCR utilizing random 5' primers and 3' oligo-dT primers with serial degenerate bases. Sequences were separated on PAGE, and analyzed for differences in RV vs. LV and cDNA fragments which were increased after RVPO were extracted from the gel and cloned into a suitable vector. Sequence analysis of these partial transcripts identified phosphofructokinase, transcripts with sequence identity to mitochondrial sequences, and some unknown sequences. These clones are actively being characterized to determine their role in the adaptive response of the heart to load.

7.6

Neonatal Rat Cardiocytes Subjected to *In Vitro* Load Stress Acutely Upregulate the Mitochondrial F_1-ATPase β-Subunit.

Terrence X. O'Brien, Donald R. Menick, Mary S. Rackley, Paul J. McDermott, George Cooper IV; Med.Univ.of SC & VAMC, Gazes Institute, Charleston, SC

The link of early transcriptional and/or post-transcriptional events to the cardiac myocyte response to load stress are incompletely understood. Since increased ATP production must occur rapidly, the nuclear-encoded β-subunit of the mitochondrial F_1-ATP synthase (β-ATPase) was examined in two *in vitro* models using neonatal rat ventricular cardiocytes stimulated with either synchronous electrical pacing at 3 Hz or phenylephrine (PE). β-ATPase mRNA levels upregulated rapidly, starting at 1^0 and reaching equilibrium between 12-18^0 with PE and beginning at 2-4^0 with peaking at 24-48^0 with electrical pacing. Also, inhibition of spontaneous contraction with either verapamil or KCl reduced levels of β-ATPase mRNA within hours of application. To explore promoter element response activity of the β-ATPase gene in cardiocytes, PCR primers designed from human 5' flanking sequence were used to isolate a fragment -392 bp upstream of transcription initiation that included several elements previously described in non-cardiac cells as contributing to constitutive activity. This was subcloned into a luciferase reporter vector in both orientations and transiently co-transfected using the calcium phosphate precipitation method, along with a CMV/β-galactosidase construct, into neonatal rat cardiocytes subsequently stimulated with PE or electrical pacing. Reporter luciferase activity showed this promoter fragment active in cardiocytes but not inducible by PE or electrical pacing. Therefore, in the acute cardiocyte response of the β-ATPase gene, there is demonstrated constitutive promoter activity independent of inducible activity. Also, a method is established to determine whether other elements may be responsible for acute cardiac β-ATPase mRNA upregulation in response to *in vitro* models of load stress.

7.7

THE RAPID UPREGULATION OF NUCLEAR AND MITOCHONDRIAL ENCODED SUBUNITS OF THE F_1 ATPase AND CYTOCHROME OXIDASE COMPLEXES IN RESPONSE TO CARDIAC LOAD.

Donald R. Menick, Terrence X. O'Brien, Diane E. McDermott, John D. Rozich, Gregg T. Schuyler, Paul J. McDermott, George Cooper IV. Medical University of SC, Gazes Cardiac Research Institute, and VAMC, Charleston, SC 29425

Differential hybridization was utilized to identify transcripts upregulated during the early induction of cardiac hypertrophic growth. One clone which was greatly upregulated corresponded to the mitochondrial encoded F_1 ATPase subunit Fo_6. Mitochondrial encoded cytochrome *b* and cytochrome oxidase subunit II were also upregulated at one and four hours of pressure overload as would be expected from the polycistronic nature of mitochondrial transcription. Transcripts for nuclear encoded subunits of the F_1 ATPase and cytochrome oxidase were probed to assess if the acute responses observed for the mitochondrial encoded subunits were also true for the nuclear encoded subunits. The nuclear encoded α and β subunits of the F_1 ATPase and cytochrome oxidase subunit IV were all dramatically upregulated at one and four hours of pressure overload. In addition, the upregulation of the Fo_6 transcirpt was examined in isolated neonatal rat ventricular cardiocytes stimulated with either phenylephrine (PE) or electrical pacing. Fo_6 is upregulated 5-10 fold within 15 minutes of PE stimulation and 4 hours of electrical stimulation. This upregulation persists for at least 18 hours with electrical stimulation. This is the first report in a higher eukaryote of the rapid coordinate upregulation of nuclear and mitochondrial transcripts for genes encoding oxidative phosphorylation complexes by a physiologic stimuli.

7.8

EFFECTS OF PRESSURE OVERLOAD HYPERTROPHY ON PASSIVE STIFFNESS AND VISCOUS DAMPING IN THE ISOLATED CARDIOCYTE.

Michael R. Zile, John M. Buckley, Ken E. Richardson, George Cooper, IV. Medical University of SC, Veterans Administration Medical Center, Gazes Cardiac Research Institute, Charleston, SC 29425.

One potential mechanism causing diastolic dysfunction during myocardial hypertrophy is an increase in the stiffness of the cardiocyte itself. We examined 2 components of cardiocyte stiffness: passive spring (K_{ps}) constant and viscous damping (C_{vd}) constant. Cardiocytes from normal cats (control) and cats with right ventricular hypertrophy (RVH), induced by pulmonary artery banding, were embedded in a 2% agarose gel. Cardiocytes were then subjected to a stepwise change in force (stress,σ) and the resultant changes in cell length (strain, ε) were measured. Changes in K_{ps} were determined by examining the slope and position of the σ–ε relation during an increase in σ applied at a constant rate. Changes in C_{vd} were determined by measuring the area between the σ-ε relation obtained during increase and decrease in force. This loop area reflects the amount of mechanical energy converted to heat energy by damping. The slope and intercept of the σ vs ε relation obtained during an increase in σ were similar in hypertrophied and control cardiocytes ($y=^-441 + 1.1e + 05x$, $r=0.99$ for control and $y=^-678 + 1.2e + 05x$ $r=0.99$ for hypertrophied cardiocytes). However, the loop area between the σ-ε relation obtained during an increase and decrease in force was significantly greater in hypertrophied (102±3) compared to control (83±4) cardiocytes ($p<0.05$). Thus, hypertrophy did not alter the spring constant but did increase the damping constant. We hypothesized that this increase in C_{vd} was caused by an increase in microtubule density. When microtubule density was decreased by treating hypertrophied cardiocytes with colchicine, the loop area (C_{vd}) decreased to normal. Thus, the increased damping in hypertrophied cardiocytes was caused, at least in part, by an increase in cardiocyte microtubules.

7.9

GROWTH EFFECTS OF ELECTRICALLY STIMULATED CONTRACTION ON ADULT FELINE CARDIOCYTES IN PRIMARY CULTURE.

Paul J. McDermott, Satoshi Kato, Charles T. Ivester, George Cooper, IV and Michael R. Zile. Medical University of SC & VA Med Center, Gazes Institute, Charleston, SC 29401

Electrically stimulated contraction of adult feline cardiocytes in primary culture acutely accelerates protein synthesis rates. The purpose of the present study was to determine the effects of long-term electrical stimulation of cardiocyte contraction on protein synthesis rates and total protein content over a 7 day time period. Adult feline cardiocytes were plated on laminin coated culture trays and maintained in a serum-free medium consisting of M199 supplemented with ascorbate, bovine serum albumin, creatine, carnitine, taurine, and 10^{-7} recombinant insulin. Cardiocytes were electrically stimulated to contract using continuous electrical pulses of alternating polarity at a frequency of 1 Hz and pulse duration of 5 msec. Non-stimulated cardiocytes are normally quiescent and were used as the control group. Protein synthesis rates were measured as the rate of incorporation of [^{3}H]-phenylalanine into total cell protein (nmol PHE/g protein/hr). Protein synthesis rate decreased by 14% in the controls between days 1 and 4 in culture and then remained stable up till day 7. In electrically stimulated cardiocytes, protein synthesis rates were significantly increased relative to same day controls by 18% and 43% on days 4 and 7, respectively ($p<0.05$). Protein content/cell was determined by measuring total fluorescence/cell using confocal microscopy of fluorescein isothiocyanate stained cells. Electrical stimulation significantly increased protein /cell which reached 52% after 7 days as compared to controls ($p<0.05$). In both control and electrically stimulated groups, cardiocytes remained rod-shaped and responsive to electrical stimulation over the 7 day period. We conclude that electrically stimulated contraction of adult cardiocytes resulted in cell growth as assessed by an increase in protein content/cell over 7 days in culture. This increase was due, at least in part, to an acceleration of steady state protein synthesis rates.

7.10

AUTOREGULATION OF TUBULIN mRNA EXPRESSION AND ORGANIZATION OF THE MICROTUBULE CYTOSKELETON IN ISCHEMIC AND DILATED CARDIOMYOPATHY.

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Monomeric tubulin is expressed in human cardiocytes and polymerizes to form microtubules (MT). High levels of unpolymerized tubulin protein in cell culture is known to diminish tubulin mRNA stability in an autoregulatory fashion. We have therefore analyzed the expression of tubulin mRNA and assembly of tubulin protein from explanted human left (LV) and right (RV) ventricles from patients with ischemic cardiomyopathy (ICM), idiopathic dilated cardiomyopathy (IDC), the cardiomyopathic hamster (CMH), and normal controls. Tubulin protein was separated into MT and unpolymerized fractions by centrifugation. Western Blot analysis shows tubulin exists almost exclusively in the unpolymerized form in human ischemic LV, and in the LV and RV from the cardiomyopathic hamster. Controls showed an even distribution MT and unpolymerized forms of tubulin. Northern blot analysis of tubulin mRNA showed markedly diminished levels of tubulin transcript in human ischemic LV compared to RV. These studies demonstrate that in cardiocytes of ICM and the CMH, tubulin is disorganized, and associated with a reduced cellular content of its own mRNA. The effect of microtubular integrity on contractile function was analyzed by measuring tension after exposure of LV and RV trabeculae to isoproterenol (ISO). Colchicine (10 μM) increased the tension response to ISO only when MT were intact. These studies demonstrate that loss of MT organization and decreased tubulin mRNA stability may contribute to cardiocyte function in heart failure.

