Offices

The Conference Office is located in the Regency Room in the Hyatt San Francisco Hotel. Telephone number: (415) 788-1234.

On-Site Registration

The 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 are nonscientist family members of registrants and may not attend scientific sessions.

Location: Grand Ballroom Foyer

Hours:	
Wednesday, November 17	2:00–8:00 pm
Thursday, November 18	8:00 am–5:30 pm
Friday, November 19	8:30 am–4:30 pm
Saturday, November 20	8:30 am–4:30 pm

Fees:	
APS Member	$230.00
APS Emeritus Member	$75.00
Nonmember	$275.00
Student	$75.00
Guest registrant	$40.00

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.

CME Credit

The APS Conference on Signal Transduction and Gene Regulation is jointly sponsored by the Federation of American Societies for Experimental Biology, and, as such, has been certified for CME Category 1 credit on an hour-for-hour basis for up to 21 hours. CME forms, which must be completed and returned for credit, will be available in the Conference Office, Regency Room. There is a $25 processing fee, payable upon submission of the application.

Program/Abstract Volume

The October issue of *The Physiologist* contains the contributed abstracts and program for the conference. Advance registrants were sent a pick-up card which may be exchanged at the Registration Desk for a copy of the volume. Replacement copies may be purchased for $20.00.

Message Center

The message board will be located in the Grand Ballroom Foyer next to 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, (415) 788-1234 and ask for the Regency Room.

Airline Reservations

Arrangements have been made with United Airlines to offer registrants special discounts off published and coach fares. Reservations may be made by calling United directly or by using your choice of travel agent. To take advantage of the discounts, you must call 1-800-521-4041 and refer to file #535AR.

Car Rental

Alamo Car Rental has been appointed the official car rental company for the conference. 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 Conference attendee and give the conference dates, I.D. #68970 and Plan Code GR to guarantee the special rate.

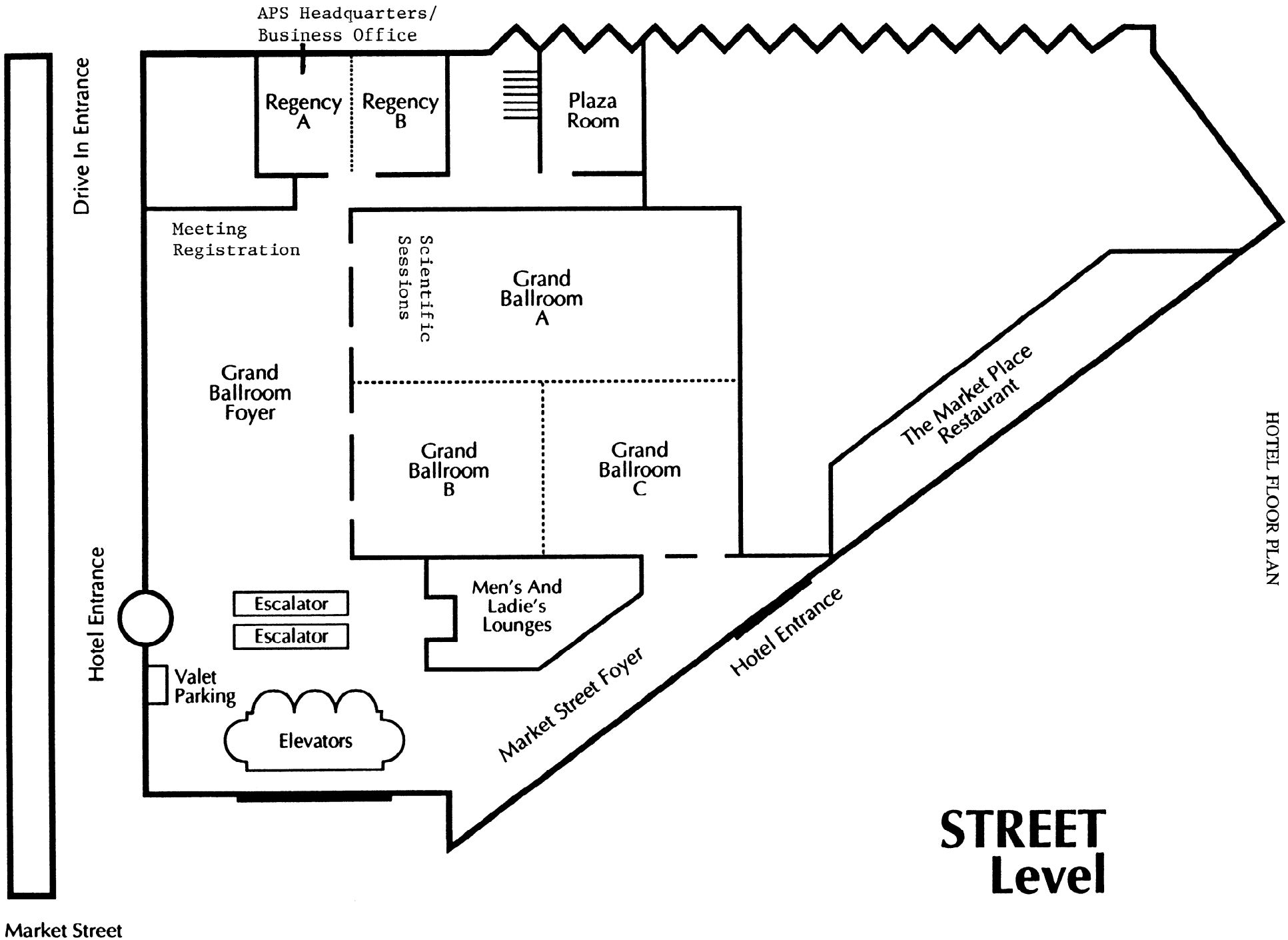
Airport Transportation

Travel time from the San Francisco Airport to the downtown area is approximately 30 minutes. The airport is served by the SFO Airporter Shuttle which departs every 20 minutes and will deliver you to the front door of the Hyatt Regency San Francisco Hotel at Embarcadero Center. You may pick up the SFO Airporter on the lower level of the airport. The cost is $8.00 one way or $14.00 round trip.

Social Program

Opening Reception. The Opening Reception will be held in the Garden Room on Wednesday, November 17 from 8:00–9:00 pm.

Banquet Lecture and Student Awards Presentation. All registrants are invited to attend the banquet on Friday evening in Grand Ballroom A. A cash bar reception is scheduled at 7:00 pm in the Grand Ballroom foyer followed by dinner at 8:00 pm. The lecture will be presented by noted authority, James M. Wilson of the University of Michigan, after dinner at 9:00 pm. The banquet is included in the registration fee; however, tickets are required for admittance. Each registrant will receive a coupon in the registration packet which must be exchanged for a diner ticket before 10:00 am on Thursday, November 18.


APS Headquarters/
Business Office
Regency A
Regency B
Plaza Room
Drive In Entrance
Meeting Registration
Scientific Sessions
Grand Ballroom A
Grand Ballroom Foyer
Grand Ballroom B
Grand Ballroom C
The Market Place Restaurant
HOTEL FLOOR PLAN
Hotel Entrance
Escalator
Escalator
Men's And Ladie's Lounges
Valet Parking
Elevators
Market Street Foyer
Hotel Entrance
STREET Level
Market Street

Signal Transduction and Gene Regulation

November 17–20, 1993
San Francisco, California

Wednesday, November 17	Thursday, November 18	Friday, November 19	Saturday, November 20
2:00–8:00 pm Registration	9:00 am–Noon Morning Symposium Signaling via G Proteins I Chair: Craig C. Malbon	9:00 am–Noon Morning Symposium Growth Factors, Tyrosine Kinases, and Regulation I Chair: John C. Cambier	9:00 am–Noon Morning Symposium Mechanisms of Gene Regulation Chair: Gary L. Johnson
7:00–8:00 pm Evening Lecture Henry R. Bourne	12:30–2:00 pm Poster Defending	12:30–2:00 pm Poster Defending	2:00–5:00 pm Afternoon Symposium Sino-American Session New Strategies for Molecular Studies of Regulation Chair: Craig C. Malbon
8:00–9:00 pm Opening Reception	2:00–5:00 pm Afternoon Symposium Signaling via G Proteins II Chair: Gary L. Johnson	2:00–5:00 pm Afternoon Symposium Growth Factors, Tyrosine Kinases, and Regulation I Chair: C. Ronald Kahn	
	5:15–6:15 pm Evening Lecture Ronald M. Evans	7:00–8:00 pm Reception 8:00–9:00 pm Dinner 9:00–10:00 pm Banquet Lecture James M. Wilson	

APS Conference

Signal Transduction and Gene Regulation

Daily Schedule

Wednesday, November 17

1. Evening Lecture

Henry R. Bourne. UCSF.

WEDNESDAY 7:00—Grand Ballroom A

Thursday, November 18

Symposium

2. Signaling via G-Proteins I

Chaired: **Craig C. Malbon**, SUNY, Stony Brook

THURSDAY—Grand Ballroom A

9:00 **Heidi E. Hamm**. Univ. of Illinois, Chicago.
9:25 Discussion.
9:30 **Brian K. Kobilka**. Stanford Med. Ctr.
9:55 Discussion.
10:00 **Melvin I. Simon.** California Inst. of Technol.
10:25 Discussion.
10:30 Break.
10:45 **Richard A. Cerrione.** Cornell Univ.
11:10 Discussion.
11:15 **Gary L. Johnson**. Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver.
11:40 Discussion.
11:45 General discussion.

Symposium

3. Signaling via G-Proteins II

Chaired: **Gary L. Johnson**, Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver

THURSDAY—Grand Ballroom A

2:00 **Arthur M. Brown**. Baylor Col. of Med.
2:25 Discussion.
2:30 **Randall R. Reed**. Johns Hopkins Univ.
2:55 Discussion.
3:00 **Sue Goo Rhee**. NIH.
3:25 Discussion.
3:30 Break.
3:45 **David E. Clapham**. Mayo Clin.
4:10 Discussion.
4:15 **Allen M. Spiegel**. NIH.
4:40 Discussion.
4:50 General discussion.

4. Evening Lecture

Ronald M. Evans. Salk Inst.

THURSDAY 5:15—Grand Ballroom A

Poster

5. G-Protein-Linked Receptors

THURSDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

1 **5.1** Depressed GTP stimulatory protein in aging monkeys. **D.E. Vatner, K. Kiuchi, N. Sato, Y-T. Shen, and S.F. Vatner.** Mass. Gen. Hosp., Harvard Med. Sch., and New England Reg. Primate Res. Ctr.

2 **5.2** Phosphorylation of the C-terminal tail of the PTH/PTHrP receptor by protein kinase C. **E. Blind, M.W. Fowlkes, S. Pratt, T-H. Chen, D. Shoback, and R. A. Nissenson**. VA Med. Ctr., San Francisco and UCSF.

3 **5.3** Effects of guanosine nucleotides and phorbol 12,13-dibutyrate on skinned muscle tissues of pregnant rat myometrium. **H. Izuma and R.E. Garfield.** Univ. of Texas Med. Branch, Galveston.

4 **5.4** Cultured astrocytic glia cells are a good model for studying regulation of $\alpha_{2A/D}$ adrenoceptors. **E.M. Richards, M.A. Reutter, M.K. Raizada, and C. Sumners**. Univ. of Florida.

5 **5.5** Insulin-promoted phosphorylation of the β2-adrenergic receptor. **M. Valiquette, S. Parent, and M. Bouvier**. Univ. of Montreal.

6 **5.6** Identification of structural elements in the PTH/PTHrP receptor critical for G-protein coupling. **Z. Huang, Y. Chen, T. Bambino, and R.A. Nissenson**. VA Med. Ctr., San Francisco and UCSF.

7 **5.7** The rat SSTR1 and SSTR2 somatostatin receptor subtypes mediate inhibition of cAMP accumulation by pertussis toxin-sensitive G-proteins. **J. Strnad, C.M. Eppler, and J.R. Hadcock**. American Cyanamid Co.

8 **5.8** Two distinct endothelin human endothelin β receptors generated by alternative splicing from a single gene. **V. Shyamala, T.H.M. Moulthrop, J. Stratton-Thomas, and P. Tekamp-Olson**. Chiron Corp., Emeryville, CA.

9 **5.9** Possible functional coupling of a GTP-binding protein to dopamine D2 receptor. **S.M. Farooqui and C.D. Gulley**. Pennington Biomed. Res. Ctr., Baton Rouge.

10 **5.10** Murine gastrin releasing peptide receptor expression in KNRK cells. **L.W. Slice, H. Wong, A. Garland, N. Bunnett, and J.H. Walsh**. UCLA, Wadsworth VA Med. Ctr., Los Angeles, and UCSF.

11 **5.11** A cloned CCK_A receptor transduces multiple signals on stimulation by full and partial agonists. **D.I. Yule, M.J. Tseng, J.A. Williams, and C.D. Logsdon**. Univ. of Michigan.

12 **5.12** Functional properties of the N-formyl peptide receptor expressed in undifferentiated HL60 cells. **E.R. Prossnitz, O. Quehenberger, C.G. Cochrane, and R.D. Ye**. Scripps Res. Inst.

13 **5.13** Use of Giα site-directed mutants to study specificity of receptor-mediated inhibition of adenylyl cyclase. **S.E. Senogles**. Univ. of Tennessee.

14 **5.14** β-Adrenergic receptor activity in liver membranes of mice with obesity diabetes syndromes. **N. Bégin-Heick**. Univ. of Ottawa.

15 **5.15** Angiotensin II activates adenylyl cyclase in AT_{1A} receptor-transfected CHO-K1 cells: role in inhibition of cell growth. **J. Du, T.J. Thekkumkara, J.C. Zwaagstra, J. Krupinski, and K.M. Baker**. Weis Ctr. for Res., Danville, PA.

16 **5.16** Hydroxylamine perturbs coupling between α_2 adrenergic receptor and G-protein. **J.B. Thompson and R.R. Neubig**. Univ. of Michigan.

17 **5.17** Elevation of intracellular Ca^{2+} can increase inositol 1,4,5-triphosphate but not inositol 1,3,4,5-tetrakisphosphate accumulation in rat brain cortical slices. **M.E. Myles and J.N. Fain**. Univ. of Tennessee.

18 **5.18** Promotor structure and tissue-specific regulation of the rat α2C-adrenergic receptor gene. **J.S. Saulnier-Blanche and S.M. Lanier**. Med. Univ. of South Carolina.