7.11

NITRIC OXIDE RELEASED FROM CAROTID SINUS SENSORY NERVES INHIBITS ACTIVITY OF BARORECEPTOR A-FIBERS.
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Baroreceptors (BR) in carotid sinuses (CS) are activated by increased arterial pressure and trigger reflex responses that oppose the rise in pressure. Nitric oxide (NO) synthase is present in CS nerves and we have shown previously that NO inhibits BR activity. In this study we tested the hypothesis that NO released from sensory endings modulates the activity of BR A-fibers. Single-fiber activity was measured from the vascularly-isolated CS in anesthetized rabbits. Capsaicin (1 μg/ml) was injected into the CS to selectively activate C-fibers and release NO. In intact CS, capsaicin decreased maximum BR activity measured at >160 mmHg from 85±7 to 55±14 spikes/s (n=5, P<0.05). After removal of endothelium with saponin perfusion, capsaicin still decreased activity from 60±5 to 43±8 spikes/s (n=5, P<0.05). Treatment of CS with hemoglobin (10 μM, n=5) to scavenge NO abolished the inhibition of BR activity by capsaicin (56±4 and 55±4 spikes/s before and after capsaicin). The persistence of the inhibitory response to capsaicin after removal of endothelium and the absence of the response after hemoglobin suggest that the inhibition of activity was mediated by NO released from sensory nerves. We speculate that NO may act in a negative feedback manner to reduce BR activity following excessive activation of C-fiber sensory afferents. The results suggest a role of NO in modulating BR mechano-electrical transduction.

7.12

PROTEIN KINASE C-EPSILON TRANSLOCATES IN RESPONSE TO BOTH ANGIOTENSIN II AND SWIMMING IN THE ADULT RAT HEART
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Recent studies suggest that hormones such as angiotensin II (ANG II), epinephrine and norepinephrine play a prominent role in cardiac hypertrophy but initiate intracellular signaling and gene expression by different receptor-mediated mechanisms. Specific isoforms of protein kinase C (PKC) play a putative role as second messengers in regulating gene transcription and muscle contraction but studies have been limited to neonatal cardiocytes or isolated perfused hearts. To determine whether PKC isoforms respond to elevated levels of circulating hormones in the intact heart, adult female Wistar rats were infused with ANG II (200 ng/min;i.v.;30 min), or swam (SW) for 30 min. Heart homogenates were purified on sepharose columns and subjected to gel electrophoresis and immunoblotting with the antibody for PKC-ε, the predominant adult rat cardiac isoform, and PKC-α. The percent of cytosolic fraction and membrane fraction relative to total PKC-ε, translocated with ANG II (33 and 67%) and with SW (23 and 77%), compared to control (58 and 42%). The amount of PKC-α in the cytosolic and membrane fraction was unchanged with ANG II and SW and was similar to control levels. Thus, two stimuli which induce hypertrophy through different receptor-mediated mechanisms may share a common pathway in regulating intracellular events.

7.13

HYPOTONIC STRETCH INCREASES THE SLOW COMPONENT OF THE DELAYED RECTIFIER POTASSIUM CURRENT IN GUINEA PIG VENTRICULAR MYOCYTES.
James G. Maylie* and William J. Groh*. Oregon Health Sciences University, Portland, OR. 97201

The effect of hypotonic stretch on the slow component (I_{Ks}) of the delayed rectifier potassium current (I_K) was studied in guinea pig ventricular myocytes with a whole cell patch clamp technique. Cells were superfused with normal Tyrode solution (296 mOsmol/l) at 23 °C and dialyzed with a patch pipette solution (mM): K-Asp 90, KCl 40, NaCl 10, K_2Phosphocreatine 10, $MgCl_2$ 1, MgATP 5, EGTA 10, $CaCl_2$ 1, HEPES 10, pH 7.1. Exposure of myocytes in the whole cell recording configuration to hypotonic solution (228 mOsmol/l; Tyrode less 35 mM NaCl) induced measurable swelling within 5 min; cell length and width increased 9±2% and 27±3%, respectively (mean±SEM, n=3). Within minutes after hypotonic stretch I_K measured during 5 s depolarization to 60 mV and the tail current (I_{tail}) on repolarization to -40 mV increased by 52±14%, p=0.01 and 31±8%, p=0.02, respectively (n=7). Similar experiments performed with 10 μM E-4031 to block the rapid component of I_K (I_{Kr}) showed hypotonic modulation of I_K and I_{tail} of 36±11% and 63±11%, respectively (n=6). Owing to the rectification of I_{Kr}, these results suggest that I_{Ks} is modulated by hypotonic stretch. Hypotonic modulation of I_{Ks} was not affected by pretreatment of myocytes for 2 hours with 10 μM cytochalasin D but was reduced by pretreatment with 10 μM phalloidin. We conclude that hypotonic stretch increases I_{Ks} and this modulation is reduced by actin stabilization with phalloidin. Stretch modulation of I_{Ks} would affect the cardiac refractory period and may thus influence the vulnerability to reentrant ventricular tachyarrhythmias.

7.14

CONTRACTILE ACTIVITY MODULATES EXPRESSION OF THE POLYMERASE I-ASSOCIATED RIBOSOMAL TRANSCRIPTION FACTOR, UBF, IN CARDIOMYOCYTE CULTURES.
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Spontaneously contracting neonatal cardiomyocytes in culture exhibit considerable hypertrophic growth relative to arrested cardiomyocytes. This growth has been shown to be fascilitated by an increased ribosome content resulting from an accelerated rate of ribosomal DNA (rDNA) transcription. One goal of our laboratory is to better define the mechanisms which link spontaneous contractile activity to increased rDNA transcription in the nucleolus. As a first step we have examined the expression of the Polymerase I-associated rDNA transcription factor, UBF. We show here that when arrested cardiomyocytes are allowed to resume contraction by reduction of the KCl concentration in the media from 50 to 5 mM, significant increases in rDNA transcription and protein accumulation occur within 24 and 48 h respectively. These changes are preceded by increased levels (4-5 fold) of UBF protein as assessed by Western analysis. Increased UBF levels are first observed 6-12 h following initiation of contractile activity and are maximal after 24 h. The increase in UBF protein in response to contractile activity is partially regulated at the pretranslational level since UBF mRNA content increases in a quantitatively and temporally similar manner. We hypothesize that UBF may be an important factor in the regulation of the accelerated rDNA transcription during neonatal cardiomyocyte hypertrophy. We are currently examining the effect of over expression of UBF on rDNA transcription in cultured neonatal cardiomyocytes.

10.1

MECHANOTRANSDUCTION ACROSS THE UROKINASE RECEPTOR.
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Past studies have focused on the enzymatic role of urokinase receptor in cell invasion. We hypothesized that the urokinase receptor may also play a mechanical role in cell migration and thus control cell invasion by modulating the cytoskeleton. Using magnetic twisting cytometry with urokinase-coated ferromagnetic beads, we applied mechanical stresses directly to the urokinase receptor or other receptors on the surface of human myogenic cells in culture. For applied stress of 40 dynes/cm^2, the stiffness measured through the urokinase receptor was 27±3 dynes/cm^2, whereas the stiffness measured through other glycosyl phosphatidylinositol (GPI) linked proteins such as Thy-1 or alkaline phosphatase was only 5±1 dynes/cm^2; the stiffness measured through the integrins was 54±5 dynes/cm^2. In addition, the stiffening response (increase in stiffness with stress) resembled those of integrins, which are linked mechanically to the cytoskeleton. Furthermore, stiffness decreased by 70% with disruption of actin microfilaments. These results demonstrate that the urokinase receptor is coupled mechanically to the cytoskeleton. Inhibition of the tripartite complex formation (urokinase receptor, urokinase, and its inhibitor) with antibodies, especially with antibodies against type-1 plasminogen inhibitor (PAI_1), led to a 2-fold increase in cytoskeletal stiffness and a dramatic decrease in cell motility. This result cannot be explained by the conventional enzymatic function of the urokinase system; rather, it is consistent with the notion that formation of the complex facilitates cell movement independent of plasminogen activation.

10.2

MECHANICAL REGULATION OF INTRACELLULAR Ca^{2+} IN ENDOTHELIAL CELLS
Joseph F. Minore* and W. Charles O'Neill, Emory University, Atlanta, Ga. 30322

Mechanical stress, specifically shear stress and direct membrane deformation, raise intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in endothelial cells (EC). The underlying mechanism is unknown, due in part to the difficulty in studying cells under these conditions and in quantitating and reproducing the stresses. We have found that hypotonic cell swelling is an additional stress that increases $[Ca^{2+}]_i$ in EC and may serve as a useful model of mechanical regulation of $[Ca^{2+}]_i$. To determine the mechanism of this rise in $[Ca^{2+}]_i$, bovine aortic EC were grown to confluence, loaded with fura-2, trypsinized into suspension, and exposed to isotonic (osm = 290) and hypotonic (osm = 220) solutions. $[Ca^{2+}]_i$ increased 49 ± 3 nM (mean ± SEM; n = 21) in swollen cells, and a similar rise was seen in monolayers. Increased $[Ca^{2+}]_i$ was apparent at 6% swelling, with progressively greater increases with further swelling, and was partially reversible. The swelling-induced $[Ca^{2+}]_i$ increase was abolished when external Ca^{2+} (Ca^{2+}_o) was removed, but was not inhibited by 10 uM La^{3+}, 20 uM Gd^{3+}, or by depolarizing the cell membrane by increasing $[K^+]_o$ to 122 mM. The agonist ATP produced a typical biphasic rise in $[Ca^{2+}]_i$ consisting of an initial peak due to internal Ca^{2+} release and a prolonged phase due to influx. The latter phase, but not the initial peak, was reduced by La^{3+}, cell depolarization, or removal of Ca^{2+}_o. We conclude that the swelling-induced rise in $[Ca^{2+}]_i$ in aortic EC is not mediated by typical Ca^{2+} channels or stretch-activated channels. Swelling may activate a nonelectrogenic influx pathway or may release Ca^{2+} from an internal store that is in rapid equilibrium with Ca^{2+}_o.

10.3

VOLUME DEPENDENT PHOSPHORYLATION OF MYOSIN LIGHT CHAIN IN AORTIC ENDOTHELIAL CELLS Janet D. Klein* and W. Charles O'Neill, Emory University, Atlanta, GA 30322

The mechanism by which cells sense their volume and activate volume-regulatory transporters is unknown. Our previous studies suggest that a volume-sensitive protein kinase activates Na-K-2Cl cotransport in shrunken endothelial cells. We examined the effect of cell volume changes on incorporation of [^{32}P]-orthophosphate into cellular proteins and have found that cell shrinkage induces rapid and reversible phosphorylation of a 19 kDa protein identified as myosin light chain (MLC) by immunoprecipitation with anti-myosin heavy chain and western blotting with anti-MLC. Peptide mapping showed one phosphopeptide, consistent with phosphorylation by MLC kinase (MLCK). Shrinkage also increased MLC phosphorylation in astrocytes and vascular smooth muscle cells. The time course of endothelial MLC phosphorylation closely matched that of Na-K-2Cl cotransport activation. ML-7, an inhibitor of MLCK, inhibited shrinkage-induced, but not basal, MLC phosphorylation and cotransport activity. Activation of protein kinase C with phorbol ester did not alter phosphorylation. Cytochalasin D, which causes cell retraction without altering cell volume also did not alter MLC phosphorylation. Cell shrinkage did not alter the [Ca_i]. These results suggest that MLCK is regulated by cell volume in a Ca-independent manner and may represent an important link between the cytoskeleton and volume-regulatory responses.

10.4

MECHANICAL LOAD ALTERS EXPRESSION OF THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR mRNA EXPRESSION IN HUMAN SMOOTH MUSCLE CELLS. J. Yamamoto*, E. Chikamatsu*, T. Fischer*, T. Iwasaki*, T. Lawrence*, M. Tsuzaki* and A.J. Banes. Dept. of Surgery, University of North Carolina, Chapel Hill, NC 27599-7050.

Inositol 1,4,5-trisphosphate (IP3) constitutes a major intracellular second messenger that transduces many growth factors and neurotransmitter signals. IP3 causes the release of [Ca^{2+}]ic from intracellular stores by binding to specific receptors that are coupled to Ca^{2+} channels. The release of [Ca^{2+}]ic from intracellular stores is a prerequisite to smooth muscle (SMC) contraction. Mechanical stimulation of target SMC by poking with a micropipet or stretching cells would release [Ca^{2+}]ic, an IP3-dependent phenomenon. Blocking the IP3 receptor with heparin blocked the load response. This study was designed to assess effects of mechanical load on expression of the IP3 receptor. **METHODS:** Oligonucleotide primers were designed to amplify a 470 bp sequence of the type 2 receptor sequence. Human SMC were isolated by collagenase digestion after removal of endothelial cells from umbilical cords. Cells were cultured in M199 with 10% fetal calf serum then subcultured to collagen bonded Flex I, flexible bottomed cutlure plates. Cells were grown to quiescence and used at day 6. Cyclic mechanical load was applied to SMC using a Flexercell Strain unit at 1 hz 0.2 strain (20% elongation) for 0, .1, .5, 1, 2, 2.5, 5, 10, 20, and 40 k cycles. cDNA was obtained through RT-PCR. The cDNA was cloned and sequenced. Total RNA was isolated. IP3 receptor mRNA expression was estimated by performing RT-PCR dilution experiments and load quantification by image analysis. **RESULTS:** The cloned cDNA human sequence showed a high degree of homology for the rat type 2 IP3 receptor sequence. Serum stimulation of quiescent SMC induced IP3 receptor message in a biphasic mode with peaks at 2 and 20 h. Mechanical load increased the IP3 receptor mRNA expression. Message expression was of low abundance. **CONCLUSION:** This is the first report concerning mechanical load stimulation of human type 2 IP3 receptor mRNA expression. Both serum and mechanical load induced IP3 receptor expression. Increased IP3 receptor expression may be related to SMC hypertrophy in response to load. Upregulation of receptor expression may regulate and amplify the cell's ability to detect and respond to load. American Heart Association.

10.5

MECHANICAL LOAD UPREGULATES CONNEXIN-43 EXPRESSION IN MC-373 OSTEOBLASTS. A. Keen*, P. Hu*, W.T. Lawrence*, and A.J. Banes. Dept. of Surgery, University of North Carolina, Chapel Hill, NC, 27599-7050.

Connexin-43 (CXN) is a 43kD protein monomer that is assembled into hexameric hemichannels at the plasma membrane into connexon structures. Register of two hemichannels results in formation of a gap junction (GJ) through which ions, including inositol phosphate (IP3) pass. GJ are essential for direct intercellular communication of chemical signals. We hypothesized that mechanical load might upregulate CNX expression to better communicate load signals among bone cells. **METHODS:** FURA-2-loaded MC-3T3 cells were challenged with an indentation of the plasma membrane with a micropipet to elicit release of intracellular calcium. In other experiments, MC-3T3 cells were plated at near confluency (50K cells/cm^2) in complete medium, allowed to attach to collagen bonded, rubbber bottomed Flex I plates for 24h, then were subjected to a maximum 10% elongation (average 4%) at 1 hz for 8 h, not loaded for 16h, then collected at 24h for quantitation of CXN-43 mRNA and protein. mRNA was measured by semi-quantitative PCR. cDNA band strength was normalized to the β actin signal in the same sample. CXN protein expression was semi-quantitated by PAGE and Western analysis by a chemiluminescence technique. **RESULTS:** CXN-43 mRNA expression did not increase substantially in the first 24 h post-load compared to the actin standard, whereas CXN protein expression increased 1.64 fold in loaded cells compared to nonloaded counterparts. A load-stimulated increase in CXN-43 protein could be observed as early as 4 h post-load and rose through 8,12,16 and 20 h. CXN-43 phosphorylation was also increased 1.4 fold in loaded cells. Expression was normalized by day 3 between load and no load groups and was similar and declined on days 5 and 7. **CONCLUSIONS:** MC-3T3 cells responded to a perturbation in their plasma membranes by releasing [Ca2+]ic and contained functional GJ as evidenced by their ability to propagate the calcium wave. Cyclic mechanical load increased CXN-43 protein expression and phosphorylation but not mRNA in osteoblasts. Increased CXN-43 protein may be a result of an increased rate of transcription, message stability or protein stability. Increased CXN phophorylation may be related to load alteration of the open-closed state of the channel. Bone cells may require a greater ability to communicate under mechanical load conditions. NIH-AR38121.

10.6

CYCLIN D1 EXPRESSION IN hSMC IS STIMULATED BY CYCLIC MECHANICAL LOAD IN A DOSE-DEPENDENT MANNER. E. Chikamatsu*, Y. Nimura*, J. Yamamoto*, P. Hu*, T. Fischer*, T. Lawrence*, M. Tsuzaki*, T. Brown and A.J. Banes. University of North Carolina, Dept. of Surgery, Chapel Hill, NC 27599-7050.

The regulation of growth control of smooth muscle cells (SMC) is critical in the treatment of vascular disease or loss of vascular patency after angioplasty. Growth factors are known to stimulate DNA synthesis in quiescent SMC. However, mechanical factors can also activate cells to divide. To understand the mechanism by which mechanical load may regulate DNA synthesis, we have measured message and protein expression of a key cell division control protein, CYCLIN D1, in quiescent SMC in response to increasing cycles of a load stimulus. **Methods:** Human SMC were obtained from umbilical cords post-delivery, isolated by collagenase digestion after removal of endothelial cells and cultured in M199 with 10% fetal calf serum. Cells were subcultured at 25k cells/cm2 to collagen bonded Flex I culture plates, grown to quiescence and used on day 6. A Flexercell Strain Unit was used to deliver a 1 hz 0.2 strain (20% elongation) stimulus for 0, .1, .5, 1, 2.5, 5, 10, 20, 40k cycles. All samples were collected at 12 h from time zero, the maximal cyclin D1 expression time. Cultures treated with M199 or serum were negative and positive controls respectively. Cyclin D1 mRNA was assayed by RT-PCR to amplify a 570 bp cDNA sequence. Cyclin D protein was identified using a cyclin D specific antibody in a Western blot. Image analysis was used to quantify bands from Southern gel and Western blots. **Results:** Quiescent hSMC expressed basal detectable cyclin message and protein. Cell cycle analysis indicated cells were in the G0 state and could be stimulated to enter S phase at 18 h with serum. Kinetics of cyclin message and protein expression in serum-treated cells indicated a steady increase that peaked at 12 and 18h respectively. Mechanical load stimulated cyclin expression with a threshold beginning between 500 and 1000 cycles (8.3 and 16.7 min) with a florid rise through 40k cycles (11.1h). Results of dilution experiments indicated that message increased 4 fold and protein increased 4-8 fold in loaded cells, suggesting that the rise in protein was due to transcriptional activation. **Conclusion:** Cyclin D1 expression is upregulated by mechanical load in a dose-dependent manner. Kinetics of expression are similar to those of serum stimulation. Mechanical load may act in conjunction with other stimulatory factors to stimulate smooth muscle cell DNA synthesis and division. NIH AR38121, Hunt Foundation.