19 **5.19** Cloning and sequence analysis of the human vasoactive intestinal peptide receptor gene. **S.P. Sreedharan, J-X. Huang, and E.J. Goetzl**. UCSF.

20 **5.20** Muscarinic receptor mediated inhibition of typical and atypical β-adrenoceptor stimulation of cyclic AMP accumulation in guinea pig ileum. **H. Reddy, A.P.D.W. Ford, and F.M. Eglen**. Syntex Res., Palo Alto, CA.

21 **5.21** Immunohistochemical localization of peptidergic nerve fibers and neuropeptide receptors in Peyer's patches of the cat ileum. **S. Ichikawa, S.P. Sreedharan, E.J. Goetzl, and R.L. Owen**. UCSF and VA Med. Ctr., San Francisco.

22 **5.22** Mechanisms of signal transduction for adenosine and ATP in pulmonary vascular bed. **A.L. Hyman and H.L. Lippton**. Tulane Med. Sch.

23 **5.23** Role of G-proteins in the vasodilator response to endothelin isopeptides in vivo. **H.L. Lippton and A.L. Hyman**. LSU Med. Sch.

24 **5.24** Mg^{2+} moves into Ca^{2+} stores during muscarinic-induced Ca^{2+} release in rat sublingual mucous acini. **G.H. Zhang and J.E. Melvin**. Univ. of Rochester.

25 **5.25** Increased mRNA levels for the L-type Ca^{2+} channels in rat myometrium prior to term and preterm labor. **N. Tezuka, M. Ali, K. Chwalisz, and R.E. Garfield**. Univ. of Texas Med. Branch, Galveston and Schering AG, Berlin, Germany.

26 **5.26** Receptor interactions of β-N-oxalyl-L-α,β-diaminopropionic acid, the Lathyrus sativus excitotoxin. **M.A. Junaid and S.L.N. Rao**. Osmania Univ., Hyderabad, India.

27 **5.27** Phosphatidylinositol-specific phospholipase C: progress on the mammalian ecto-enzyme. **O.H. Griffith, K.K. Hedberg, G.B. Birrell, J.J. Volwerk, and M. Ryan**. Univ. of Oregon.

28 **5.28** Rapid analysis of inositol phosphates using solid phase extraction. **P.A. Whitson, H.M. Huls, and Y-M. Chen**. NASA-Johnson Space Ctr. and KRUG Life Sci., Houston.

29 **5.29** Endotoxin tolerance differentially down-regulates macrophage thromboxane β2 and IL-6 production. **H. Chen, J.A. Cook, and P.V. Halushka**. Med. Univ. of South Carolina.

30 **5.30** The effect of adenosine on the NMDA receptor mediated calcium influx in turtle cerebral cortical sheets. **L.T. Buck and P.E. Bickler**. UCSF.

31 **5.31** Ser phosphorylation levels affect signaling by CD69, a type II single-transmembrane, G-protein-associated receptor. **R. Testi, R. Trotta, R. DeMaria, S.F. Zeigler, G. Torelli, L.L. Lanier, and A. Santoni.** Univ. of Rome, Italy, Immunex, Inc., Seattle, WA, and DNAX Res. Inst., Palo Alto, CA.

Poster

6. G-Proteins

THURSDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

32 **6.1** βγ subunits of G-proteins activate phospholipase C-β2. **A. Katz, D. Wu, and M.I. Simon**. California Inst. of Technol.

33 **6.2** Small GTP-binding proteins in plasma membranes of sea urchin spermatozoa. **P. Cuéllar-Mata, L.E. Castellano, G. Novoa-Martínez, G. Martínez-Cadena, and J. García-Soto**. Univ. of Guanajuato, Mexico.

34 **6.3** Occurrence of Gα proteins in the fungus Phycomyces blakesleeanus spores. **G. Martinez-Cadena, A. Gonzalez-Hernandez, G. Novoa-Martinez, and J. Garcia-Soto**. Univ. of Guanajuato, Mexico.

35 **6.4** Ethanol does not affect expression of Gsα and Giα in murine embryonic palate mesenchyme cells. **W.M. Weston, K. Shah-Quazi, R.M. Greene, and M.M. Pisano**. Thos. Jefferson Univ.

36 **6.5** Enhanced contractile responses of arteries from diabetic rats to direct stimulation of G-proteins. **L. Weber, W. Abebe, and K.M. MacLeod**. Univ. of British Columbia.

37 **6.6** Localization of rap1 and investigation of its role in rat parotid gland. **N.J. D'Silva, D. Di Julio, K.L. Jacobson, and E.L. Watson.** Univ. of Washington.

38 **6.7** Supression of tumorigenicity by rap1A/krev-1 gene in human hepatoma cells. **Y-L. Lin and C-K. Chou**. Natl. Yang-Ming Med. Col., and VA Gen. Hosp., Taipei, Taiwan.

39 **6.8** Pregnancy alters uterine artery constriction to G-protein and protein kinase C activation. **M. Cipolla and G. Osol**. Univ. of Vermont.

40 **6.9** Linkage of serotonin receptor with a Gαz-like guanine nucleotide binding protein in rat stomach fundus. **H-Y. Wang and E. Friedman**. Med. Col. of Pennsylvania.

41 **6.10** cDNA cloning and analysis of a new G-protein β subunit. **A.J. Watson and M.I. Simon**. California Inst. of Technol.

42 **6.11** Random mutagenesis of Goα. **V.Z. Slepak, A.M. Aragay, M.W. Quick, and M.I. Simon**. California Inst. Technol.

43 **6.12** Coupling G-protein α subunits with seven-pass-membrane receptors in Xenopus oocytes. **A.M. Aragay, M.W. Quick, N. Davidson, H.A. Lester, and M.I Simon**. California Inst. of Technol.

44 **6.13** The specificity of G-protein γ subunit binding to β is conferred by γ1 residues 36-49. **D.J. Spring and E.J. Neer**. Brigham & Women's Hosp. and Harvard Med. Sch.

45 **6.14** A unique 30/32 kD GTP-binding protein dimer in mammalian brain: purification, properties and tissue distribution. **L. Zeng and M.M. Rasenick**. Univ. of Illinois.

46 **6.15** Fluorescence resonance energy transfer suggests that $G\alpha_{i1}$ binds to tubulin αβ dimers. **M.E. Knight and M.M. Rasenick**. Univ. of Illinois.

47 **6.16** Chimeric G-proteins expressed in COS-1 cells define domains on Gsα which interact with tubulin for the β-adrenergic activation of adenylyl cyclase. **J.S. Popova, G.L. Johnson, and M.M. Rasenick**. Univ. of Illinois and Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver.

48 **6.17** Isolation and characterization of a 66 kDa GTP-binding protein, G_{ir}, associated with the insulin receptor, from human placenta. **T.K. Varma and S.K. Srivastava**. Univ. of Texas Med. Branch, Galveston.

49 **6.18** Cholera toxin catalyzes ADP-ribosylation of heterotrimeric $G_{s\alpha}$ and not the free G_s-subunit. **M. Toyoshige, S. Ikuya, and R.V. Rebois**. NIH.

50 **6.19** Activation of the stimulatory guanine nucleotide binding protein can occur without subunit dissocation. **S. Okuya, N.S. Basi, and R.V. Rebois**. NIH.

51 **6.20** GTP binding during G_s activation does not promote subunit dissociation. **N.S. Basi and R.V. Rebois**. NIH.

52 **6.21** G-protein βγ subunits are specific for distinct rhodopsins. **J.K. Northup, H. Tamir, and D. Wildman**. NIMH.

53 **6.22** The G_s heterotrimer activates adenylylcyclase in cell membranes. **R.V. Rebois and S. Okuya**. NIH.

54 12:30 **6.23** Regulated vesicles of neuroendocrine and neuronal cells contain different heterotrimeric G-proteins. **G. Ahnert-Hilger, K. Spicher, T. Schäfer, G. Schultz, and B. Wiedenmann**. Univ. Klinikum Steglitz, FU Berlin, Germany and Friedrich Meischer-Institut, Basel, Switzerland.

55 **6.24** Tissue specific developmental expression of $G_{\alpha s}$-isoforms in the rat. **N. Fraeyman and E. Van de Velde**. Univ. of Ghent, Belgium.

56 **6.25** Addition of amino acids to the N-terminus of the stimulatory G-protein α-subunit inhibits ADP-ribosylation by choleragen. **D.R. Warner and R.V. Rebois**. NIH.

57 **6.26** Heterogeneity of bovine brain G-protein γ-subunit and their carboxyl methylation reaction. **H. Sohma, H. Hashimoto, and T. Akino**. Sapporo Med. Univ., Japan.

58 **6.27** Hypertrophic agonists stimulate the mitogen-activated protein kinase cascade in cardiac myocytes. **P.H. Sugden, A. Lazou, P.E. Glennon, A. Clerk, M.B. Andersson, C.J. Marshall, and M.A. Bogoyevitch**. Natl. Heart & Lung Inst., London, UK.

59 **6.28** Interleukin-1 increases protein kinase A activity by a cAMP-independent mechanism in AtT-20 cells. **A.R. Gwosdow, N.A. O'Connell, and A.B. Abou-Samra**. Mass. Gen. Hosp. and Shriners Burns Inst., Boston.

60 **6.29** Glucose regulates acetyl-CoA carboxylase gene expression in the pancreatic β-cell line INS-1. **M. Prentki, T. Brun, K-H. Kim, and E. Roche**. Univ. of Geneva, Switzerland.

61 **6.30** Effect of hemorrhagic shock on gluconeogenesis. **S. Maitra, M. Edwards, W. Pan, and E. Geller**. SUNY at Stony Brook.

62 **6.31** Endotoxin tolerance is associated with decreased macrophage cyclooxygenases-1 and -2. **J. Geisel, J.A. Cook, and P.V. Halushka**. Med. Univ. of South Carolina.

63 **6.32** Detection of estrogen receptors in bone in vivo. **K.C. Westerlind, G. Sarkar, M.E. Bolander, and R.T. Turner.** Mayo Clin.

Poster

7. G-Protein-Linked Effectors

THURSDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

64 **7.1** Aldosterone stimulates protein acylation and G-protein synthesis in A6 cells. **M.D. Rokaw, P.M. Palevsky, and J.P. Johnson**. Univ. of Pittsburgh and VA Med. Ctr., Pittsburgh.

65 **7.2** Role of protein kinase C in angiotensin II-induced mitogenesis of neonatal rat cardiac fibroblasts. **G.W. Booz, H.H. Singer, and K.M. Baker**. Weis Ctr. for Res., Danville, PA.

66 **7.3** Na^+-selective channels in type II pneumocytes are regulated by G-proteins and arachidonic acid. **P.J. Kemp, G.G. MacGregor, and R.E. Olver**. Univ. of Dundee and Ninewells Hosp. & Med. Sch., Dundee, Scotland, UK.

67 **7.4** Effects of cyclic guanosine monophosphate on pregnant rat longitudinal smooth muscle. **R.E. Garfield and H. Izumi**. Univ. of Texas Med. Branch, Galveston.

68 **7.5** Purification and reconstitution of a G-protein-activated phospholipase C from squid photoreceptors. **J. Mitchell and J.K. Northup**. Univ. of Toronto and NIMH.

69 **7.6** Activation of phosphatidylinositol 3-kinase by the G_i-coupled acetylcholine muscarinic M_2 receptor. **M. Russell, S. Winitz, and G. Johnson**. Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver.

70 **7.7** Protein kinase C-α mediates both potentiation and desensitization of adenylylcyclase in human neurotumor SK-N-MC cells. **X-M. Zhou, P.K. Curran, J. Baumgold, M. Pak, and P.H. Fishman**. NIH and Geo. Washington Univ. Med. Ctr.

71 **7.8** Protein histidine phosphorylation in eukaryotes. **H.R. Matthews, K. Pesis, A.N. Hedge, S.K. Sharma, and M.R. Das**. Univ. California, Davis.

72 **7.9** Cloning of a novel rhodopsin-activated phospholipase C from squid retina. **H. Tamir, M.J. Brownstein, and J.K. Northup**. NIMH.

Poster

8. G-Protein-Linked Signaling and Gene Expressions

THURSDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

73 **8.1** A divergence in the MAP kinase regulatory network defined by MEK kinase. **C.A. Lange-Carter and G.L. Johnson.** Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver.

74 **8.2** A requirement for both heterotrimeric G-proteins and Ras in thombin signaling. **V.J. LaMorte, E. Kennedy, J.H. Brown, and J.R. Feramisco**. UCSD.

75 **8.3** Hormonal regulation of transmembrane signaling in neonatal rat ventricular myocytes. **S.W. Bahouth and E.A. Park**. Univ. of Tennessee.

76 **8.4** Involvement of calcium in the cholera toxin stimulated-prolactin gene expression in GH_3 cells. **J-H. Lin and F-F. Wang.** Yang-Ming Med. Col., Taipei, Taiwan.

77 **8.5** Implication of CK-2 and ERKs in mitogenic and cytotoxic signaling. **P. Agostinis, W. Merlevede, and J.R. Vandenheede**. K.U. Leuven, Belgium. W

78 **8.6** Developmental changes in cyclic nucleotides, G proteins and muscarinic receptors in heart cells. **R. Joyner, F. Rishi, and R. Kumar**. Emory Univ.

79 **8.7** Mechanism of cyclic strain-induced cAMP production in endothelial cells. **V.G. Manolopoulos and P.I. Lelkes.** Univ. of Wisconsin and Sinai Samaritan Med. Ctr., Milwaukee.

80 **8.8** Regulation of substance P receptor gene expression in astrocytoma cells. **T.R. Bai, D. Zhou, and B. Walker**. Univ. of British Columbia.

81 **8.9** Ras is required for the full mitogenic response to thyrotropin in Wistar rat thyroid cells. **E. Kupperman, W. Wen, and J. Meinkoth**. UCSD.

82 **8.10** Non-linear propagation of agonist-induced calcium waves in astroglia. **S. Yagodin, C.A. Sheppard, L.A. Holtzclaw, and J.T. Russell**. NIH.

83 **8.11** Extracellular ATP activates multiple second messenger systems and increases immediate-early gene expression in cardiac fibroblasts. **J-S. Zheng, M.O Boluyt, L. Song, W.H. Adler, L. O'Neill, M.T. Crow, and E.G. Lakatta**. NIA, NIH.

84 **8.12** Angiotensin II and phorbol esters stimulate c-fos gene expression in rat hepatocytes: possible roles of thyrosine phosphorylation and c-fos mRNA stabilization **C. González-Espinosa and J.A. García-Sáinz**. Univ Natl. Autonoma of Mexico, Mexico City.