10.7

CHARACTERIZING WHOLE-CELL MECHANOSENSITIVE CURRENTS IN CHICK HEART. Hai Hu* and Frederick Sachs*, Biophysical Sciences, SUNY-Buffalo, Buffalo, NY 14214

Whole-cell mechanosensitive currents (MSC) can be recorded from embryonic chick heart cells, using perforated patches and a second micropipette to press on the cells (Hu and Sachs, Bioph. J., 66:A170). The current is completely blocked by 100μM Gd^{3+} (Figure 1, A: 1.67μm step-displacements of the stimulation pipette; B: current before Gd^{3+}; C: current after Gd^{3+}; inward current downward, holding potential -59mV). The current is reproducible as shown in Figure 1 (B). Sometimes we see adaptation of the current, i.e., the current decays with time during one mechanical stimulus reaching a finite steady state value. Some preparations do not have whole-cell MSC and in these preparations single channel recordings also do not exhibit MS channel activity.

The current seems to be cation selective. Figure 2 shows MSC at -40mV (B) and +20mV (C) with extracellular Cl^- substituted with isethionate. Trace (A) represents the displacement of the stimulation pipette, with steps 2.5μm for (B) and 1.67μm for (C). At positive potentials the currents are outward, probably carried by K^+. The reversal potential of MSC in normal saline is -16.4±1.3mV (n=5) and changes to -14.0±3.3mV (n=3) with Cl^- substitution. These data suggest that Cl^- does not contribute to MSC. When the bath solution contains high K^+ substituted for Na^+, MSC is greatly increased (Figure 3, Na^+ data not shown). This suggests that MS channels are more permeable to K^+ than Na^+. Ca^{2+} is not necessary to observe MSC. Our results suggest that the whole-cell MSC is the macro-counterpart of MS channels revealed in single channel studies.

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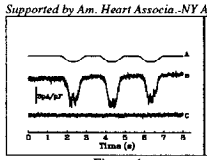
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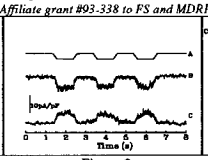
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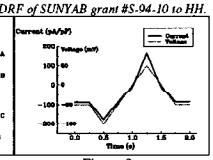


Figure 3

10.8

ROLE OF G-PROTEINS IN MECHANICAL SIGNAL TRANSDUCTION. Siva R.P. Gudi, Nan-Q. Li and John A. Frangos. Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802

Cells have highly specialized force sensing mechanisms such as those involved in hearing and touch which can convert a mechanical stimulus into electrical and biochemical responses. Fluid flow itself is another such stimulus that exists in the cellular environment. Indirect evidence suggests that flow regulation of vascular tone via the endothelium and smooth muscle cells involves mechanical signal transduction via G-proteins. Human Umbilical Vein Endothelial Cells (hUVECs) grown on glass slides were subjected to fluid flow in parallel plate flow chambers. Fluid shear-induced GTP binding in hUVECs was assayed by preincubating the cells with a GTP photoreactive analogue, 8-azido GTP (α-^{32}P) and subjecting to UV crosslinking after fluid flow (10 dynes/cm^2) for 10 sec. Autoradiographic analysis of these cells lysate show a 42 kDa protein being labeled by GTP upon flow stimulation. Further this protein was identified with anti G_α antibodies and by immunoprecipitation. These experiments suggest that G_α subunits $G_{\alpha q}$ and $G_{\alpha i3}$ may be involved in the flow induced signal transduction pathway in endothelial cells. In other supporting experiments, G-proteins purified from bovine brain were reconstituted into PE:PS liposomes and subjected to fluid shear. These was an increase in G-protein mediated GTP hydrolysis upon the onset of shear. These experiments suggest the involvement of G-proteins as mechanochemical signal transducers.

This work is supported by HL40696-07.

10.9

MECHANICAL STRAIN IS SENSED BY VSM CELLS THROUGH AN INTERACTION WITH EXTRACELLULAR MATRIX PROTEINS. Emily Wilson, Krishnankutty Sudhir and Harlan E. Ives. Div. of Nephrology and the Cardiovascular Research Institute, University of California, San Francisco.

Cyclic mechanical strain (1 Hz) increases DNA synthesis in vascular smooth muscle (VSM) cells via the autocrine production and release of PDGF into the media. Cells plated on collagen, fibronectin or vitronectin showed 5-10 fold increases in DNA synthesis in responses to mechanical strain: cells on laminin or elastin did not respond. To further evaluate the role of the ECM-integrin system, we have utilized an RGD containing peptide GRGDTP and an inactive control peptide GRGESP. RGD peptide completely blocked the strain-induced increase in DNA synthesis; RGE peptide had no effect. RGD could theorctically prevent growth factor secretion or growth factor response. RGD did not alter the mitogenic response to either PDGF or α-thrombin. However, RGD eliminated the secretion of growth factors (mitogenicity of conditioned media) following mechanical strain. RGD also reduced by 50% the strain-induced expression of a PDGF-A promoter (890 bp)-CAT construct that was transiently transfected into VSM cells. RGE did not alter the activity. Thus interaction with matrix proteins is essential for induction and secretion of growth factors in response to mechanical strain.

10.10

MECHANICAL STRAIN INCREASES mRNA FOR PLATELET-DERIVED GROWTH FACTOR (PDGF) -B CHAIN IN VASCULAR SMOOTH MUSCLE CELLS. Yunn-Hwa Ma and Harlan E. Ives. Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

Previous studies from our laboratory indicate that cyclic mechanical strain induces proliferation of vascular smooth muscle (VSM) cells. This is associated with increased secretion of PDGF- A and B chains into the medium. The goal of the present study was to determine whether mechanical strain alters PDGF-B chain mRNA levels. Neonatal rat VSM cells grown on silicon elastomer plates coated with collagen I were subjected to cyclic strain (60 cycle/min). Steady state levels of PDGF-B mRNA were determined by Northern blot analysis and normalized using mRNA for β2-microglobulin on the same blots. The size of PDGF-B mRNA was approximately 3.5 to 4 kb. The level of PDGF-B mRNA started to increase after 6 hr of exposure to mechanical strain and peaked at 24 hr with an increase of 1.7 to 4.4 fold (n=3) over basal level. Thymidine incorporation of the same batch of cells increased by 2.8 to 3.6 fold after 24 hr exposure to mechanical strain. After 48 hr of strain, the expression level of PDGF-B mRNA and thymidine incorporation was 1.3 to 5 and 1.9 to 2.2 fold of control, respectively. These results suggest that increased secretion of PDGF-B from VSM cells in response to mechanical stain is due, at least in part, to increased message expression. Further studies are aimed at determining whether PDGF-B mRNA is increased at the level of transcription.

10.11

ACTIVATION OF CYCLIC AMP SIGNALING INHIBITS VOLUME-SENSITIVE OSMOLYTE EFFLUX IN CARDIAC MYOCYTES. James I. Smith, Eric S. Moore, Dahna Leiser and Melvyn Lieberman*. Dept. of Cell Biology, Div. of Cellular Physiology and Biophysics, Duke University Medical Center, Durham NC 27710.

Heart cell swelling is attenuated by volume-sensitive transport systems that mediate the efflux of osmolytes (ions and amino acids) and result in a regulatory volume decrease (RVD) (*Am. J. Physiol.*, 264:C136, 1993). Although cAMP production is not stimulated during cell swelling (*FASEB J.*, 8:A619, 1994) we hypothesized that volume-sensitive transport mechanisms may be regulated by cAMP-dependent pathways. Relative changes in the volume of cell aggregates (~100μm; n=6-9) were measured by video microscopy and correlated with osmolyte efflux (assessed by release of ^{3}H-taurine; n=6-9) over 20 min. Peak volume (mean±s.e.) increased as the osmolarity of the perfusate decreased (see table) followed by RVDs that coincided with increasing taurine efflux. Upon reperfusion with isosmotic medium (290mOsm), cells returned to progressively less of their original volume (depending upon severity of the swell) reflecting the increased loss of organic osmolytes. Elevating cAMP content with 0.5mM isobutylmethylxanthine (IBMX) or 10μM forskolin led to the attenuation of RVD coincident with a decrease of taurine efflux. Results show that when cardiac myocytes are swollen, activation of cAMP-dependent pathways will prevent volume regulation in part by inhibiting osmolyte efflux. *Supported in part by NIH grant #HL27105.*

Osmolarity	Peak Swell	Post-RVD	290 mOsm	% Taurine Efflux
230 mOsm	1.19 ± 0.02	1.14 ± 0.02	0.97 ± 0.02	8.8 ± 0.6
180 mOsm	1.38 ± 0.02	1.22 ± 0.02	0.92 ± 0.02	38.2 ± 0.3
80 mOsm	1.86 ± 0.07	1.16 ± 0.03	0.74 ± 0.02	90.6 ± 0.6
180+IBMX	1.34 ± 0.03	1.30 ± 0.01	0.99 ± 0.01	26.2 ± 2.1
180+Forsk	1.36 ± 0.01	1.34 ± 0.02	0.98 ± 0.01	27.4 ± 0.9

10.12

CYTOSKELETAL REARRANGEMENT ACTIVATES THE SWELLING-INDUCED CHLORIDE CURRENT IN CARDIAC MYOCYTES. Jianping Zhang, Randall, L. Rasmusson and Melvyn Lieberman*. Department of Cell Biology, Division of Cellular Physiology and Biophysics, Duke University Medical Center, Durham, NC 27710

Whole-cell patch clamp revealed a swelling-activated chloride current I_{Cl} in cultured chick heart myocytes (Zhang *et al*; *J. Physiol.* 472:801-820, 1993). We now investigate whether this swelling-activated chloride conductance is associated with a signal transduction pathway that involves deformation of the cytoskeletal network. Pharmacological reagents that alter the dynamic assembly and disassembly of actin filaments were applied in whole-cell patch clamp experiments. When heart cells were pretreated (≥16hrs) and perfused with an F-actin depolymerizing reagent, cytochalasin B (15μM), I_{Cl} was markedly reduced (138±17pS/pF, at +60mV, n=4; vs. control, 1306±138pS/pF). Removal of cytochalasin B from the hypoosmotic solution caused a gradual increase in I_{Cl} towards the control levels. Similar results were observed when cells were pretreated (2hrs) with another F-actin depolymerizing reagent, pentoxifylline (1mM) and then perfused. Under these conditions, I_{Cl} was reduced to 206±43pS/pF (at +60mV, n=9). These results agree with the observations in which disruption of F-actin in intact heart cells attenuated the regulatory volume decrease (RVD) during hypoosmotic swelling. An alternative approach to test the involvement of cytoskeletal deformation was to study the effect of F-actin stabilizing reagent, phalloidin, on I_{Cl}. Pretreatment of cells with phalloidin (30μM, ≥18hrs) and inclusion of phalloidin (19μM) in the pipette solution substantially attenuated I_{Cl} (423±99pS/pF, n=4). In conclusion, cell swelling appears to initiate signal transduction mechanisms that involve rearrangement of cytoskeletal network. *Supported by NIH grant HL27105 and The Walter P. Inman Fund.*

10.13

LOAD-ACCELERATED PROTEIN SYNTHESIS OF STRETCHED ADULT CARDIOCYTES DEPENDS UPON STRETCH-ACTIVATED CALCIUM (Ca) INFLUX. Robert L. Kent* and George Cooper, IV.
VA Medical Center & Gazes Cardiac Research Institute, Cardiology Division, Medical University of South Carolina, Charleston, SC 29401

The mechanisms whereby hemodynamic load accelerates protein synthesis in cardiac hypertrophy are unknown. A role for stretch-activated Ca influx in the signal pathway linking load to enhanced protein synthesis was examined in the isolated adult cardiac myocyte, or cardiocyte. Feline cardiocytes were plated onto a laminin-coated deformable membrane and maintained for 24 hrs in serum- and mitogen-free culture with 1.8 mM Ca. Cardiocyte stretch was proportional to membrane deformation until 10%, which increased the myoplasmic fluorescence of the Ca-selective dye fluo-3 by 50%. This fluorescence with cardiocyte stretch was abolished by washing the cardiocytes in culture medium for 1 hr with 0.1 mM Ca with or without EGTA, or by 20 μM gadolinium, a blocker of stretch-activated ion channels. Acceleration of protein synthesis was also proportional to membrane stretch ($r^2 = 0.94$), and was enhanced by 45% after 4 hrs of 10% stretch when compared with that of non-stretched cardiocytes; this was measured by scintillation counting of [^{3}H]phenylalanine incorporation into protein in culture medium with 0.4 mM unlabeled phenylalanine. This anabolic effect of load was reduced to the level of that in slack cardiocytes by washing them with 0.1 mM Ca for 1 hr or by gadolinium, but it was unaffected by 5 μM verapamil. The low Ca medium did not affect the acceleration of protein synthesis by insulin. This anabolic effect of load was not reduced by saralasin, an angiotensin II blocker. Direct angiotensin II (1 nM to 10 μM) treatment of these cardiocytes had no anabolic effect. These results indicate that extracellular Ca influx through stretch-activated ion channels is necessary for transduction of load into accelerated myocardial protein synthesis.

10.14

LIGAND-INDUCED Ca^{+2} UPTAKE VIA THE ACETYLCHOLINE RECEPTOR IS INHIBITED REVERSIBLY BY PLURONIC F-68. Edward J. Massaro*,**, L. Aloi*, Y.C. Izu, J. Niggel, F. Sachs. *Center for Biochemical Engineering, Duke Univ., Durham, NC, **U.S. EPA, Health Effects Research Laboratory, Research Triangle Park, NC, 27711, Dept. of Biophysical Sciences, SUNY-Buffalo, NY.

The nonionic surfactant Pluronic F-68 (PLU), a block copolymer of polyoxyethylene and polyoxypropylene (avg. MW = 8,350), protects cells against damage in stirred and/or sparged bioreactors. The mechanism of protection is poorly understood. We have observed that PLU reversibly blocks Ca^{+2} uptake via the acetylcholine receptor (AchR) in FURA-loaded human embryonic kidney cells (HEK 293) transfected with mouse AchR subunits. Relative increase in intracellular Ca^{+2} was monitored fluorometrically (ratio method) at room temperature (22 ± 2°C) in HEPES-buffered (Ph = 7.4) saline solution. The cells (on cover slips) were incubated (flow-through cuvette) in the presence of 0.2-0.3% PLU for 5.0 min prior to addition of Ach (50 μl of 500 μM Ach). In the absence of PLU, intracellular Ca^{+2} increased immediately after Ach addition. In the presence of PLU, Ca^{+2} uptake was blocked. Flushing the cuvette with 50 ml PLU-free buffer and reexposure to Ach restored Ca^{+2} uptake (until the cells no longer respond to Ach). In addition, suspensions of Sf-9 insect cell subjected to trituration exhibited a 30% increase in $[Ca^{+2}]_i$ compared to a 13% increase in the presence of 0.2% PLU. However, at concentrations as high as 0.3%, PLU had no apparent effect on Ca^{+2} uptake via stretch-activated (hypoosmotic stress) channels of GH_3 cells. Supported in part by DAAL0392G0014.

(This abstract of a proposed presentation does not necessarily reflect EPA policy.)

10.15

PERCOLATION AS A POSSIBLE MODEL FOR BIOLOGICAL SIGNALING. Gabor Forgacs*, Clarkson University, Potsdam, NY 13699 -5820 and Central Research Institute for Physics, Budapest, Hungary

Random filamentous networks show up at various scales (nuclear matrix, microfilament networks, intermediate filament network, extracellular matrix) and developmental stages in a multicellular organism. They participate in a number of vital biological processes. We propose yet another role for these networks: biological signal transduction. We introduce the notion of percolation clusters, a concept well-known and widely used in physical and engineering sciences, and relate it to the various macromolecular assemblies, in particular to the cytoskeletal meshworks. Using properties of percolating networks, we develop a possible combined mechano-chemical signal transduction process. Similarities and differences between biological percolation networks and tensegrity structures introduced by Ingber are discussed. The results of computer simulations are presented to support the model, specific predictions are made and experiments are suggested. This work was supported by NSF, under Grant IBN 93-17633.

10.16

MECHANICAL STIMULATION OF NEURITES OF NODOSE BARORECEPTOR NEURONS IN CULTURE INDUCES A WHOLE CELL CURRENT. J.T. Cunningham, L. Fankhauser, R.E. Wachtel, & F.M. Abboud. Depts. of Int. Med., Anest., VAMC and Cardiovascular Center, Univ. of Iowa, 52242.

The purpose of this experiment was to study the mechanism of mechanotransduction of baroreceptor neurons. Male Harlan-Sprague rats were anesthetized and DiI was injected into the adventitia of the aortic arch. A week later the nodose ganglia were removed and the neurons were dissociated and cultured on coverslips. Within 2 to 3 days neurite outgrowth was evident on many neurons and whole cell patch clamp experiments were performed. The recording solution was (in mM): 106 KCl, 14 KOH, 10 HEPES, and in some experiments nystatin. The extracellular medium was (in mM): 140 NaCl, 2 $CaCl_2$, 1 $MgCl_2$ 1 KCl, 10 HEPES, and 2 g/l glucose. The neurites were deformed with a pneumatic ejection of extracellular solution delivered via a glass pipette (7-15 μm i.d. tip) placed 25 -50 μm from the neurite. In voltage clamp experiments with holding potentials of -70 mV, neurite deformation with a 50 ms injection at 5-10 psi induced an inward current (1.7 to 0.6 nA) in 5 out of 6 DiI labeled putative baroreceptor neurons. In 3 experiments these currents were suppressed by gadolinium (20 μM), a putative blocker of stretch-activated channels. Similar results were obtained in perforated patch experiments. These currents were observed without significant changes in seal resistance. Our results indicate that stretch-activated conductances in baroreceptor neurons may represent the mechanism of mechanoelectrical transduction associated with deformation of baroreceptor terminals. (Supported by HL 14388).

10.17

CHRONIC, INTERMITTENT MECHANICAL STRAIN (CMS) INDUCES WHOLE CELL CONDUCTANCE INCREASES VIA MODULATION OF MECHANOSENSITIVE CHANNELS IN OSTEOBLAST-LIKE CELLS. R.L. Duncan* and K.A. Hruska. Renal Division, Jewish Hospital of St. Louis, MO 63110.

We have previously characterized a PTH-stimulated, mechanosensitive, non-selective cation channel (SA-cat) in UMR-106.01 osteoblast-like cells which we postulate may act as a mechanotransducer for the osteogenic response of bone to physical strain. Using patch clamp techniques, we studied the response of SA-cat channels to CMS applied via the Flexercell apparatus. Chronically strained cells subjected to 8,000 to 15,000 microstrain demonstrated significantly larger increases in whole cell conductance when subjected to addition mechanical perturbation during the patch than non-strained control cells (69.0 ± 5.7% vs. 14.1 ± 3.1%, $p<0.001$). The increase in whole cell conductance could be blocked by the SA-cat channel inhibitor, gadolinium, and corresponded to a 3-5 fold increase in SA-cat channel open probability. Single channel studies demonstrated that CMS increased the number of open channels in response to strain and induced spontaneous channel activity in 37% of the patches of chronically strained cells. Graded increases in negative patch pressure demonstrated that SA-cat channels in chronically strained cells were activated at significantly lower levels of mechanical strain than non-strained controls. These data suggest that chronic, cyclic strain reduces the activation threshold of the SA-cat channel and further strengthens our hypothesis that this channel may act as a mechanotransducer for activation of bone remodeling by physical strain. (Supported by NASA Grant NAG 2-791)

10.18

MECHANOSENSING IN *ARABIDOPSIS*: TOWARDS A GENETIC ANALYSIS BASED ON MECHANO-INDUCIBLE LUMINESCENT PHENOTYPES. George A. Karlin-Neumann and Ronald W. Davis. Biochemistry Dept., Stanford University Medical School, Stanford, CA 94305-5307.