Friday, November 19

Symposium

9. Growth Factors, Tyrosine Kinases, and Regulation I

Chaired: **John C. Cambier**, Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver

FRIDAY—Grand Ballroom A

9:00 **C. Ronald Kahn**. Joslin Diabetes Ctr., Boston.
9:25 Discussion.
9:30 **Morris White**. Joslin Diabetes Ctr., Boston.
9:55 Discussion.
10:00 **Morris J. Birnbaum**. Harvard Med. Sch.
10:25 Discussion.
10:30 Break.
10:45 **Daryl K. Granner**. Vanderbilt Univ.
11:10 Discussion.
11:15 **Graeme I. Bell**. Univ. of Chicago.
11:40 Discussion.
11:45 General discussion.

Symposium

10. Growth Factors, Tyrosine Kinases, and Regulation II

Chaired: **C. Ronald Kahn**, Joslin Diabetes Ctr., Boston

FRIDAY—Grand Ballroom A

2:00 **Lewis T. Williams**. UCSF.
2:25 Discussion.
2:30 **John C. Cambier**. Natl. Jewish Ctr. of Immunol. & Resp. Med., Denver.
2:55 Discussion.
3:00 Break.
3:15 **Liliana Attisano**. Mem. Sloan Kettering Cancer Ctr.
3:40 Discussion.

3:45 **Craig C. Malbon**. SUNY, Stony Brook.
4:10 Discussion.
4:15 General discussion.

11. Banquet Lecture

Chaired: **James M. Wilson**. Univ. of Pennsylvania.

FRIDAY 9:00 PM—Grand Ballroom A

Poster

12. Growth Factor Receptors

FRIDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

1 **12.1** Signal transduction by the type II and type I TGFβ receptors. **P.I. Knaus, M.B. Hille, H.Y. Lin, A. Moustakas, and H.F. Lodish.** Whitehead Inst. Biomed. Res., Cambridge, MA.

2 **12.2** Cloning of columbid prolactin receptor, a unique member of the cytokine/growth hormone/prolactin receptor family. **X. Chen and N.D. Horseman**. Univ. of Cincinnati.

3 **12.3** Mapping the receptor binding/activation residues of human EGF by protein engineering. **S.K. Niyogi, S.R. Campion, D.K. Takaki, M.R. Hauser, and J.S. Cook.** Oak Ridge Natl. Lab.

4 **12.4** Transfection of the HER2 receptor into CHO and 293 cells does not produce cells responsive to heregulin stimulation. **D. Antoniucci, S. Chan, K. Fok, L. Alajoki, M. Hirst, and H.G. Wada**. Molec. Devices Corp., Menlo Park, CA.

5 **12.5** Malonyl CoA: a marker for hyperinsulinemia-insulin resistance. **A.K. Saha, T.G. Kurowski, and N.B. Ruderman**. Boston Univ. Med. Ctr.

6 **12.6** EGF-induced growth inhibitor or stimulation of A431 and subline cells is directly correlated with receptor tyrosine kinase concentration but not with PLC γ activity. **J-K. Chen and S.S. Lin**. Chang Gung Med. Col., Taoyuan, Taiwan.

7 **12.7** Heparin inhibits serum stimulation of mitogen-activated protein kinase in vascular smooth muscle. **E.M. Langan, III, J.R. Youkey, and H.A. Singer.** Weis Ctr. for Res., Danville, PA.

8 **12.8** Developmental changes in insulin signal transduction in fetal rat hepatocytes. **J.R. Smith-Hall, L.V.V. Faur, D.C. DeSante, and D.E. Peavy**. Indiana Univ. and VA Med. Ctr., Indianapolis.

9 **12.9** LY294002 is a potent and specific inhibitor of phosphatidylinositol 3-kinase. **C.J. Vlahos, W.F. Matter, and R.F. Brown**. Lilly Res. Labs.

10 **12.10** Evidence for neurotrophin receptor expression and mutability in the dorsal column nuclei of the brainstem of the rat. **D.R. Foschini, D.P. Crockett, and M.D. Egger**. UMDNJ-R.W. Johnson Med. Sch., Piscataway, NJ.

11 **12.11** Osmotic enhancement of mitotic stimulation in rat thymocytes. **Y. Zilberman, V. Melnikov, and Y. Gutman**. Hebrew Univ., Jerusalem, Israel.

Poster

13. Tyrosine Kinases

FRIDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

12 **13.1** Activation of mitogen-activated protein kinases by angiotensin II and growth factors in vascular smooth muscle from spontaneously hypertensive and Wistar-Kyoto rats. **P.A. Lucchesi, R.A. Redden, and B.C. Berk**. Emory Univ.

13 **13.2** Identification of prolactin-induced tyrosine phosphorylated 125 kDa protein as focal adhesion kinase. **S.E. Fenton and L.G. Sheffield**. Univ. of Wisconsin.

14 **13.3** Prolactin inhibits epidermal growth factor-stimulated 42 kDa MAP kinase tyrosine phosphorylation and activity. **L.G. Sheffield and S.E. Fenton**. Univ. of Wisconsin.

15 **13.4** Tyrosine kinase regulates the Ca^{2+} current activated by thapsigargin and carbachol in jurkat cells transfected with the human muscarinic receptor 1. **S.C. Chung and P. Gardner**. Stanford Univ.

16 **13.5** Differences in protein interactions with the insulin receptor. **D.A. Martin, M. McCaleb, and J. Livingston**. Yale Univ. and Miles Pharmaceuticals, Inc.

17 **13.6** Implication of tyrosine kinase in the cold-mediated contraction of newborn lamb cerebral arteries. **L.C. Wagerle, G. Speziali, and P. Russo**. Temple Univ.

18 **13.7** Tyrphostins inhibit CCK8, guanosine 5'-(3-O-thio)triphosphate-induced inositol 1,4,5-triphosphate production and amylase secretion in pancreatic acinar cells. **A. Piiper, D. Stryjek-Kaminska, W.F. Caspary, and S. Zeuzem**. Univ. of Frankfurt, Germany.

19 **13.8** Mouse osteopontin expressed in E. coli is autophosphorylated on tyrosine residues. **R.A. Saavedra, S. Ashkar, and M.J. Glimcher**. Harvard Med. Sch. and Children's Hosp., Boston.

Poster

14. Tyrosine Kinases and Gene Expression

FRIDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

20 **14.1** The intracellular signaling pathway for vascular smooth muscle cell migration in response to PDGF involves calcium/calmodulin-dependent protein kinase II. **M.T. Crow, R.R. Pauly, J-S. Zheng, and E. Lakatta**. NIA, NIH.

21 **14.2** Differential display identifies changes in transcription pattern of PC12 cells in response to NGF stimulation and K-ras transduction. **R. Somogyi, M.G. Alessandri, S. Almeida, J. Strohkorb, X. Wen, J. Barker, and D.L. Simpson.** NIH.

22 **14.3** Signal induction and coordination of cellular enzymatic activities: coordination of protein phosphorylation and glycosylation. **A.A. Hakim**. Kankakee, IL.

Poster

15. Mechanisms of Gene Regulation

FRIDAY—Exhibit Hall

Authors will be in attendance from 12:30 to 2:00 pm.

Board #

23 **15.1** Regulation of cellular retinoic acid-binding proteins -I and -II gene expression by retinoic acid and transforming growth factor-β in primary cultures of developing palate cells. **P. Nugent, K. Shah-Quazi, and R.M. Greene**. Thos. Jefferson Univ.

24 **15.2** Differential regulation of immediate early genes in rat uterine epithelium by estrogen and progesterone. **R.M. Bigsby and L. Aixin**. Indiana Univ.

25 **15.3** Regulation of expression: classical and non-classical HLA antigen expression by trophoblasts. **M.H. Chiang, G.T. Colbern, and E.K. Main**. CPMC Res. Inst., San Francisco.

26 **15.4** Hypoxia increases β2-adrenergic receptor mRNA in mammalian cells. **J.F. Schmedtje, Jr., and W.L. Liu**. Univ. of Texas Med. Branch, Galveston.

27 **15.5** Regulation of gastric inhibitory peptide gen expression by a glucose meal. **M.M. Wolfe, L.A. Jarboe, and C-C. Tseng**. Brigham & Women's Hosp. and Harvard Med. Sch.

28 **15.6** Multiple levels of basic fibroblast growth factor regulation in catecholaminergic cells. **M.K. Stachowiak, J. Moffett, A. Joy, R. Florkiewicz, and E.K. Stachowiak**. Barrow Neurol. Inst., Phoenix and Whittier Inst., La Jolla.

29 **15.7** The roles of dyad symmetry elements in basal and acetylcholine-stimulated expression of the tyrosine hydroxylase gene. **E. Kim, S. Maltchenko, and M.K. Stachowiak**. Barrow Neurol. Inst., Phoenix.

30 **15.8** The potential involvement of an inducible orphan nuclear receptor, T1S1, in transcriptional regulation of muscle creatine kinase gene in skeletal muscle cells. **W-L. Yang and R.W. Lim.** Univ. of Missouri.

31 **15.9** Evidence for the involvement of an Id-like protein, HLH462, in the regulation of skeletal muscle specific gene expression. **B. Chen, B.H. Han, and R.W. Lim**. Univ. of Missouri.

32 **15.10** Involvement of a labile repressor activity in modulation of endotoxin tolerance. **K.E.A. LaRue and C.E. McCall.** Bowman Gray Sch. of Med.

33 **15.11** Extracellular Ca^{2+} modulates the prereplicative intracellular cyclic AMP surges in EGF-stimulated primary neonatal rat hepatocytes. **U. Armato, M. Ribecco, C. Guerriero, and J.F. Whitfield**. Univ. of Verona, Italy and Natl. Res. Council of Canada, Ottawa.

34 **15.12** Activation of the type -B natriuretic factor gene in mouse P19 and D3 stem cell cultures induced for cardiac myogenesis. **P.H. Boer, J. Phipps, and Z. Rassi.** Univ of Ottawa Heart Inst. and Natl. Res. Council of Canada, Ottawa.

35 **15.13** Positive regulation of the cdc2 promotor by c-myc overexpression. **T.L. Born, J.A. Frost, and J.R. Feramisco**. UCSD.

36 **15.14** An in vitro model to study signaling mechanisms of programmed cell death. **R. Halaby, R.A. Lockshin,**

and Z.F. Zakeri. Queen's Col./CUNY, Flushing, NY and St. John's Univ., Jamaica, NY.

37 **15.15** Alterations in $\alpha_{1\beta}$-adrenoceptor mRNA expression during cyclic stretch is cell-type specific in cultured vascular smooth muscle. **M.S. Lundberg, D.N. Sadhu, K.S. Ramos, and W.M. Chilian**. Texas A&M Univ.

38 **15.16** Minimally oxidized low density lipoprotein-induced cAMP levels inhibit ELAM expression and neutrophil binding to endothelial cells. **F. Parhami, A.M. Fogelman, M.C. Territo, and J.A. Berliner**. UCLA.

Saturday, November 20

Symposium

16. Mechanisms of Gene Regulation

Chaired: **Gary L. Johnson**, Natl. Jewish Ctr. for Immunol. & Resp. Med., Denver

SATURDAY—Grand Ballroom A

9:00 **Donald D. Brown**. Carnegie Inst. of Washington, Baltimore.
9:25 Discussion.
9:30 **Gregor Eichele**. Baylor Col. of Med.
9:55 Discussion.
10:00 Break.
10:30 **Richard Firtel**. UCSD.
10:55 Discussion.
11:00 **Harvey F. Lodish**. Whitehead Inst. for Biomed. Res., Cambridge, MA.
11:25 Discussion.
11:30 General discussion.

Symposium

17. New Strategies for Molecular Studies of Regulation

Chaired: **Craig C. Malbon**, SUNY, Stony Brook

SATURDAY—Grand Ballroom A

2:00 **Hsien-yu Wang**. Natl. Defense Med. Ctr., Taipei, Taiwan.
2:25 Discussion.
2:30 **Klim King**. Inst. of Biomed Sci., Taipei, Taiwan.
2:55 Discussion.
3:00 **Günter Schultz**. Free Univ. of Berlin, Germany.
3:25 Discussion.
3:30 **Christopher Moxham**. SUNY, Stony Brook.
3:55 Discussion.
4:00 General discussion.

FORTY YEARS OF MEMBRANE CURRENT IN NERVE

Guest Editor: Daniel Gardner, Cornell University Medical College

This coordinated set of modern reviews commemorates the fortieth anniversary of the publication of the Hodgkin, Huxley and Hodgkin, Huxley and Katz papers. It examines, from a contemporary perspective, the near-universal influence of these pivotal physiological works. Present and future views of ions, channels, and membranes in all tissues reflect the quantitative description of currents in squid axon.

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Signal Transduction and Gene Regulation

November 17–20, 1993
San Francisco, California

Sessions with Contributed Abstracts

Thursday

Friday

5.1

DEPRESSED GTP STIMULATORY PROTEIN (G_s) IN AGING MONKEYS. D.E. Vatner, K. Kiuchi*, N. Sato*, Y-T. Shen, and S.F. Vatner., Children's Service, Mass General Hospital, Harvard Medical School, New England Reg Primate Res Center, Southborough MA 01772

To examine age-related cardiovascular function in a model free from atherosclerosis, 4 young adult (3±1 yrs) and 8 old (16±1 yrs) monkeys (macaca cyclopis) were chronically-instrumented with a left ventricular (LV) pressure gauge, aortic and left atrial catheters, and aortic flow probe. There were no significant differences in baseline hemodynamics. However, inotropic responses to isoproterenol (ISO, 0.1 μg/kg) were significantly depressed in older monkeys, i.e., smaller increases ($p<0.05$) in LV dP/dt in response to ISO (young: +4253±553 vs. old: +2129±168 mmHg/sec). ß-adrenergic receptor density (^{125}I cyanopindolol) was decreased with age (young: 49±4 vs. old: 29±2 fmol/mg, $p<0.05$). G_s reconstituted activity was depressed ($p<0.05$) in older monkeys (young: 4.4±0.3 vs. old: 2.7±0.2 pmol/10 min/μg). Also, manganese-stimulated adenylyl cyclase activity decreased with age (young: 138±7 vs. old: 74±13 pmol cAMP/min/mg, $p<0.05$). Thus, inotropic responses to sympathomimetic amines were attenuated in aging conscious monkeys. The mechanism of depressed inotropic responsiveness to sympathomimetic amines involves down-regulation of ß-adrenergic receptors, depressed adenylyl cyclase catalytic unit activity, and depressed G_s functional activity.