Plants exhibit morphogenetic, cellular and biochemical responses to mechanostimulation. In the small, genetically tractable mustard plant, *Arabidopsis thaliana*, gentle flexing or rubbing leads to decreased elongation of stems and leaves and to delayed flowering. These changes are preceded by the very rapid and transient mechano-induction of five *TCH* ("touch") genes, one of which encodes calmodulin (*TCH1*) and two others (*TCH2* & *TCH3*) putative Ca^{2+}- binding proteins (Braam and Davis, 1990). A fourth gene, *TCH4*, encodes a putative cell wall-modifying enzyme, a xyloglucan *endo*transglycosylase. We have created transgenic lines that constitutively express the Ca^{2+}-sensitive jellyfish photoprotein, aqueorin, and with these, have shown that mechanostimulation elicits a brief and nearly instantaneous Ca^{2+} spike in young seedlings, preceding the gene expression changes. Using a low light (VIM) camera, we have established conditions for identifying mutant seedlings that exhibit altered luminescence kinetics following stimulation. We anticipate that this screen will yield mutants with defects in the Ca^{2+} channels and pumps believed to be responsible for Ca^{2+} signaling and homeostasis in plants, and may also help to identify the nature of the mechanoreceptor(s). A second class of conditionally luminescent transgenic plants contains a *TCH4* promoter fused to the firefly luciferase (Luc) gene and to a second histochemical reporter, the β-glucuronidase (GUS) gene; this *TCH4:Luc/GUS* line enables us to monitor mechano-inducible gene expression as transient luminescence and as stable histochemical staining. This latter class of transgenics has shown that the *TCH4* gene is activated transiently both at sites of experimental mechanical manipulation and during the course of development at sites where endogenous biomechanical forces appear to be at work, e.g. in newly expanding leaves, stomates and at the sites of emerging root laterals. The *TCH4:Luc/GUS* seedlings will be screened for mutants exhibiting either constitutive luminescence or diminished mechano-induced luminescence, potentially reflecting mutations in the mechanosensory signaling pathway leading to nuclear gene induction.

10.19

ISOLATION OF A MECHANOSENSITIVE SUBPOPULATION OF VAGAL SENSORY NEURONS IN VITRO. G.Hajduczok. Department of Physiology, Univ. at Buffalo School of Medicine, Buffalo, NY 14214.

The nodose ganglion (NG) is a vagal sensory ganglion containing a heterogeneous population of neurons transmitting both mechano- and chemosensory information centrally. The cell bodies of aortic baroreceptor (BR) neurons are located in the NG. BR neurons were labeled by microinjection of the fluorescent dye DiI into the aortic arch of rats and the ganglia harvested 2 weeks post-injection and cultured. BR neurons were identified by the presence of label. The increase of cytosolic free calcium ($[Ca^{2+}]_i$) in response to mechanical stimulation in fura-2 loaded cells was determined by applying an external pressure pulse of normal Ringer's via a glass microelectrode (1-3 μm tip diameter). Baseline $[Ca^{2+}]_i$ was 77±10 nM (n=72, $\bar{x}$ ± SD) in BR neurons and 75±15 nM (n=64) in non-labeled (NL) neurons. A single pressure pulse increased $[Ca^{2+}]_i$ to a peak of 854± 184 nM in 69 of 72 BR neurons and was blocked by gadolinium (25 μM, 31/33 cells). Pressure application increased $[Ca^{2+}]_i$ in only 41 of 64 NL neurons to a peak of 823±198 nM. $[Ca^{2+}]_i$ did increase in non-responding NL neurons when stimulated chemically with angiotensin II or 5-HT. The data indicate that BR neurons represent a mechanically sensitive subpopulation of vagal sensory neurons which can be used to study the signal transduction pathways involved in baroreceptor function.

10.20

OSMOTIC ACTIVATION OF MECHANOSENSITIVE CHANNELS IN VESICLES ISOLATED FROM *XENOPUS* OOCYTES. Henry Sackin and Wolfgang Schütt*. Dept. of Physiol. & Biophysics, Cornell Univ. Medical College, New York, N.Y. USA

Excision of cell-attached patches from stage V,VI oocytes into 2 mM calcium solutions yielded a high percentage of membrane vesicles contained wholly within the patch pipette. Use of a 60x objective, together with a pipette tip parallel to the focal plane of the microscope, permitted continuous visualization of the larger edge of these vesicles, whose total length averaged 10.8 ± 3 μm (n=7). The endogenous mechanosensitive 26 pS channel could be readily identified in this vesicle preparation, despite attenuation of current amplitudes by 59 ± 6% (n=6) and significantly slower rise times than in either cell-attached or excised patches. Application of 10 mmHg negative pipette pressure stretched the vesicles in their linear (pipette) axis by 1.2 ± 0.1 μm or 13 ± 3% and increased channel open probability (P_o) from 0 to 0.2 ± 0.03. (n=4). A 50% decrease in bath osmolarity swelled the vesicles, increasing their linear dimension by 2.0 ± 0.6 μm or 21 ± 2%, simultaneous with an increase in P_o from 0 to 0.13 ± 0.05 (n = 10) in 5 ± 1 min. Maintained bath hypotonicity (relative to pipette) produced discrete "jumps" in vesicle surface area (and volume) that coincided with quantized increases in P_o until the vesicle broke (> 30 min). In cell-attached patches on intact oocytes, a 50% decrease in bath osmolarity increased P_o of these channels from 0 to 0.05 ± 0.01 (n=10) in 10 ± 2 min. Hence, mechanosensitive channels retained their essential characteristics in isolated vesicles that contained only a minimum of cytosolic material (particularly when these vesicles were formed from pre-existing excised patches). This suggests that mechanosensitivity is an intrinsic property of the channel and its adjacent membrane environment, independent of cytoplasmic constituents. (NIH, DK38596)

10.21

STRETCH STIMULATES PROLIFERATION OF RENAL EPITHELIAL CELLS. G. A. Tanner, P. F. McQuillan, M. R. Maxwell, J. K. Keck, J. A. McAteer. Indiana U. Sch. Medicine, Indianapolis, IN 46202

It has been suggested that in renal cystic disease, fluid accumulation within cyst lumens stretches cyst walls and in this way stimulates cell proliferation. To test this idea, we stretched confluent monolayers derived from a Madin-Darby canine kidney cyst. Cells were grown on collagen-coated Flexcell dishes and subjected to 0 - 30% stretch for 1 - 48 hr. The percentage of cells in S-phase (labeling index = LI) was determined by measuring bromodeoxyuridine incorporation. Stretch (25%) for 12 - 24 hr approximately doubled the LI. After 48 hr, population density was significantly ($P<0.001$) increased, from 41.9 ± 0.2 to 48.2 ± 0.5 cells/10,00 μm^2 ($\bar{Y}$ ± SEM, n = 12). LI increased linearly with applied stretch, from 7.2 ± 0.3% (n = 36) with no stretch to 16.2 ± 1.0% (n = 6) with 30% stretch. Stretch had to be maintained for eight or more hours to produce an increase in LI at 18 hr. No evidence was obtained for release of a soluble growth factor by stretched monolayers in co-culture experiments. The increase in LI induced by stretch was abolished by treatment with cytochalasin B, an actin microfilament disrupting agent, but was unaffected by 50 μM gadolinium, a stretch-activated cation channel blocker. We conclude that prolonged stretch stimulates renal epithelial cells to synthesize DNA, and may contribute to cell proliferation and cyst enlargement in renal cystic disease.

10.22

Ca^{2+} DEPENDENT MECHANOSENSITIVITY IN CARDIAC FIBROBLASTS. Peter Kohl*, Andre Kamkin*, Irina Kiseleva* and M.J. Lab. Dept. of Physiology, University of Oxford, OX1 3PT, UK.

Electrically non-excitable fibroblasts (F) in the sino-atrial node region of amphibian and mammalian heart have been described to be mechanosensitive (Kohl et al., Exp Physiol, 1992 & 1994). Computer simulations show that these cells can increase the spontaneous depolarization rate of adjacent pacemaker cells in a stretch dependent manner and, thus, be a cellular substrate for the positive chronotropic response of the heart to stretch. In this investigation we attempted to analyse the mechanism of mechano-transduction in more detail. F. were studied in isolated preparations of right rat atrium using floating microelectrodes. *Gadolinium* (20-100 μM, blocker of stretch activated channels) added to the perfusate abolished the appearance of membrane depolarizations induced by artificial stretch. Intracellular application of BAPTA (0.1-1 μM, Ca^{2+} chelator), Ryanodyne (0.1 0.5 μM, inhibitor of sarcoplasmic reticulum) or Thapsigargin (0.1-0.5 μM, inhibitor of sarcoplasmic Ca^{2+} pump) reduced the amplitude of stretch induced depolarizations by 45% to 80%. Kodama et al. (In: Ion channels and cellular function of the heart, Tokyo 1994) demonstrated that the positive chronotropic response of multicellular preparations of sino-atrial node tissue was inhibited by Ryanodyne and Thapsigargin by 50% to 74%. These data correspond with our findings and support the suggestion that F may play an important role in cardiac regulation. The mechanism of this particular mechano electric feedback is based on the operation of stretch activated channels and subsequent changes in intracellular Ca^{2+} concentration. Supported by Boehringer Ingelheim, BHF, Alexander v. Humboldt-Stiftung.

10.23

MECHANOSENSITIVE ACTIVATION OF CA^{2+} TRANSPORT PATHWAYS IN HT29 HUMAN COLON CANCER CELLS. Lin J. Hymel, Yunfeng Qin*, and Tibor Ertl*. Depts. of Physiology and Medicine, Tulane University School of Medicine, New Orleans, LA 70112.

Subconfluent cultures of the HT29 human colon cancer cell line grown on glass coverslips were loaded with 2 μM Fura-2 acetoxymethylester for 30 min at room temperature, washed, and incubated for further 30 min at 37°C before determination of $[Ca^{2+}]_{in}$. Cytosolic $[Ca^{2+}]$ was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was repeatedly observed for both cell monolayers mounted in a cuvette and single cells studied in a microscope-based photometry system. Similar manipulation of a wide variety of cell types, including the human pancreatic cancer line CFPAC-1, was without effect on resting $[Ca^{2+}]$ levels. Pretreatment of HT29 cells with 1 μM thapsigargin, a specific inhibitor of the SERCA ATPases, eliminated the Ca^{2+} transient upon solution exchange. These results indicate that the mechanical effect of exchanging solutions caused transient release of Ca^{2+} from thapsigargin-sensitive intracellular stores. Additional studies of HT29 cells grown on collagen-coated elastomer plates demonstrated that an average of 10% surface elongation (Flexercell Apparatus, Flexcell International Corp.) stimulated $^{45}Ca^{2+}$ influx 8-fold during the first 5 min. Thus, it appears that a cell surface Ca^{2+} influx pathway is also activated by mechanical deformation in HT29 cells. The exceptional mechanosensitivity of both cell surface and ER Ca^{2+} transport mechanisms may be related to cell proliferation mechanisms in this cancer cell line. (Supported by a grant from the Louisiana Board of Regents).

10.24

OSMOTIC STIMULATION OF AXONAL ELONGATION IS MEDIATED BY AN INCREASE IN TENSION SENSITIVITY OF GROWTH. Steven R. Heidemann, Phillip Lamoureux *and Chingju Lin*. Dept. Physiology, Michigan State Univ., E. Lansing, MI 48824

We tested for a possible relationship between osmotic (Bray et al., J. Cell. Sci. 98:507) and tensile (Zheng et al. Neurosci. 11:1117) stimulation of elongation of cultured chick sensory neurons. Neurites were tethered by their growth cone to force-calibrated glass needles and towed at a constant force, which varied between 150-300 udynes for 6 different neurites, producing a uniform rate of elongation between 75-145 um/hr. After 1 hr. of towing, the culture medium (L-15) was diluted 1:1 with a medium similar in composition to L-15 but lacking inorganic salts. Within 15 min. after the decrease in the osmolarity of the medium, the elongation rate of the neurite increased between 38% and 115% at the same constant towing force. That is, the decrease in osmolarity of the medium increased the sensitivity of neurites to tension-induced elongation. We are currently studying whether this presumed osmotic effect may be due to a decrease in extracellular [Ca++]. We previously proposed a tensegrity mechanism, a complementary force balance between microtubules and actin, to explain tension-induced axonal elongation. The presumed osmotic effect we observe could be due to a swelling and weakening of the actin network induced by water influx.

10.25

EFFECTS OF FLUID FLOW ON OSTEOCYTES. J. Klein-Nulend[1,3*], N.E. Ajubi[1,2*], A. van der Plas[2*], C.M. Semeins[1*], P.J. Nijweide[2*], E.H. Burger[1*], [1]ACTA-Free Univ., Dept. Oral Cell Biology, Amsterdam, [2]Univ. Leiden, Dept. Cell Biology, Leiden, The Netherlands. [3]Support: fellowship of the Royal Netherlands Academy of Arts and Sciences.

The primary function of the skeleton is to carry mechanical loads. A bone organ can adapt to changes in mechanical loading with changes in internal architecture. However, the mode of conveyance of the mechanical signal to the bone remodeling process is still unknown. When compact bone is loaded, fluid is squeezed from the osteocytic network towards the Haversian channels. Because of their central localization in the bone matrix, osteocytes are thought to function as mechanosensors. We tested the hypothesis that the stress-induced FF through the canaliculi and lacunae of the osteocytes acts as the mechanical stimulus that activates the osteocytes. The effect of FF was studied on isolated osteocytes purified from periosteal cell suspensions using immunomagnetic separation, and was compared to the effect on osteoblasts and fibroblasts. The release of prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_{1\alpha}$ ($6kPGF_{1\alpha}$, the stable metabolite of PGI_2) as signal molecules was measured. Pulsating (5 Hz) FF was applied in a parallel-plate flow chamber (max fluid shear stress 0.5±0.02 Pa; peak stress rate 0.4 Pa/sec). FF for 1 h elevated PGE_2 release in osteocytes, but not in osteoblasts or fibroblasts. $6kPGF_{1\alpha}$ release was not changed by FF in all 3 cell types. Control levels of PGE_2 and $6kPGF_{1\alpha}$ release from all 3 cell types were of the same order of magnitude. These data suggests that osteocytes are sensitive to very small pulsating fluid shear stress, but osteoblasts or fibroblasts are not. This supports the hypothesis that the osteocyte in particular functions as a mechanosensor of local strain in bone.

10.26

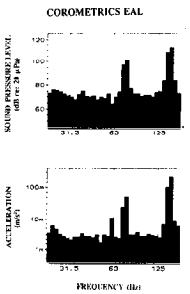
FOCAL CONTACTS AND LAMELLAR PROTRUSION ARE INCREASED AFTER MECHANICAL WOUNDING BY AGENTS THAT INHIBIT PHOSPHOINOSITIDE BREAKDOWN. G.E. Moeller, L.E. Hinman & P.J. Sammak. Dept. of Pharmacology, University of Minn., Mpls., Mn. 55455.

In a mechanical model of wounding, confluent monolayers of bovine pulmonary endothelial cells are wounded by dragging a needle through the serum-starved culture. Both $[Ca^{2+}]_i$ and tyrosine kinase-mediated growth factors stimulate wound-induced motility. Use of this model allows us to separate the relative contributions of different intracellular signaling pathways towards wound-induced cell behavior. For example, the actual wounding event produces a $[Ca^{2+}]_i$ pulse >1μM that propagates out 10 cell diameters from the wound and returns to baseline levels after 5-10 minutes. This $[Ca^{2+}]_i$ transient stimulates motility only when present during wounding. In contrast, growth factors such as 10% FCS, 10μM Insulin and 10 ng/ml bFGF stimulate motility when added before, during and after wounding. Reducing the calcium transient resulted in decreased lamellar protrusion and focal contacts as detected by interference reflection microscopy and a 50% slowdown in average wound closure rate. Application of 10μM insulin during wounding resulted in an increase in focal contacts along the wound edge and a 50% increase in average motility. Phosphoinositidyl signalling mediates some growth factors effects via PIP_3 and mobilizes calcium via IP_3/DAG. To test the importance of these pathways for motility, we applied 1μM lithium, an uncompetitive inhibitor of inositol phosphate breakdown products, during wounding. Lithium increased lamellar protrusion at the wound edge, focal contacts at cell edges throughout the wounded area and motility 300%. Beryllium, a competitive inhibitor of the same monophosphatase, duplicated this pattern. Both compounds effectiveness was limited to the time period surrounding wounding. Wortmannin, an irreversible inhibitor of PI-3-kinase, reduced substrate adhesion, cell-cell contacts and motility. The PLC inhibitor U-73122 and polycationic neomycin which binds PIP_2 both reduced wound-induced motility and lithium-associated stimulation. Therefore, signaling through phosphoinositide metabolites may coordinate lamellar protrusion and focal contacts formation induced by wounding or wound factors.

10.27

EFFECT OF ABDOMINAL VIBROACOUSTIC STIMULATION ON ACCELERATION LEVELS AT THE FETAL HEAD IN SHEEP. Robert M. Abrams, Aemil J.M. Peters*, and Kenneth J. Gerhardt*. University of Florida, Gainesville, FL 32610.

A vibroacoustic stimulator such as the electronic artificial larynx (EAL) is commonly used by obstetricians as an adjunct to a formalized test to determine human fetal reactivity and thus fetal health. The EAL is placed on the maternal abdomen over the fetal head and activated for up to five seconds. Intrauterine sound pressure level (SPL) may exceed 125 dB provoking in many normal fetuses an increase in heart rate variability. Sheep are excellent animal models for human pregnancy research. Understanding the vibratory and acoustic characteristics of mechanical stimulators at the head of fetal sheep may provide important information about whether or not human fetuses hear and/or feel these stimuli. We measured acceleration levels at the fetal skull with piezoresistive accelerometers (Model EGA-125-10DX, Entran, Inc., Fairfield, NJ) and sound pressure levels near the head with a miniature hydrophone (Model 8103, Bruel& Kjaer, Marlborough, MA). Animals were anesthetized and supine throughout the procedure and fetuses were returned to the uterus and the abdominal wall was closed. An EAL (Corometrics Medical Systems, Inc., Wallingford, CT) was pressed against the flank with a force that depressed the skin by 3 cm. At the fundamental frequency of the EAL (77 Hz) the intrauterine SPL was 101 dB (re: 20 μPa) and acceleration was 53 mm/sec^2. The levels at the first overtone (154 Hz) were 113 dB and 224 mm/sec^2 (see Figure). These values were well above the noise floors which were approximately 70 dB and 3 mm/sec^2 through the frequency regions of interest. Both mechanoreceptors and auditory receptors may play roles in evoking a response to vibroacoustic stimulations. Supported in part by NIH grant HD-20084.