5.2

PHOSPHORYLATION OF THE C-TERMINAL TAIL OF THE PTH/PTHrP RECEPTOR BY PROTEIN KINASE C. Eberhard Blind*, Mary W. Fowlkes*, Stacy Pratt*, Tsui-Hua Chen*, Dolores Shoback*, Robert A. Nissenson, Endocrine Unit, VAMC and University of California, San Francisco, CA 94121.

Some G-protein-coupled receptors are desensitized by phosphorylation in their cytoplasmic domains by serine/threonine kinases. The present studies were conducted to determine whether desensitization of the PTH/PTHrP receptor elicited by protein kinase C (PK-C) activation might involve direct phosphorylation of the receptor by PK-C. In vitro transcribed RNA derived from the PTH receptor plasmid "OK-O" was injected into *Xenopus laevis* oocytes and 48 hours later the effect of bPTH(1-34) (3 μg/ml for 20 min) on Ca_i^{++} mobilization was estimated by measurement of $^{45}Ca^{++}$ efflux. Activation of PK-C by addition of Phorbol-12-myristate-13-acetate (PMA, 200 nM) 20 min prior to PTH resulted in 80 % inhibition of PTH-stimulated $^{45}Ca^{++}$ efflux (522 ± 132 cpm vs. 2610 ± 173 cpm per 20 min in nonpretreated oocytes). Previous studies have suggested that the C-terminal tail of the PTH/PTHrP receptor may play an important role in Ca_i^{++} signalling. We expressed the 129 amino-acid C-terminal tail of the rat PTH/PTHrP receptor in *E. coli* as the carboxyl portion of a recombinant glutathione S-transferase (GST) fusion protein. Purification of bacterial extracts by glutathione affinity chromatography yielded a major 42 kDa product (the expected size of the fusion protein) as well as 5 lower M_r proteolytic products that contained GST and varying amounts of the receptor's C-terminal tail. Addition of purified PK-C in the presence of [γ-^{32}P]ATP resulted in Ca^{++}/ phospholipid-dependent phosphorylation of each of these fusion proteins, but not of GST itself. In summary, activation of PK-C with PMA produced desensitization of PTH/PTHrP receptor-mediated Ca_i^{++} signalling in *Xenopus oocytes*. This may be due to direct phosphorylation of the C-terminal tail of the receptor by PK-C, the precise sites of which remain to be determined.

5.3

EFFECTS OF GUANOSINE NUCLEOTIDES AND PHORBOL 12,13-DIBUTYRATE ON SKINNED MUSCLE TISSUES OF PREGNANT RAT MYOMETRIUM. Hidetaka Izumi* and R.E. Garfield. University of Texas Medical Branch, Galveston, Tx, 77551.

In this study the phenomenon of agonist-induced Ca^{2+} sensitization of the contractile proteins as investigated in pregnant rat myometrium. After application of carbachol or oxytocin, spontaneous myometrial contractions were much greater than before treatment with these compounds. In the ß-escin treated skinned myometrial fibers (membrane permeable conditions), various concentrations of Ca^{2+} (0.1 μM-10 μM) produced phasic followed by tonic contractions. Phorbol 12,13-dibutyrate (PDB, 1 nM) (activator of protein kinase C, PCK) increased the tonic parts of these Ca^{2+}-induced contractions and these were antagonized by [1-(5-isoquinolinesulfonyl)-2-methylpiperazine, diHCl] (H-7, inhibitor of PKC). PDB (1 nM) also increased the contractions induced by cumulatively applied concentrations of Ca^{2+}. However, contractions induced by Ca^{2+} were inhibited by pre-treatment with higher PDB concentrations (1 mM). GTPγS (0.1-100 μM), carbachol (0.1mM-1 mM) and oxytocin (0.1 nM-1 μM) also increased the tonic parts of 0.3 μM Ca^{2+}-induced contractions and these were antagonized by 1 mM GDPβS. GTPγS (10 μM) and oxytocin (0.1 μM) also increased the contractions induced by increasing concentrations of Ca^{2+}. Maximum responses to Ca^{2+} were enhanced by application of PDB, GTPγS, carbachol and oxytocin. Ca^{2+} sensitization to either GTPγS or PDB was not inhibited respectively by GDPßS or by H-7. These results suggest that PDB and GTPγS increase Ca^{2+} sensitivity to contractile proteins in pregnant rat myometrium. The increased sensitivity is possibly mediated via two independent pathways (ie. with or without phosphorylation of myosin light chain). In addition, this study suggests that agonists such as oxytocin have an important role to change sensitivity of the contractile apparatus and control uterine contractility.

5.4

CULTURED ASTROCYTIC GLIA CELLS ARE A GOOD MODEL FOR STUDYING REGULATION OF ALPHA$_{2A/D}$ ADRENOCEPTORS. Elaine M. Richards*, Michael A. Reutter, Mohan K. Raizada and Colin Sumners. University of Florida, Gainesville, FL 32610.

We are interested in the regulation of alpha$_2$-adrenergic receptors (α_2R) in the central nervous system, and are using primary cultures of neurons and astrocytic glia for this purpose. Astrocytic glia cultured from 1-day-old, 21-day-old and adult (3–9-month-old) rats express $\alpha_{2A/D}$ receptors. This has been demonstrated by mRNA analysis by α_{2D}-specific DNA probes (RG20), by Western blotting with α_{2A}-specific antibodies, ^{3}H-yohimbine binding studies and their functional connections to at least one second messenger by measurement of intracellular cyclic 3',5'-adenosine monophosphate (cAMP) levels. Functional $\alpha_{2A/D}$R numbers are greater in astrocytic glia cultured from 21-day-old or adult rats compared to those from 1-day-old rats, by Northern blotting, Western blotting, binding studies or cAMP analysis. Insulin downregulates α_2R of astrocytic glia cultured from 1-day-old rats in a time- and dose-dependent fashion. We are currently investigating whether this also occurs in astrocytic glia cultured from adult rats. This is a useful model system because the cells express the receptor in large numbers but are neither transformed nor transfected and therefore perhaps closer to a normal physiological state. (EMR is an American Heart Association, Florida Affiliate, Inc., Research Fellow. Supported in part by NS-19441.)

5.5

INSULIN-PROMOTED PHOSPHORYLATION OF THE β_2-ADRENERGIC RECEPTOR. Manon Valiquette*, Stéphane Parent* and Michel Bouvier* Dépt. de Biochimie et Groupe de Recherche sur le Système Nerveux Autonome, Univ. de Montréal, Montréal, Qc, Canada, H3C 3J7.

Several studies have reported a modulatory influence of insulin on β-adrenergic-stimulated adenylyl cyclase activity. Such cross-talk regulation between the insulin and the β-adrenergic signalling pathways could contribute to the fine tuning of their antagonistic actions on glucose and lipid metabolism. Molecular mechanisms involved in this regulatory process remain largely unknown. In this study, we aimed to characterize molecular events mediating insulin actions at the level of the β_2-adrenergic receptor (β_2AR). The ability of insulin to promote phosphorylation of the human β_2AR was therefore assessed in Chinese hamster fibroblasts (CHW), in which the human β_2AR cDNA was transfected. Cells were metabolically labelled with $^{32}P_i$ and the β_2AR purified by affinity chromatography. Treatment of the cells with insulin (1μM) for 30 min induced a 1.5 fold increase in the incorporation of $^{32}PO_4$ in the β_2AR. Following purification, the receptor was hydrolysed and $^{32}P_i$-labelled phosphoamino acid residues separated by electrophoresis on cellulose plates followed by ascending chromatography. This phosphoamino acid analysis revealed that the phosphorylation on tyrosine as well as on serine and threonine residues was increased by insulin treatment. In addition, immunoblots using a polyclonal anti-phosphotyrosine antibody, confirmed the presence of phosphotyrosine residues in β_2AR purified from either control or insulin treated cells. Quantitative analysis of the immunoreactivity revealed that insulin treatment increased the phosphotyrosine content of the receptor by 2.4 fold. In an effort to determine which tyrosine residues were phosphorylated upon insulin treatment, we first assessed the mutant [Ala^{350}, Ala^{354}]β_2AR in which Tyr-350 and Tyr-354, located in the carboxyl tail of the β_2AR, were replaced by alanines. This mutation which was previously shown to impair agonist-promoted down-regulation of the receptor number (Valiquette et al., Proc. Natl. Acad. Sci. 87, 5089-5093) had no effect on the phosphorylation pattern. This suggests that Tyr-350 and Tyr-354 do not correspond to the site(s) of tyrosine phosphorylation in the β_2AR. Further studies will be required to identify the residues which represent the site of insulin-promoted phosphorylation of the β_2AR.

5.6

IDENTIFICATION OF STRUCTURAL ELEMENTS IN THE PTH/PTHrP RECEPTOR CRITICAL FOR G-PROTEIN COUPLING. Zhengmin Huang*, Ying Chen*, Tom Bambino*, and Robert A. Nissenson, Endocrine Unit, VA Medical Center, Dept. of Medicine and Physiology, UCSF, San Francisco, CA 94121

High affinity agonist binding and adenylate cyclase (AC) activation by the parathyroid hormone (PTH)/ PTH-related protein (PTHrP) receptor requires coupling of G-proteins. To identifying structural determinants in the opossum kidney (OK) PTH/PTHrP receptor critical for signaling, we utilized tandem alanine scanning mutagenesis to replace stretches of four consecutive amino acid residues with alanines. Seven such mutant receptors scanning the entire third intracellular loop (IC-3) were constructed and transiently expressed in COS-7 cells. To our surprise, most of the mutant receptors retained high affinity bPTH(1-34) binding and AC stimulation indistinguishable from that of the wild type (WT) receptor. However, two mutation stretches in the juxtramembrane regions of IC-3 (one on each end) resulted in PTH receptors with WT PTH binding affinity (IC_{50}= 1nM) but with reduced efficiency of coupling to AC (EC_{50}= 1.3nM vs. WT EC_{50}= 0.1nM). To assess the function of the 127 aa C-terminal tail (IC-4), we constructed a series of mutant OK PTH/PTHrP receptors with progressive C-terminal truncations and expressed them in COS-7 cells. Receptors with a tail as short as 16 residues retained high affinity PTH binding and AC activation similar to WT. However, receptors with an 8-residue tail displayed decreased PTH binding (20% of WT) and slightly decreased AC (70% of WT). Complete truncation of IC-4 resulted in a loss of both functions. Immunochemistry revealed that WT receptors were expressed diffusely in transfected COS cells whereas mutant receptors lacking IC-4 displayed distinct perinuclear localization. In summary: 1) most side-chains in IC-3 of the PTH/PTHrP receptor are not essential for high affinity PTH binding or AC activation; 2) side-chains from eight AA residues in the juxtamembrane regions of IC-3 contribute to activation of AC but may not be required for receptor-G-protein-association *per se*; 3) most of the C-terminal tail is not required for high affinity PTH binding or AC activation; and 4) a small portion of the tail (16 residues) adjacent to the membrane may contain specific information required for proper expression and function of the PTH/PTHrP receptor.

5.7

THE RAT SSTR1 AND SSTR2 SOMATOSTATIN RECEPTOR SUBTYPES MEDIATE INHIBITION OF cAMP ACCUMULATION BY PERTUSSIS TOXIN-SENSITIVE G-PROTEINS. Joann Strnad*, C. Mark Eppler*, and John R. Hadcock*. American Cyanamid Co., Princeton, NJ. 08543-0400

The rat SSTR1 and SSTR2 somatostatin receptor subtypes have been cloned and stably expressed in Chinese Hamster Ovary (CHO-K1) cells. To determine whether or not these receptor subtypes couple to G-proteins, we examined the ability of GTPγS and pertussis toxin to alter somatostatin (S-14) binding in membranes prepared from cells transfected with each subtype. In the presence of 100 μM GTPγS, [^{125}I] S-14 binding to SSTR1 and SSTR2 decreased to 25% and 43% of the controls, respectively. Cyclic AMP accumulations showed S-14 to inhibit forskolin stimulated cAMP accumulation by 40% and 75% in cells expressing SSTR1 and SSTR2, respectively, and this inhibition was dose dependent (ED_{50} of 350 pM and 340 pM, respectively). S-14 binding studies with pertussis toxin and immunoprecipitations with antisera raised against $G_{i\alpha1,2}$, but not $G_{s\alpha}$, also suggest that SSTR1 couples to G-proteins. From these data, we conclude that the rat SSTR1 and SSTR2 somatostatin receptor subtypes expressed in CHO-K1 cells are coupled to G-proteins and mediate inhibition of cyclic AMP accumulation in intact cells.

5.8

TWO DISTINCT ENDOTHELIN HUMAN ENDOTHELIN B RECEPTORS GENERATED BY ALTERNATIVE SPLICING FROM A SINGLE GENE. Venkatakrishna Shyamala*, Thomas H. M. Moulthrop, Jennifer Stratton-Thomas and Patricia Tekamp-Olson . Chiron Corporation, 4560 Horton St., Emeryville CA 94608.

A novel variant of endothelin B receptor (ETB) has been found in human brain, placenta, lung and heart by reverse transcriptase polymerase chain reaction. This variant receptor referred to as ETB1 has an additional 30 nucleotide sequence with splice sites at both ends. This results in a ten amino acid increase in the length of the second cytoplasmic loop of ETB. Polymerase chain reaction on genomic DNA indicates that this sequence is part of the 134 bp intron which separates the second and third exons and is contiguous with the third exon of the ETB gene. Through Southern analysis of chromosomal DNA and genomic PCR we suggest that this variant arises by alternative RNA splicing of the single copy ETB gene. The insert sequence in ETB gene is absent in bovine, rat, and porcine DNA, and is unique to human DNA. Functional expression of the ETB and ETB1 in COS cells indicates that both receptors encode fully functional endothelin receptors. Ligand binding properties using ET3 and ET1 indicate no difference between the two receptors. Studies to determine other biological functions are under way.

5.9

POSSIBLE FUNCTIONAL COUPLING OF A GTP-BINDING PROTEIN TO DOPAMINE D2 RECEPTOR. Shakeel M. Farooqui* and Charles D. Gulley* Section of Neurobiology, Pennington Biomed. Res. Ctr., Baton Rouge, LA 70808.