REGULATION OF CELL SHAPE AND FUNCTION BY THE EXTRACELLULAR MATRIX

11.1

DYNAMICS OF MECHANICAL STRESS-REGULATED MODULATION OF CELL SHAPE, MICROFILAMENTS, AND MACROMOLECULAR BIOSYNTHESIS IN FIBROBLASTS. Howard Doong, Raphael C. Lee, and Satish C. Tripathi, University of Chicago, Dep. of Organismal Biology and Anatomy and Surgery, Div. Plastic Surgery, Chicago, IL 60637.

Mechanical stress factors are known to act as epigenetic regulators of connective tissue modelling and repair. Our laboratory has developed an *in vitro* model system that enables better understanding of molecular mechanism(s) involved in the mechanical stress-mediated extracellular matrix remodelling and repair. Our model consists of a tissue equivalent comprising a fibroblast-populated collagen matrix (FPCM) in which static and dynamic mechanical stresses can induce uniaxial alignment of both the cells and their biosynthetic products, e.g., collagen. These FPCMs are tissue equivalents that permit control of cell density as well as extracellular matrix composition and organization. They are translucent to permit observation of cellular geometry and intercellular connections. In addition, this model allows uniform mechanical stress to be imposed without damage to the tissue.

FPCMs were prepared from a mixture of neonatal human foreskin fibroblasts (2.5, 5, 10, and 20 million cells/FPCM), type I collagen, Dulbecco's modified Eagle's medium (DMEM), and 10% fetal bovine serum (FBS). The collagen gels were casted in 80x40 mm pyrex dishes containing 2 porous polyethylene posts that were rigidly held 2 cm apart. The FPCMs were maintained in DMEM containing 10% FBS, 10mM fructose, and 5% CO_2 environment over a period of 2 to 8 weeks. During this period, the FPCMs formed uniaxially-oriented matrices due to contraction of gel between 2 rigid posts. The FPCMs were exposed to both static and dynamic mechanical stretch using a custom-built programmable mechanical spectrometer. FPCMs were subjected to 2 to 18 gram mechanical (static) loads and changes in both cell shape and, the length of FPCM (measure of strain) were recorded using FPCM-stretching chamber and three-dimensional confocal microscope (3-DCM). FPCMs were subjected to 0.01 to 1.0 Hz loading (dynamic) and changes in cell shape recorded with 3-DCM for each cycle of stretching and relaxing. The cell shape and size before and after dynamic loading was compared to determine degree of deformation. Filamentous actin was stained with N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-phallacidin. Biosynthesis of collagen and glycosaminoglycan were determined by ^{3}H-proline and ^{35}S-sulfate (10 and 20 μCi/mL) incorporation. Collagenase activity was determined by SDS-PAGE. Cell growth and viability were measured by ^{3}H-thymidine incorporation and trypan blue exclusion.

Results from these studies suggested cell-density-dependent effect of mechanical force on breaking strength and Young's Modulus values for FPCMs and nearly linear relationship between the age of FPCM vs. its breaking strength. Mechanical stress tended to redirect cellular filamentous actin while, biosynthesis of proteoglycans was not particularly affected. Mechanical stress induced growth of fibroblasts as measured by ^{3}H-thymidine incorporation with no effect on cell viability. These results suggest FPCMs as an excellent model system for mechanistic studies in wound repair and remodelling.

11.2

Human culture-derived macrophages contain a K^+ channel that modulates the cytoskeleton and is activated by membrane stretch and cytokines. Samuel N Breit, Michelle R Bootcov, Terence J Campbell, Peter W French, Donald K Martin. *Centre for Immunology, and Departments of Clinical Pharmacology and Cardiology, St Vincent's Hospital and University of NSW, Sydney. NSW. 2010. Australia.*

A variety of stimuli, (including cytokines and adhesion to surfaces and matrix proteins) can regulate macrophage function, in part through changes in Ca^{2+} dependent second messengers. Whilst fluctuation in intracellular Ca^{2+} is an important modulator of cellular activation, little attention has been paid to the roles of other ions whose cytoplasmic concentrations can be rapidly regulated by passage through ion channel.

In order to understand the role of ion channels in macrophage function, studies have been undertaken utilizing patch clamping methods and human culture derived macrophages (MNC) grown under serum free conditions. The major ionic current in these cells is carried by an outwardly rectifying K^+ channel with a single-channel conductance of 106pS, open probability of 0.06 at maximal activity of the single channels in resting cells and whole-cell conductance for outward current of 9.8nS. This suggests that there are only around 1550 outwardly rectifying K^+ channels present in the unstimulated MNC at an average channel density of 1 per μm^2. These channels are open infrequently in resting cells but are activated immediately by (i) adhesion of mobile cells onto a substrate; (ii) stretch applied to isolated membrane patches in Ca^{2+} free buffers; (iii) by intracellular Ca^{2+} (EC50=0.4μm); (iv) the cytokines IL-2 and IL-6. Furthermore, an inorganic (barium) and an organic (4-aminopyridine) blocker of this channel altered the distribution of the cytoskeletal proteins actin, tubulin and vimentin, which correlated with a reversible alteration to the morphology of the cells.

We have identified an outwardly rectifying K^+ channel that appears to be involved in both cytokine and adherence mediated macrophage activation and also participates in the maintenance of cytoskeletal integrity and cell shape.

11.3

A STUDY OF PARAMETERS AFFECTING FIBROBLAST MORPHOLOGY IN RESPONSE TO AN APPLIED MECHANICAL FORCE. Rosalind A. Grymes[1] and Christine Sawyer*[2]. Life Science Division[1,2] and Bionetics Corp.[2], NASA-Ames Research Center, Moffett Field, CA 94035

A precisely controlled stretch/relaxation regimen (20% elongation at 6.6 cycles/min) was applied to normal human fetal, neonatal and aged dermal fibroblasts cultured on flexible membranes. Culture conditions included poly (NH_2) or collagen type I coated substrate membranes; control cultures were grown on the same pliable material in the absence of applied stretch. Direct observation and immunofluorescence analyses revealed a progressive change in cell body orientation limited to the stretched dermal fibroblast cultures. Monolayers gradually (over 4 days) acquired a symmetric, radial distribution equivalent to the biaxial array of the applied force. At high seeding density, alignment was inhibited in the fetal cell cultures. This cell strain required collagen type I coating for optimal attachment to the flexible membrane, preferring growth in three-dimensional cell 'balls' on the poly(NH_2) coated substrate. Neonatal cells also required the collagen type I coating, but both neonatal and aged dermal fibroblasts aligned efficiently at all seeding densities examined. The randomly oriented neonatal cells on the unstretched control membranes spontaneously detached at confluence, as a single cell sheet. Their aligned counterparts did not detach until the applied stretch stimulus was removed. Low concentrations of cytochalasin D (62.5 ng/ml) disrupted the stretch-related alignment response. Rhodamine phalloidin staining visualized fewer actin stress fibers in stretched, aligned cells than in controls. Both intercellular interactions and cytoskeletal integrity mediate the response to mechanical strain. Normal rabbit corneal stroma fibroblasts (NRC) were also analyzed, and failed to orient under these conditions. This cell type may require a different regimen, or a longer time period, to demonstrate alignment behavior. Supported by NASA Space Biology RTOP 199-40-22 and the NASA-ARC Director's Discretionary Fund

11.4

PREDICTIONS OF A MODEL FOR CELL / INTERCELLULAR MATRIX INTERACTIONS IN SHORTENING SMOOTH MUSCLE. Richard A. Meiss. Indiana University School of Medicine, Indianapolis, IN 46202

Most smooth muscles consist of small cells embedded in an extracellular matrix (ECM) of connective tissue, and the mechanical function of both the individual cells and the whole tissue is constrained and modified by multiple interconnections. A model to account for some of these interactions has been developed (Meiss, R. A., *Adv Exp Med. Biol.* **304**: 425-434, 1991), which proposes that active shortening is limited by the tendency of the tissue to resist radial expansion, and that this resistance is manifest in the axial stiffness of the muscle. The model parameters relate to the behavior of the radial and axial elasticity and to the effect of tissue cross-sectional area. This paper analyzes the relative sensitivity of the model to manipulations of these parameters in order to develop experimental strategies for testing model assumptions. The mechanical end-point for the analysis is the strong length-dependence of stiffness at short lengths; this is shown to be weakly sensitive to axial elastic components, moderately sensitive to tissue cross-sectional area, and extremely sensitive to radial elastic components. This suggests that experimental changes in cross-sectional area or tissue volume should produce significant changes in shortening behavior, and that manipulations (partial enzymatic digestion, etc.) that affect ECM connections should also produce large and detectable changes in shortening behavior. Since the model is much more sensitive to radial than to axial elastic component alterations, it should be possible to differentiate between alternative experimental outcomes.

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11.5

Modulation by reversible inhibition of β1 integrin by antisense oligonucleotides of myoblast attachment to EMC and proliferation potential.

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Myoblasts gene-engineered *in vitro* and then injection *in vivo* are safe, efficient options for gene therapy. While isolation of satellite cells is routinely achieved, their proliferation potential *in vitro* remains a limiting factor of cell transplantation in clinical settings. We are studying the role of reversible inhibition by antisense oligonucleotides of the synthesis of integrins (surface proteins which mediate interactions between cells and extracellular matrices and are known to modulate cell motility and proliferation) on satellite cells proliferation. Addition of antisense oligonucleotides to myoblast cultures has been used to specifically inhibit expression of the b1 integrin subunit gene. Here we show that the effects of multiple pulses of a phosphorothioate oligo deoxinucleotides antisense on myoblast proliferation and detachment from substrata (gelatin or rat tail collagen) are dose-dependent. A single pulse shows not consistent effects on myoblast proliferation, while in the presence of continuously administered antisense relative and absolute numbers of myoblasts in treated muscle culture are slightly increased. On the other hand we have not evidence of inhibition of myoblast fusion in the tested conditions. Results suggests a potential role of integrin antisense strategies on modulation of proliferation potential of myoblasts.

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