The multitude of dopamine-mediated pharmacological and physiological effects are due to its interaction with dopamine receptors, classified as D1 through D5. The dopamine D2 receptor (DR) from rat and human brain has been cloned and sequenced; however, the identity of the chemical make up of functional DR remains unclear. A full length cDNA clone for DR codes for a 47 kDa core protein, which is fully functional when expressed in cultured cells. However, the molecular weight (MW) of DR, purified to homogeneity by affinity chromatography, ranges between 95-110 kDa. The receptor core protein undergoes post-transcriptional modifications which result in ligand binding and a 2-2.5 fold increase in MW. The apparent increase in the MW was investigated for the coupling of a GTP-binding protein with receptor complex. Here we provide evidence for a possible covalent coupling of DR with a GTP-binding protein. UV cross linked [α-^{32}P]GTP labeled striatal proteins were identified by SDS-PAGE and phosphor-Image analysis. [α-^{32}P]GTP specifically labeled 4 major proteins (MW 110, 49, 43 and 40). The labeling of 110 and 49 kDa proteins was increased by D2, but not D1 agonist and specifically blocked by (-)sulpride, a D2 antagonist. Addition of an inactive isomer, (+)sulpride had little effect on the labeling of 110 and 49 kDa proteins. Immunoprecipitates of ^{32}P-labeled striatal proteins with DR antibody enriched three proteins of MW 110 and 88 and 74 kDa. The identity of DR associated 110 kDa G-protein (DRAG) was investigated by Western Blotting using specific G-protein subunit antibodies. Amino terminal but not carboxy terminal Gβ subunit peptide antibody specifically labeled a 110 kDa protein. Partial purification of DR on wheat germ agglutinin affinity column resulted in enrichment of 110 kDa DRAG detected by DR and NH_2 terminal Gβ antibody. These data suggest that 110 kDa DRAG may contain DR core protein. Supported by US Army grant # DAMD17-92-V2009.

5.10

MURINE GASTRIN RELEASING PEPTIDE RECEPTOR EXPRESSION IN KNRK CELLS. Lee W. Slice, Helen Wong, Adella Garland[1], Nigel Bunnett[1]*, and John H. Walsh*. CURE: VA/UCLA, Wadsworth VAMC, Sawtelle and Wilshire Blvds., Los Angeles, CA 90073 and [1]Departments of Surgery and Physiol., UCSF, San Francisco, CA 94143-0660.

A rat kidney epithelial cell line (KNRK) was transfected with a murine gastrin releasing peptide (GRP) receptor expression vector containing a "flag" epitope (sequence: DYKDDDDK) at its amino terminus. Expression of the flag-GRP receptor was detected by western blots of whole cell lysates using antibodies against the rat GRP receptor. Functional analysis of the flag-GRP receptor was done by measuring the binding affinity to ^{125}I labeled GRP. Scatchard plot analysis indicated that the transfected KNRK cells contained 27,000 receptors per cell with a K_d of 0.76 nM for GRP binding. GRP binding induced intracellular calcium mobilization. Fluorescent spectrophotometry using the calcium indicator, Fura-2, measured an EC_{50}= 1 nM to GRP binding for $[Ca^{++}]_i$ response. GRP induced receptor internalization was also measured. An acid wash procedure (0.2 N acetic acid, 0.5 M NaCl) was used to remove surface bound GRP. Cell associated GRP that is resistant to acid washing was considered internalized along with the receptor. KNRK cells expressing flag-GRP receptors were pre-incubated with ^{125}I GRP at 4 C for one hour. Internalization is blocked at 4 C. The temperature was raised to 37 C . After 5 minutes, 34% of the total bound GRP was resistant to acid washing. By 10 minutes, 59% of the bound GRP was internalized. At 20 and 30 minutes, the amount of internalized GRP was 70% of the bound total. The presence of 0.45 M sucrose significantly decreased the amount of internalized GRP with the highest levels occurring at 30 minutes (12% of bound GRP). Supported by NIH DK 444923.

5.11

A CLONED CCK_A RECEPTOR TRANSDUCES MULTIPLE SIGNALS ON STIMULATION BY FULL AND PARTIAL AGONISTS. *D.I.Yule,* M.J. Tseng, J.A. Williams and C.D. Logsdon.

CHO cells, stably transfected with the cloned rat CCK_A receptor were used to study signal transduction events initiated by CCK-8 and the partial agonist JMV-180. In single CHO-CCK_A cells, loaded with fura-2, superfusion of CCK-8 (10 pM-1 nM) resulted in an increase in $[Ca^{2+}]_i$. At CCK-8 concentrations below 100 pM the signal consisted of $[Ca^{2+}]_i$ oscillations. At higher concentrations, CCK-8 induced a typical biphasic response consisting of a large peak followed by a lower sustained plateau. Superfusion of JMV-180 also resulted in an increase in $[Ca^{2+}]_i$; in contrast to acinar cells this increase did not consist exclusively of $[Ca^{2+}]_i$ oscillations. Both CCK-8 and JMV-180 increased polyphosphoinositide hydrolysis, although JMV-180 stimulated the formation of only 10% as much [^{3}H]inositol phosphates. $[Ca^{2+}]_i$ signals stimulated by both CCK-8 and JMV-180 were blocked by the aminosteroid U73122. CCK-8 (1- 10 nM) increased the formation of cAMP in CHO-CCK_A cells. This increase was not mimicked by JMV-180 (10 μM). Furthermore no cAMP formation could be detected when cells were incubated with both JMV-180 and CCK-8. CCK-8 induced a 3 fold increase in arachidonic acid release, while JMV-180 caused no measurable increase and concurrent incubation blocked the stimulation induced by CCK-8 alone. These data indicate that in CHO-CCK_A cells, unlike acinar cells, both CCK-8 and JMV-180 increase $[Ca^{2+}]_i$ by similar mechanisms. However, the CCK_A receptor can differentially recognize and then activate discrete transduction pathways on binding of these two agonists.

5.12

FUNCTIONAL PROPERTIES OF THE N-FORMYL PEPTIDE RECEPTOR EXPRESSED IN UNDIFFERENTIATED HL60 CELLS. Eric R. Prossnitz*, Oswald Quehenberger,* Charles G. Cochrane* and Richard D. Ye*. The Scripps Research Institute, La Jolla CA 92037

Differentiated HL60 cells respond to challenge with ligand by superoxide production, degranulation and actin polymerization with subsequent chemotaxis and phagocytosis. The capabilites of undifferentiated HL60 cells have not been well characterized due to the absence of cell surface receptors. In order to investigate these properties, undifferentiated HL60 cells were transfected with the N-formyl peptide receptor (FPR). Expression of the recombinant FPR gene product in FPR-transfected HL60 cells and the absence of the endogenous FPR in vector-transfected HL60 cells was demonstrated. FPR-transfected HL60 cells retained their ability to undergo granulocytic differentiation with dibutyryl cAMP. Furthermore, incubation of FPR-transfected HL60 cells with fMLP resulted in limited differentiation. Binding studies of FPR-transfected HL60 cells demonstrated the presence of two binding affinities (Kd's = 0.6 and 33 nM), contrasting the single high affinity state of the FPR expressed in mouse L cell fibroblasts (Kd = 1 nM). FPR-transfected HL60 cells displayed fMLP-dependent calcium mobilization and actin polymerization, both of which were sensitive to pretreatment with pertussis toxin. Stimulation of HL60 cells with ATP resulted in pertussis toxin insensitive calcium mobilization but was ineffective in producing actin polymerization. The results described here show for the first time that undifferentiated HL60 cells can respond to chemoattractant receptor stimulation with many of the properties of the mature neutrophil. Transfected HL60 cells will provide an excellent system to study the characteristics of chemotactic receptors as well as the functional properties of myeloid cells.

5.13

USE OF Giα SITE-DIRECTED MUTANTS TO STUDY SPECIFICITY OF RECEPTOR-MEDIATED INHIBITION OF ADENYLYL CYCLASE.
Susan E. Senogles, Dept. of Biochemistry, University of Tennessee, Memphis, TN 38163.

Site directed mutations of Gi1, Gi2, and Gi3 α subunits were constructed which substituted glycine for cysteine at the C terminal cysteine position, the residue which becomes modified by pertussis toxin. After transfection into cells, the mutated Giα subunits are resistant to the covalent ADP-ribosylation by pertussis toxin, and are functional after treatment with pertussis toxin. GH4C1 cells, a pituitary tumor derived cell line, were used to generate stably transfected cell lines of each of the alternate splice forms of the D2 dopamine receptor, with the three individual Gi α mutant subunits. The ability of dopamine agonists to mediate inhibition of adenylyl cyclase in individual clones was assessed in untreated control cells and cells which had been treated overnight with 20 ng/ml pertussis toxin. Using clonal lines generated by transfection with the short form of the D2 dopamine receptor and Gi2α, dopamine agonists inhibited adenylyl cyclase with the same affinity before and after toxin treatment, as did clonal lines transfected with the long form of the dopamine receptor and Gi3α. Inhibition of adenylyl cyclase was not observed with clonal lines transfected with either form of D2 dopamine receptor and Gi1α. In contrast, somatostatin appeared to inhibit adenylyl cyclase in clonal lines transfected with Gi1α and Gi3α, but not Gi2α. These results suggest that several receptors may couple to the same effector through distinct G proteins. (Supported by NIH NS28811)

5.14

BETA-ADRENERGIC RECEPTOR ACTIVITY IN LIVER MEMBRANES OF MICE WITH OBESITY DIABETES SYNDROMES. Nicole Bégin-Heick, University of Ottawa, Ottawa On. Canada K1N 6N5

Catecholamine stimulation ellicits three times more adenylyl cyclase (AC) activity in membranes isolated from livers of ob/ob mice compared to lean mice. By contrast, similar levels of AC activation are found in db/db mice and their lean controls. Binding studies with 125I-iodopindolol show that the number of beta-adrenergic receptor binding sites is also elevated ca. three fold in the ob/ob as compared to lean samples, but not in samples from db/db mice compared to their lean controls. Both mutations (*ob* and *db*) produce hypercortiscism. To find out if hypercortiscism is responsible for beta-receptor hyperactivity, groups of ob/ob mice were adrenalectomized (ADX) or sham (SHM) operated. ADX restored both catecholamine-stimulated adenylyl cyclase activity and receptor activity to levels observed in lean controls, but these activities remained elevated in the SHM group. These results show that the consequences of hypercortiscism on liver beta-adrenergic receptor activity are different in the two mutants, suggesting that the *ob* mutation leads to hypersensitivity of the liver beta-adrenergic receptor cascade to glucocorticoids.

5.15

ANGIOTENSIN II ACTIVATES ADENYLYL CYCLASE IN AT_{1A} RECEPTOR-TRANSFECTED CHO-K1 CELLS: ROLE IN INHIBITION OF CELL GROWTH. Jing Du, Thomas J. Thekkumkara, John C. Zwaagstra, John Krupinski and Kenneth M. Baker. Weis Center for Research, Danville, PA. 17822

Angiotensin II (AII) inhibits adenylyl cyclase (AC) activity in rat adrenal, liver and kidney, whereas it stimulates AC in hypertrophied rat cardiomyocytes, bovine adrenocortical cells and rat fetal skin fibroblasts. In AT_{1A} receptor-transfected CHO-K1 cells (T3CHO/AT_{1A}), AII (10^{-7}M) stimulated cAMP accumulation 21-56 fold above control. This stimulation was dose dependent (EC_{50}= 3.3 nM) and inhibited by the AT_1 nonpeptide receptor antagonist EXP 3174. AII-induced cAMP accumulation could be inhibited ($P<0.05$) with calmodulin antagonists TFP (30μg/ml) and W7 (80μg/ml) by 32 and 37%, respectively. However, neither 100 μM ATP, which also stimulated the release of calcium from intracellular stores in these cells, nor the calcium ionophores A23187 or ionomycin could mimic the stimulation of cAMP accumulation that is caused by AII. Thus, the AII-induced cAMP accumulation is not simply a result of the activation of a calmodulin-stimulated AC. While the precise mechanism of this effect remains unclear, the AII-induced stimulation of cAMP accumulation may be responsible for the inhibition of [^{3}H]thymidine incorporation and cell proliferation in T3CHO/AT_{1A} cells. A 24 hr exposure of these cells to 1μM AII inhibited [^{3}H]thymidine incorporation by 68%. This effect was blocked by the antagonist EXP3174. 10μM forskolin or 1mM dibutyryl-cAMP inhibited thymidine incorporation by 55% and 25% respectively, suggesting a link between cAMP accumulation and growth inhibition in these cells. This system provides a useful model to study AT_{1A} receptor-coupled AC stimulation and AII mediated growth inhibition.

5.16

HYDROXYLAMINE PERTURBS COUPLING BETWEEN α_2 ADRENERGIC RECEPTOR AND G PROTEIN . Joseph B. Thompson* and Richard R. Neubig*. University of Michigan, Ann Arbor, MI 48109.

The role of fatty acylation in G protein coupled receptor function is not well understood. We used mild hydroxylamine (NH_2OH) treatment to attempt chemical removal of thioester fatty acids (e.g. palmitoylation) from membranes containing the α_2 adrenergic receptor. Membranes from CHO and MDCK cell lines overexpressing the α_2 adrenergic receptor were treated with 1M NH_2OH for 2 hours at pH 7.4. Binding of the α_2 agonist UK 14,304 (UK 1 nM) and α_2 antagonist yohimbine (YOH 20 nM) were measured. The ratio of UK to YOH binding, a measure of G protein coupling, decreased by 44 ± 3 % (SEM) with treatment at 37° but no effect was seen at 0° (n=9). This was due to both a decrease in UK binding and an increase in YOH binding. Scatchard analysis revealed changes in the B_{max} but not the K_d of YOH and UK binding. The increase in antagonist binding was unexpected and could have been due to relief of receptor sequestration, so we studied partially purified α_2 receptor (in 0.1 % digitonin). NH_2OH treatment stabilized YOH binding against a 73 ± 4 % (SEM) decrease in binding observed at 37° within 2 hours (n=3). NH_2OH has two effects: 1) a decrease in α_2 adrenergic receptor-G_i coupling and 2) a stabilization of soluble α_2 receptor. Since recent data (Kennedy and Limbird, JBC, 268:8003, 1993) suggests that palmitoylation of the α_2 receptor on cys 442 is not required for G protein coupling, Our data suggests that thioester fatty acylation at other sites on the receptor or on the G protein may be important in regulating receptor G protein interactions.
(Supported by NIGMS/MARC grant F31 GM-12945 and NIH grant GM-39561)

5.17

ELEVATION OF INTRACELLULAR Ca^{2+} CAN INCREASE INOSITOL 1,4,5-TRISPHOSPHATE BUT NOT INOSITOL 1,3,4,5-TETRAKISPHOSPHATE ACCUMULATION IN RAT BRAIN CORTICAL SLICES. Marvin E. Myles* and John N. Fain*, The University of Tennessee, Memphis, 38163.

The present studies were designed to investigate the hypothesis that the formation of inositol 1,3,4,5-tetrakisphosphate (IP_4) in cortical slices cannot be activated by an indirect elevation in intracellular Ca^{2+} but rather requires agonist stimulation. Stimulation by carbachol results in increased inositol 1,4,5-trisphosphate (IP_3) and IP_4 production. Veratridine, which indirectly elevates intracellular Ca^{2+}, inhibited carbachol stimulation of IP_3 and IP_4 accumulation. Ouabain, but not EGTA, reversed the inhibition due to veratridine. NMDA and kainate, which activate ion channels and elevate Ca^{2+}, also inhibited carbachol stimulation of IP_3 and IP_4 accumulation. EGTA, but not ouabain, reversed the inhibition due to NMDA. All agents that elevate intracellular Ca^{2+} (veratridine, NMDA, kainate, high K^+ or calcium ionophore), transiently stimulate total IP_3 accumulation but only carbachol, a muscarinic cholinergic agonist, is able to increase IP_4 accumulation. These results suggest that the major activator of the kinase that converts inositol 1,4,5-trisphosphate to IP_4 is some factor other than the level of intracellular Ca^{2+}.

5.18

PROMOTER STRUCTURE AND TISSUE-SPECIFIC REGULATION OF THE RAT α2C-ADRENERGIC RECEPTOR GENE. J.S. Saulnier-Blache* and S.M. Lanier*. Medical University of South Carolina. Dept Pharmacology, Charleston, SC, 29425-2251.

Alpha2-adrenergic receptors (α2C-AR) exist as three subtypes (A/D, B and C) encoded by distinct genes that are expressed in a tissue-specific and developmentally regulated manner. Both radioligand binding studies and RNA blot analysis indicate a wide tissue distribution of the α2A/D-AR in rat whereas a more restricted expression is observed for the α2B-AR (liver, neonatal lung) and α2C-AR (central nervous system). Mechanisms responsible for tissue specific expression of these receptor subtypes remain unclear.

To address this issue, we have attempted to define regulatory elements accounting for the predominant expression of the α2C-AR subtype in rat brain. The α2C-AR gene was isolated from a rat genomic library and a 4.5 kb DNA fragment 5' to the translational start codon was subcloned for further analysis. Sequencing to date (1.1 kb) revealed a high level of GC nucleotides (>75%), a CAAT box, two GC boxes and the absence of TATA box.

To identify sites of transcriptional initiation and potential regulatory element involved in the tissue-specific expression, we generated a series of 5' upstream gene fragments for use in RNAse protection assays (RPA) and gel shift analysis. Analysis of brain RNAs by both RPA and primer extension indicated the existence of at least two sites of transcription initiation located -380 and -977 nt 5' to the translational start codon. This is consistent with the existence of two α2C-AR mRNA species in rat brain. In gel shift assays with gene segments near the -380 transcription start site, we identified a DNA element (located between -278 and -403 nt upstream to the AUG codon) recognized by nuclear protein(s) present in tissues expressing the α2C-AR (olfactory bulb, hippocampus, cerebellar cortex, caudate putamen) but absent in tissues where the α2C-AR is not expressed (pituitary, liver).

Our data suggest that the -278/-403 gene segment may be involved in the restricted expression of the α2C-AR gene in the central nervous system.
(Supported by NIH, CTR and MUSC).

5.19

CLONING AND SEQUENCE ANALYSIS OF THE HUMAN VASOACATIVE INTESTINAL PEPTIDE RECEPTOR GENE. S. P. Sreedharan*, J. -X. Huang*, and E. J. Goetzl*. Departments of Medicine and Microbiology/Immunology, University of California Medical Center, San Francisco, CA 94143-0724.

Vasoactive intestinal peptide (VIP) is a 28-amino acid neuroendocrine peptide of the central and peripheral nervous systems belonging to the secretin-glucagon family. VIP regulates epithelial water and ion transport, relaxes arterial smooth muscle, promotes neuronal growth and survival, and modulates lymphocyte function. A cDNA clone (HVR) encoding a high-affinity VIP receptor was recently obtained from human HT29 colonic adenocarcinoma cells (Sreedharan, S.P. et al., 1993, BBRC, 193, 546-556). The 457 amino acid protein (MW = 52 kDa) encoded by HVR has homology to other members of the secretin-glucagon-PTH, seven transmembrane spanning, G protein-coupled receptor family. A human placental genomic library was screened using nucleic acid probes derived from HVR, and four genomic clones were obtained (HVRG1-4). DNA sequence analysis of the exon/intron structure and 5' flanking region of the gene indicate that the receptor coding sequence is composed of at least 11 exons, ranging from 60 bp to 1.4 kbp. All the exon/intron splice junctions conform to the GT-AG rule. The 15 kb HVRG1 genomic clone contains exons 2-11, while a 5 kb Eco RI insert encompassing exon 1 is present in the genomic clones HVRG2-4. (Supported by NIH grants DK 44876 and AI 29912)

5.20

MUSCARINIC RECEPTOR MEDIATED INHIBITION OF TYPICAL AND ATYPICAL β-ADRENOCEPTOR STIMULATION OF CYCLIC AMP ACCUMULATION IN GUINEA-PIG ILEUM Helen Reddy,* Anthony P.D.W. Ford,* and Richard M. Eglen.* Syntex Research, Institute of Pharmacology, Palo Alto, CA 94304.

Typical and atypical β-adrenoceptors mediate relaxation of guinea-pig ileum *in vitro* (Bond and Clarke, 1988, *Br.J. Pharmacol.*,95,723). M_2 muscarinic receptors may modulate the responsiveness of ileum to β-adrenoceptor agonists by inhibition of adenylyl cyclase (Griffin and Ehlert, 1992, *J. Pharm. Exp. Ther.*, 263,221). This study investigated the effect of β-adrenoceptor and muscarinic agonism on cyclic AMP accumulation measured in [^{3}H]adenine incorporated - slices prepared from guinea-pig ileal longitudinal smooth muscle. Isoproterenol and BRL 37344 (sodium-4-[2-[-hydroxy-2-(3-chlorophenyl) ethylamino]propyl] phenoxyacetate sesquihydrate), an atypical β-adrenoceptor agonist, both increased cyclic AMP accumulation with -log EC_{50} estimates of 6.6 and 5.8, respectively. Maximal stimulation, above basal cyclic AMP levels, to BRL 37344 (10 μM) was 26% of the maximum obtained to isoproterenol (10 μM). Cis-dioxolane, a non-selective muscarinic receptor agonist, inhibited basal, isoproterenol- and BRL 37344 -stimulated cyclic AMP accumulation. Antagonist studies were performed to characterize the receptor mediating this inhibition. Isoproterenol (10 μM)-stimulated cyclic AMP accumulation was inhibited in a concentration-dependent manner by cis-dioxolane (-log EC_{50}=7.3). Atropine, pirenzepine, methoctramine and para-fluoro hexahydrosiladifenidol yielded - log K_B estimates of 9.0, 6.3, 7.1 and 6.5, versus cis-dioxolane. These results are consistent with M_2 muscarinic receptors mediating inhibition of β-adrenoceptor stimulated adenylyl cyclase. The interaction between ileal β-adrenoceptors and M_2 muscarinic receptors, in terms of contraction, requires elucidation.

5.21

IMMUNOHISTOCHEMICAL LOCALIZATION OF PEPTIDERGIC NERVE FIBERS AND NEUROPEPTIDE RECEPTORS IN PEYER'S PATCHES OF THE CAT ILEUM. Sanae Ichikawa,* Sunil P. Sreedharan,* Edward J. Goetzl,* and Robert L. Owen.* University of California and DVA Medical Center, San Francisco, CA 94121.

The localization of peptidergic nerves and receptors for neuropeptides in Peyer's patches of the cat ileum was determined immunohistochemically using biotinylated rabbit antibodies and the streptavidin-biotin (SAB) method. Numerous vasoactive intestinal polypeptide (VIP)-, calcitonin gene-related peptide (CGRP)- and substance P (SP)-immunoreactive nerve fibers were distributed throughout the lamina propria of the crypts and villi. Immunoreactive nerve fibers also ran around the margin of Peyer's patch lymphoid follicles, but only a few such fibers were seen in the subepithelial layer of follicles. These fibers occasionally extended into the margin of follicles. Compared with crypts and villi, Peyer's patches were sparsely innervated. Nerve fibers were found mainly around lymphatics and high endothelial venules at the edges of follicles. Receptors for VIP were frequently localized over the crypts and the margins of the follicles, in the vicinity of high endothelial venules and immunocytes. The co-distribution of nerve fibers containing VIP, CGRP, and SP, and receptors for VIP and SP in ileal lymphoid tissue suggests that neuropeptides may regulate lymphocyte, lymphatic and venous endothelial cell surface adhesive proteins, in patterns that control lymphocyte traffic into and out of Peyer's patches.

5.22

MECHANISMS OF SIGNAL TRANSDUCTION FOR ADENOSINE AND ATP IN PULMONARY VASCULAR BED. Albert L. Hyman and Howard L. Lippton. Tulane Medical School,New Orleans, LA 70112

The purpose of the present study was to investigate the contribution of pertussis toxin (PTX)-sensitive guanine nucleotide (G) proteins in the pulmonary vascular response to adenosine and ATP in the intact cat under conditions of controlled pulmonary blood flow and left atrial pressure. Adenosine, ATP, and β-γ-ATP increased lobar arterial pressure in a dose-dependent manner. The pulmonary vasoconstrictor response to adenosine was abolished by BW 1433U, a specific purinergic receptor (P_1) inhibitor, PTX pretreatment, indomethacin and ONO 3708, a thromboxane A_2 (TxA_2) receptor antagonist. These data suggest that the pulmonary vasoconstrictor response to adenosine depends on activation of P_1 purinergic receptors coupled to PTX-sensitive G proteins and subsequent metabolism of liberated arachidonic acid to form TxA_2. Because each blocking agent studied produced similar reductions in the pulmonary vasoconstrictor response to β-γ-ATP, the present data suggest that ATP constricts the pulmonary vascular bed, in part, by hydrolysis to adenosine. Moreover, the present study suggests that both A_1 purinoceptors that are linked to PTX-sensitive G proteins as well as $P_{2\alpha}$ purinoceptors receptors that are independent of PTX-insensitive G proteins mediate the pulmonary vasoconstrictor response to ATP in vivo.

5.23

ROLE OF G PROTEINS IN THE VASODILATOR RESPONSE TO ENDOTHELIN ISOPEPTIDES IN VIVO. Howard L. Lippton and Albert L. Hyman. LSU Medical School, New Orleans, LA 70112

The purpose of the present study was to determine the influence of pertussis toxin (PTX) on the pulmonary (PUL) and systemic vasodilator responses to endothelin (ET) isopeptides in the intact cat under conditions of constant PUL blood flow and left atrial pressure. When pulmonary vasomotor tone was actively increased by intralobar arterial infusion of U46619, intralobar arterial bolus injections of ET-1, ET-2 and ET-3 decreased lobar arterial pressure and systemic vascular resistance in a dose-dependent manner. The PUL vasodilator responses to ET-1 and ET-2 were abolished by PTX-pretreatment whereas PTX-pretreatment did not alter the PUL vasodilator response to ET-3 and cromakalim, a specific K_{ATP} (ATP-sensitive potassium) channel activator, and the systemic vasodilator responses to all ET isopeptides studied. Glipizide, an inhibitor of K_{ATP} channels, inhibited the PUL vasodilator responses to ET-1, ET-2 and ET-3, whereas the systemic vasodilator responses to these isopeptides were not changed. The present data are the first to provide a functional correlate in vivo suggesting the existence of different signal transduction mechanisms for two PUL vascular ET receptor subtypes. However, both ET receptor subtypes promote vasodilation in the adult PUL vascular bed by activating K_{ATP} channels. The present data also suggest that the intracellular signalling for the mechanisms mediating the vasodilator responses to ET-1 and ET-2 in the systemic and PUL vascular beds differ.

5.24

Mg^{2+} MOVES INTO Ca^{2+} STORES DURING MUSCARINIC-INDUCED Ca^{2+} RELEASE IN RAT SUBLINGUAL MUCOUS ACINI. Guo H. Zhang* and James E. Melvin* (SPON: J. Arreola). Dept. Dent. Res., Univ. Rochester, NY 14642

The Mg^{2+} movement during muscarinic stimulation in the BAPTA (50 μM)-loaded sublingual acini was monitored by using dual wavelength microfluorometry of the Mg^{2+}-sensitive dye mag-fura-2. Stimulation with carbachol (CCh) induced a transient decrease in $[Mg^{2+}]_i$ (~29%) in Ca^{2+}-containing medium, $[Mg^{2+}]_i$ returning to the resting level in 2-3 min. In Ca^{2+}-free medium CCh-induced a sustained decrease (~40%) in $[Mg^{2+}]_i$. TMB-8 (50 μM) completely blocked this decrease, suggesting that the $[Mg^{2+}]_i$ decrease is dependent on the filling state of the IP_3-sensitive Ca^{2+} stores. Re-introducing Ca^{2+} resulted in a complete recovery in $[Mg^{2+}]_i$. The absence of extracellular Mg^{2+} did not alter this Ca^{2+}-dependent $[Mg^{2+}]_i$ recovery. Blocking Ca^{2+} influx with La^{3+} or Ni^{2+} inhibited $[Mg^{2+}]_i$ recovery. Thapsigargin (TG) induced a slow decrease in $[Mg^{2+}]_i$ (~25% in 5 min). Addition of TG after CCh did not induce further $[Mg^{2+}]_i$ decrease and did not affect the Ca^{2+}-induced $[Mg^{2+}]_i$ recovery. These results suggest that during muscarinic agonist-stimulated Ca^{2+} release, Mg^{2+} moves into the Ca^{2+} store via the IP_3-sensitive Ca^{2+} release pathway to maintain charge balance. (Supported by NIH DE10655, DE08921, and STRC #381-01).

5.25

INCREASED mRNA LEVELS FOR THE L-TYPE CA^{2+} CHANNELS IN RAT MYOMETRIUM PRIOR TO TERM AND PRETERM LABOR. N. Tezuka*[1], M. Ali*[2], K. Chwalisz*[3] and R.E. Garfield[4], Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas 77555[1,2,4] Schering Ag, Berlin, Germany[3].

Ca^{2+} for myometrial contractility enters the cells mainly through voltage-dependent channels (VDC). The aim of this study was to measure levels of mRNA to the α1-subunit of the L-type Ca^{2+} channel (VDC) throughout pregnancy to determine if alterations are associated with term or preterm labor. Myometrial tissues were examined at various stages of pregnancy, at term delivery and during preterm labor on day 18 following antiprogesterone treatment (ZK98.299). Myometrial tissues were removed from the uterus of timed-pregnant rats and RNA extracted. Levels of mRNA for the Ca^{2+} channel were measured with reverse transcription-PCR using a set of amplimers designed to correspond to the sequence reported by Mikami et al., (Nature 340, 230-233, 1989). Our results showed two distinct PCR products: one of the expected size at 372 base pairs (bp) and a smaller at 340bp. The two products followed the same increasing trend during late gestation. mRNA levels were low on day 14 and 15, raised gradually to 6.9 - fold at term on day 22, but decreased during labor. Similarly in animals treated with ZK98.299 the message increased after 8 hours then declined during preterm delivery. We conclude that the increase in mRNA for Ca^{2+} channels to high amounts prior to both term and preterm birth reflect an increase in expression and abundance of VDC in preparation for the muscle to contract. The decrease in VDC mRNA during labor indicates a rapid decline in synthesis during a period when the channels are likely at their highest levels. Progesterone withdrawal appears to be responsible for the control of VDC mRNA.

5.26

RECEPTOR INTERACTIONS OF β-N-OXALYL-L-α,β-DIAMINOPROPIONIC ACID,THE LATHYRUS SATIVUS EXCITOTOXIN. *M.A. JUNAID &* S.L.N.RAO,Department of Biochemistry,Osmania University,Hyderabad-500007,INDIA.

β-N-Oxalyl-L-α,β-diaminopropionic acid(ODAP)is an excitotoxic amino acid present in the seeds of Lathyrus sativus,excessive consumption of which has been implicated in the neurodegenerative disorder neurolathyrism. Recent studies have shown that ODAP acts as an agonist at certain glutamate receptors in the CNS and generates metabotropic effects through inositol lipid metabolism. However,there have been no studies to examine whether ODAP does in fact bind to receptors in the CNS. We have,for the first time,using chemically synthesized (2.3) ^{3}H ODAP as the ligand confirmed that the L.sativus excitotoxin interacts with membrane bound receptors in the CNS. Ligand binding studies with synpatic membranes has not only shown that the binding of ^{3}H ODAP varies between different CNS regions but also between a susceptible & a resistant species. It has also been observed that ^{3}H ODAP not only interacts with glutamate receptors, but also with another as yet unidentified class of receptors. Non-glutamate type of ^{3}H ODAP interactions were found to be more predominant in a susceptible animal species whereas in a resistant species the interactions are more at the glutamate receptors. An as yet unidentified endogenous factor(s) has been partially purified and shown to displace most of the ^{3}H ODAP bound to synaptic membranes. The results thus suggests that neurotoxicity of ODAP may simply not be related to its interactions at glutamate receptors alone.

5.27

PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C: PROGRESS ON THE MAMMALIAN ECTO-ENZYME. O. Hayes Griffith*, Karen K. Hedberg*, G. Bruce Birrell*, Johannes J. Volwerk*, and Margret Ryan*. University of Oregon, Eugene, OR 97403.

Phosphatidylinositol-specific phospholipase C (PI-PLC) isozymes located inside mammalian cells are activated upon ligand binding to surface receptors, generating two second messengers. Recently, a PI-PLC has been detected *on* the surface of certain mammalian cells (ecto-PI-PLC). The occurrence and properties of this member of the PI-PLC family are being investigated: It is a true ecto-enzyme, anchored to the membrane with the activity directed outside of the cell. It is not cleaved by bacterial PI-PLC, indicating that it does not have a simple GPI-anchor. The enzyme activity can be isolated after biotinylation of intact cells followed by solubilization of plasma membranes and incubation with avidin beads. Two patterns of expression of this activity have been observed among the cell lines examined. In the first, seen in contact-inhibited mouse fibroblast lines, the expression of activity is minimal or undetectable in subconfluent growing cultures but abruptly develops as the culture becomes confluent and quiescent. Transformed fibroblasts do not exhibit significant activity regardless of the state of confluence of the culture. In the second pattern of expression, seen in both primary rat astrocytes and transformed C6 rat glioma cells, the activity per dish of growing cells is linear with cell number and not transformation-sensitive. The activity is Ca^{2+} dependent, is inhibited by Zn^{2+}, and has a broad pH optimum, which in 3T3 L1 cells peaks in the range of 7.6 to 8.0. While the function of ecto-PI-PLC is not yet known, the evidence suggests a correlation with cell growth in mouse fibroblasts. Supported by PHS Grants CA 11695 and GM 25698.

5.28

RAPID ANALYSIS OF INOSITOL PHOSPHATES USING SOLID PHASE EXTRACTION Peggy A. Whitson, Helen M. Huls, and Yu-ming Chen. NASA Johnson Space Center, Biomedical Operation & Research Branch and KRUG Life Sciences, Houston, TX 77058

In response to an extracellular signal, the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate is enzymatically cleaved to form the second messengers diacylglycerol and inositol 1,4,5-trisphosphate (IP3). IP3 can then be converted to other inositol phosphates (IPs), including monophosphate (P1), diphosphate (IP2), trisphosphate (IP3 isomer) and tetraphosphate (IP4). Inositol phosphates have typically been radiolabeled for detection and separated by either open bed anion-exchange chromatography or HPLC. In this study, we developed a rapid separation method using Sep-Pak Accell Plus QMA cartridges (Waters). Different species of inositol phosphates were sequentially eluted from solid phase cartridges with different concentrations of ammonium formate. Inositol, IP1, IP2, IP3, and IP4 were eluted separately with water and with 0.06 M, 0.1 M, 0.2 M, and 0.4 M ammonium sulfate, respectively. This method requires less salt to elute the IPs than open bed chromatographic and HPLC methods. Since salt interferes with liquid scintillation fluors, this method provides greater sensitivity. The effect of atrial natriuretic peptide (ANP) on the generation of inositol phosphates in brain microvessel endothelial cells (BMEC) was examined using this rapid IP detection method. Results showed that ANP stimulated IP1, IP2 and IP3 accumulation in the presence of guanosine 5'-r-thiotriphosphate (GTPrS). ANP alone had little or no effect on the formation of IPs. In the presence of GTPrS, ANP-stimulated IPs exhibit an inverse dose response relationship. These results are consistent with observations of Berl et al. (Am. J. Physiol. 260:F590-F595, 1991) in cultured rat inner medullary collecting tubule cells. The solid phase extraction method described here is a fast, sensitive, and reliable analytical method for measuring inositol phosphates from cell lysates.

5.29

ENDOTOXIN TOLERANCE DIFFERENTIALLY DOWN-REGULATES MACROPHAGE THROMBOXANE (TX)B_2 AND IL-6 PRODUCTION. Hong Chen*, James A. Cook and Perry V. Halushka*. Medical University of SC, Charleston, SC 29425.

Endotoxin (LPS) tolerance (TOL) is an acquired state of resistance to LPS shock induced by prior sublethal LPS exposure. Cellular changes that contribute to LPS TOL include diminished LPS stimulated macrophage (MØ) arachidonic acid (AA) and certain cytokines. In the present study synthesis of the AA metabolite TXB_2 and the cytokine IL-6 was determined in rat peritoneal MØ taken at various times after induction of LPS TOL. Rats were given an i.p. injections of *S. enteritidis* LPS on day 1(100μg/kg) and 2 (500 μg/kg) respectively. On each study day beginning at day 5 the cells were harvested for *in vitro* stimulation with LPS or purified lipid A. Data from day 5 are shown in the table below. Data are expressed as mean ± S.E. *P<0.05 compared to respective CON.

MØ Stimulant	CON TXB_2(ng/ml)	MØ IL-6(pg/well)	LPS TOL TXB_2	MØ IL-6
Basal	3.1±0.4	0.4±0.0	0.8±0.0*	0.3±0.0
LPS (50μg/ml)	15.5±2.1	20.7±4.7	1.5±0.2*	17.4±2.0
Lipid A(1μg/ml)	10.1±1.4	3.2±0.4	1.1±0.2*	1.5±0.3*
LipidA(10μg/ml)	16.5±0.8	0.9±0.2	1.7±0.3*	0.7±0.1*

MØ from LPS TOL exhibited suppressed LPS and lipid A induced *in vitro* MØ TXB_2 production. In contrast to TXB_2, IL-6 levels were not significantly depressed in LPS TOL MØ at day 5. Despite potent induction of TXB_2 by lipid A in control MØ, lipid A did not induce IL-6. These data demonstrate that LPS TOL differentially alters MØ TXB_2 and IL-6, and that a polysaccharide component of LPS may be necessary for optimal IL-6 induction. Supported in part by NIH GM 27673.

5.30

THE EFFECT OF ADENOSINE ON THE NMDA RECEPTOR MEDIATED CALCIUM INFLUX IN TURTLE CEREBRAL CORTICAL SHEETS. Leslie T. Buck and Phillip E. Bickler. Department of Anesthesia, University of California, San Francisco, Ca. 94143-0542

During normoxia glutamate (glu) and the glu receptor family of ion channels play a key role in mediating rapid excitatory synaptic transmission in the central nervous system. During hypoxia glu accumulates and produces neurotoxic elevations in $[Ca^{2+}]_i$, mediated largely by the N-methyl-D-aspartate (NMDA) subfamily of glu receptors. In mammalian brain slices adenosine has been shown to decrease the magnitude of the hypoxia (glu) induced $[Ca^{2+}]_i$ increase, thereby delaying tissue injury. In the anoxia-tolerant turtle *Chrysemys picta bellii*, where an anoxia-induced 12 fold increase in cerebral [adenosine] has been observed, the effects of adenosine on neuronal Ca^{2+} sequestration could be dramatic. We therefore investigated the influence of adenosine on the activity of NMDA receptors in normoxic turtle cerebral cortical sheets. Cortex sheets were pre-loaded with the Ca^{2+} sensitive dye Fura-2. Adenosine (100 μM) pre-incubation for 20, 60, and 100 min resulted in a 26, 91, and 97% reduction in the magnitude of the NMDA (150 μM) elicited Ca^{2+} influx (30° C), respectively, compared to control. The NMDA response (ΔCa^{2+} 950 nM) from control cortex sheets did not change significantly over this time course. These data demonstrate that adenosine significantly decreases the magnitude of the NMDA mediated Ca^{2+} influx, likely through its interaction with plasma membrane adenosine receptors and intracellular second messengers. This work was supported by a grant from the Foundation for Anesthesia Education and Research to P.E.B.

5.31

SER PHOSPHORYLATION LEVELS AFFECT SIGNALLING BY CD69, A TYPE II SINGLE-TRANSMEMBRANE, G-PROTEIN-ASSOCIATED RECEPTOR

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CD69 is a 28-32 kD constitutively phosphorylated surface homodimer expressed on a variety of hemopoietically derived cells. Crosslinking of CD69 molecules generates signals which include Ca^{2+} influx and PLA2 activation, possibly through a G-protein, resulting in as diverse cellular events as degranulation and aggregation in platelets or transcriptional control of lymphokine gene expression in activated T lymphocytes. The possilble relevance of constitutive phosphorylation for signal generation from CD69 was evaluated. In activated peripheral blood T cells, phosphorylation was shown to be limited to Ser residues by phosphoaminoacid analysis. Two hours treatment with okadaic acid and staurosporin increased and decreased CD69 phosphorylation respectively, without affecting surface expression. Treatment with okadaic acid severely inhibited the ability of CD69 to induce Ca^{2+} influx upon crosslinking, while staurosporin enhanced it. Similar treatments did not affect signaling through CD2 and CD28, which are not phosphorylated nor undergo phosphorylation by pharmacological treatment. These results suggest that Ser phosphorylation levels on CD69 are under continuous enzymatic control and may be important in signal generation ability. The analysis of a number of CD69 mutants in which each Ser of the intracytoplasmic domain has been changed to Ala will identify critical Ser residues in this system, and help define the role of Ser phosphorylation in receptor signal transduction.

G-PROTEINS

6.1

βγ SUBUNITS OF G-PROTEINS ACTIVATE PHOSPHOLIPASE C-β2

Arieh Katz*, Dianqing Wu*, Melvin I. Simon* California Institute of Technology, Pasadena, CA, 91125.

Many hormones and neurotransmitters activate phospholipase C (PLC) via G-proteins. The G-protein mediated pathways leading to the activation of PLC can be distinguished by their sensitivity to Pertussis toxin (Ptx). There is growing evidence for the involvement of the Gq class α subunits in the Ptx-insensitive pathways of PLC activation. Less is known about the Ptx-sensitive pathways of PLC regulation, these pathways have been described in a variety of cell types such as, neutrophils, platelets and macrophage cells. Employing cotransfection assays in Cos cells, we have demonstrated that Gβγ subunits specifically activate the β2 isoform of PLC. This activation of PLC-β2 by Gβγ subunits is restricted to cells in which beta and gamma subunits are produced together and form dimers. Furthermore, only free Gβγ complexes will activate PLC-β2, coexpression of Gαi2/3 prevented this activation by binding the free βγ complex and neutralizing its activity. This activation of PLC-β2 by free Gβγ may be the basis of the pertussis-toxin-sensitive phospholipase C activation. To test this idea we have reconstituted by cotransfection the C5a and fMLP receptors which activate PLC in a Ptx-sensitive manner, together with either Gαi2 or Gαi3 in addition to Gβ1γ2 and PLC-β2. Cells expressing these components display a Ptx-sensitive, ligand dependent activation of PLC-β2 hence, demonstrating that C5a and fMLP receptors can interact with Gαi2 and Gαi3 leading to the release of Gβγ which subsequently activates PLC-β2. These findings that both the α subunits and the βγ subunits activate PLC suggests that complex circuits of PLC signaling may be generated depending on the nature of the G-protein subunits and the PLC isoforms expressed in each cell type.

6.2

SMALL GTP-BINDING PROTEINS IN PLASMA MEMBRANES OF SEA URCHIN SPERMATOZOA.

Patricia Cuéllar-Mata*, Laura E. Castellano*, Guadalupe Novoa-Martínez*, Guadalupe Martínez-Cadena* and Jesús García-Soto*. IIBE, Facultad de Química, Universidad de Guanajuato, Guanajuato, Gto. 36000, MEXICO.

Plasma membranes isolated from heads and flagella of sea urchin spermatozoa were used to examine the presence of small GTP-binding proteins. Membrane proteins were separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose and the blot probed for the presence of GTP-binding proteins using (α-^{32}P)GTP. Three major bands with molecular mass of 28, 25 and 24 kDa, respectively, were identified on head and flagellar membranes. Radiolabeling by (α-^{32}P)GTP was inhibited by nonradioactive GTP or GDP in a dose-dependent manner. Maximal inhibition by either GTP or GDP was at 50 nM. GMP, UTP and ATP were also used to further confirm the specificity of (α-^{32}P)GTP binding. These nucleotides in excess (100 nM) did not interfere with (α-^{32}P)GTP binding. Thus, these results demonstrate that the plasma membrane of sea urchin sperm have three GTP-binding proteins of low molecular weight. Detection of these proteins could help to elucidate the signal transduction pathways involved in the physiology of spermatozoa.

(Supported by Conacyt).

6.3

OCCURRENCE OF Gα PROTEINS IN THE FUNGUS Phycomyces blakesleeanus SPORES.

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The presence of Gα -proteins in a membrane mixed fraction (MMF) of dormant spores of the fungus Phycomyces blakesleeanus was demonstrated using four experimental approaches. First, when the proteins were separated by SDS-PAGE and transferred to nitrocellulose, the presence of two bands corresponding to $G_{\alpha s}$ (48 and 53 kDa), and a band of 41 kDa corresponding to $G_{\alpha i}$ were detected with specific antibodies against G_s and G_i, respectively. Second, the proteins of 48 and 53 kDa were ADP-ribosylated by cholera toxin. Third, (^{32}P)GTP-binding of the MMF by a photoaffinity labeling method yielded two bands of 48 and 53 kDa. Finally, when we used $G_{\alpha s}$ and $G_{\alpha i}$ probes, we found by Southern blot that these proteins were codified by the fungus DNA. Under conditions for ADP-ribosylation by pertussis toxin and by experiments of GTP-binding, we did not detect $G_{\alpha i}$ (41 kDa). Phycomyces spores must be activated in order to germinate. Immediately after the activation treatment, the cytoplasmic cAMP levels elevate suggesting that this second messenger might be the trigger of germination. The occurrence of Gα-proteins in this stage of development could indicate that adenylate cyclase activity could be regulated by these proteins. (Supported by CONACYT)

6.4

ETHANOL DOES NOT AFFECT EXPRESSION OF Gsα AND Giα IN MURINE EMBRYONIC PALATE MESENCHYME CELLS.

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Chronic exposure to ethanol has been shown to inhibit the ability of a number of different cell types to produce cAMP in response to hormone treatment, including murine embryonic palate mesenchyme (MEPM) cells. In several cases this effect has been linked to either inhibition of expression of the stimulatory G protein α subunit (Gsα) or overexpression of the inhibitory G protein α subunit (Giα) as a result of ethanol treatment. In this study we demonstrate that this is not the case for MEPM cells. Treatment of cells for 48 hours with 200 mM ethanol had no effect on expression of either Gsα or Giα as determined by Northern and Western blot analyses. This same treatment had previously been shown to inhibit PGE_2-stimulated cAMP production in MEPM cells. These results demonstrate that desensitization of adenylate cyclase to hormone stimulus in response to chronic ethanol treatment is accomplished in MEPM cells without alteration of G protein expression. The data further indicate that alteration of G protein expression may not be a necessary condition for ethanol-induced hormone desensitization in general. Supported in part by NIH grants DE05550 and DE08199 to RMG and DE10239 and DE10323 to MMP. WMW is the recipient of NRSA DE05593.

6.5

ENHANCED CONTRACTILE RESPONSES OF ARTERIES FROM DIABETIC RATS TO DIRECT STIMULATION OF G-PROTEINS. Lynn Weber*, Worku Abebe* and Kathleen M. MacLeod*. University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Previous studies from this laboratory have demonstrated that α_1-adrenoceptor-mediated increases in tension and phosphoinositide metabolism are enhanced in arteries from diabetic rats. In the present investigation, contractile responses elicited via direct stimulation of GTP-binding proteins (G-proteins) with sodium fluoride (NaF) in the presence of 10 μM aluminum chloride were obtained in mesenteric arteries from three month streptozotocin-diabetic (60 mg/kg i.v.) male Wistar rats and age-matched control rats. NaF (1-30 mM) produced slowly developing, concentration-dependent contractions in mesenteric arteries from both control and diabetic rats, the magnitude of which were reduced in the absence of extracellular calcium. Maximum contractile responses of mesenteric arteries from diabetic rats to NaF were significantly enhanced in both the presence and absence of extracellular calcium when compared to responses of arteries from control rats. Incubation of arteries from control and diabetic rats with the intracellular calcium store depletor, ryanodine (30 μM), with the calcium channel blocker, nifedipine (3 μM), and with the selective protein kinase C inhibitor, calphostin C (0.5 μM), significantly inhibited maximum contractile responses to NaF. These data suggest that there is increased activation of G-proteins or of the effectors coupled to these G-proteins in mesenteric arteries from diabetic rats. This may be responsible for the enhanced contractile responses of these arteries to α_1-adrenoceptor stimulation. (Supported by the Medical Research Council of Canada and the Heart and Stroke Foundation of B.C & Yukon.)

6.6

LOCALIZATION OF RAP1 AND INVESTIGATION OF ITS ROLE IN RAT PAROTID GLAND. Nisha J. D'Silva*, Dennis Di Julio*, Kerry L. Jacobson* and Eileen L. Watson*, U. of Washington, Seattle, WA 98195

Rap1 is a small GTP-binding protein that has been identified in various cell types. The aims of the present study were to identify and localize rap1 in the rat parotid gland by immunoblot analysis and immunohistochemistry; to investigate whether rap1 translocates from the granule membrane during stimulation of acini; and to determine in-vitro whether rap1 can be phosphorylated and whether phosphorylation contributes to its translocation. Rap1 was identified in rat plasma membrane-enriched (PM), cytosol (CY) and secretory granule membrane (SGM) fractions by use of rap 1 affinity purified polyclonal antibody. Rap1 was present in highest concentration in the SGM. The immunohistochemistry studies showed positive staining in the cytoplasm, which was most prominent in the apical region of the acinar cells where the secretory granules are located. Immunoreactivity was abolished using rap1 antibody preincubated with a 10-fold excess of the cognate peptide. For the translocation studies, rat parotid acini, prepared as described previously (Watson et al., Mol. Pharmacol. 38:54, 1990) were treated with 10μM isoproterenol for 20 min., and CY and PM fractions from stimulated and unstimulated acini were isolated. Rap1 was found, by densitometric analysis of immunoblots, to be increased by 39% to 56% in the stimulated versus unstimulated CY, and unchanged in the PM fractions. In-vitro phosphorylation of rap1 in SGM fractions with protein kinase A was performed as described (Lazarowski et al., J. Biol. Chem. 265:13118, 1990). Under these conditions rap1 was found to be a phosphoprotein, but did not move off the SGM during phosphorylation. The data presented suggest that rap1 is associated with the SGM, and may play a role in salivary gland exocytosis. (This work was supported by NIDR grants DE07023 and DE 5249.)

6.7

Suppression of Tumorigenicity by rap1A/krev-1 Gene in Human Hepatoma Cells Yea-Lih Lin[1*], Chen-Kung Chou[2*] 1. Institute of Biochemistry, National Yang-Ming Medical College, Taipei, Taiwan ,R.O.C. 2. Department of Medical Research, Veterans General Hospital, Shih-Pai, Taipei, Taiwan, R.O.C.

ras genes constitute a gene family encoding a series of closely related proteins with guanine nucleotide-binding activities. These prteins have been implicated in the regulation of a diverse array of cellular processes. The mammalian rap1A/krev-1 gene was isolated by Kitayama et al. (1989) from a human fibroblast cDNA exprssion library based on its ability to cause reversion of the transformed phenotypes of v-ki-ras-transformed DT fibroblast. This gene encodes a 21-25 kDa protein, shares 50% sequence similarity with ras , The biological function of Rap1A was demonstrated to compete with Ras for binding to Ras-GAP by Hata et al. (1990) and Frech et al. (1990), but the physiological significance of the inhibition of Ras action by Rap1A is still uncertain.

We have previously demonstrated that Ras proteins play a crucial role in insulin stimulated cell proliferation and c-jun expression in human hepatoma Hep3B cells. In order to further clarify the effect of Ras proteins in the growth control of Hep3B cells, we transfected the rap1A/krev-1 gene into these cells and selected stable transfectants. Morphology of the rap1A transfectants was changed to more flat than the parental Hep3B cells.The growth rate of the Rap1A transfectants remained unchanged, but the saturation density was only 60% of those of Hep3B cells. We injected hypodermically both Hep3B and rap1A transfected cells into Balb/c mice and observed the tumor formation of Hep3B cells after 6 weeks. The rap1A transfectants totally lost its original tumorigenecity. We also investigated the insulin stimulated cell proliferation and c-jun expression in the rap1A transfected cells. These cells lost its proliferative response to insulin ,but the c-jun expression was still inducible . Compare to our previous statements, the rap1A gene might have its own biological effects other than interfering the Ras actions to regulate the cell proliferation in Hep3B cells.

6.8

PREGNANCY ALTERS UTERINE ARTERY CONSTRICTION TO G PROTEIN AND PROTEIN KINASE C ACTIVATION Marilyn Cipolla* and George Osol. Univ. Vt. Coll. Med., Burlington, VT 05405

Uterine artery sensitivity to α-adrenergic agonists is increased significantly during pregnancy. This response may be due to an enhancement of vasoconstriction to G protein and/or protein kinase C (PKC) activation, two well-recognized components of the adrenergic signal transduction cascade. Uterine radial arteries (100-250 μm) from non-pregnant (NP) and late pregnant (da 20, LP) Sprague-Dawley rats were isolated, cannulated, and pressurized to 50 mmHg in a specialized arteriograph; lumen diameter was measured continuously with a video-electronic system. The sensitivity of LP arteries to the constrictor effects of NaF, which activates G proteins non-specifically, was increased approximately two-fold (EC_{50}: NP= 2.3 ± 0.3 mM; LP = 1.2 ± 0.1 mM, $p < 0.05$; $n = 13$), and threshold concentrations (EC_{10}) in LP arteries were one tenth of those required in NP vessels (0.1 vs. 1.0 mM). Direct stimulation of PKC with (-)-Indolactam-V (IND) did not parallel the effects of G protein activation, as LP arteries were approximately 2.3x *less* sensitive to IND (EC_{50}: NP = 60 ± 10 nM; LP=140±18 nM, $p<0.05$; $n=8$). Together, these results provide the first evidence for specific and divergent modulation of signal transduction events during gestation. [AHA EI 92001470]

6.9

LINKAGE OF SEROTONIN RECEPTOR WITH A Gαz-LIKE GUANINE NUCLEOTIDE BINDING PROTEIN IN RAT STOMACH FUNDUS H.-Y. Wang* and E. Friedman. Department of Psychiatry, Medical College of Pennsylvania/EPPI, Philadelphia, PA 19129.

The fundal serotonin (5-HT) receptor is a member of the G-protein-linked receptor superfamily. However, the associated transduction pathway has not been fully examined. Experiments were performed to examine the coupling mechanism associated with this fundal 5-HT receptor. In the fundus but not in the corpus of the rat stomach, 5-HT stimulated [^{35}S]GTPγS binding to a protein which was recognized by anti-Gαz antiserum in a Mg^{2+}-dependent fashion. Serotonin also enhanced the binding of [α-^{32}P]GTP to the fundal protein and increased the hydrolysis of GTP to GDP in fundal membranes. This fundal GTP binding protein is 25-29 kDa in size whereas the brain Gαz protein is a 41 kDa protein. Unlike Gαz protein, the fundal GTP binding protein does not associate with the ß subunit of G proteins. Mixing experiments revealed that the fundal G protein does not appear to be a proteolytic product of a 41 kDa Gαz protein. Trypsin digestion of fundal GTP binding protein and brain Gαz produced different immunoreactive fragments. The activation of [^{35}S]GTPγS binding by serotonergic agonists and its inhibition by pharmacological antagonists corresponded to the known actions of these agents on contraction of fundal muscle. These results provide evidence that the stomach fundal 5-HT receptor is coupled directly or indirectly to a Gαz-like protein which may mediate the 5-HT-induced contraction in this tissue.

6.10

cDNA CLONING AND ANALYSIS OF A NEW G-PROTEIN β SUBUNIT. A. John Watson* and Melvin I. Simon* California Institute of Technology, Pasadena CA 91125

cDNA clones encoding a new heterotrimeric G-protein β-subunit protein were isolated from mouse brain RNA by a combination of PCR, anchored PCR and conventional screening techniques. The cDNA clone ($G\beta_5$) contains an open reading frame that potentially encodes a protein of 352 amino acids with a calculated molecular weight of 38,727 daltons. This is slightly larger than the four known G-protein β proteins, all of which contain 341 amino acids and have molecular weights between 35 and 36 kDa. The $G\beta_5$ mRNA is predominantly expressed in rodent brain as two transcripts of 1.7 and 2.1 kilobase pairs. This is again in contrast to the four previously identified β subunits, all of which are expressed more or less ubiquitously. Analysis of the predicted amino acid sequence of $G\beta_5$ indicates that the encoded protein is approximately 50% identical to the known β subunits. The four previously cloned mammalian β subunits are highly homologous to each other, displaying over 90% sequence identity. Interestingly, $G\beta_5$ appears to be most closely related to a brain-specific G-protein β subunit found in the fruit fly, *Drosophila.* This raises then possibility that this particular β subunit has been evolutionarily conserved and has a specialized function in central nervous system signal transduction.

6.11

RANDOM MUTAGENESIS OF Goα. Vladlen Z. Slepak*, Anna M. Aragay*, Michael W. Quick*, and Melvin I. Simon* California Institute of Technology, Pasadena, CA 91125

Nucleotide binding properties of the G protein α subunit Goα were probed by mutational analysis in recombinant *E.coli*. Thousands of random mutations generated by PCR were screened by *in situ* [^{35}S]GTPγS binding on the colony lifts following transformation of bacteria with modified $G_o\alpha$ cDNA. Clones that did not bind the nucleotide under these conditions were characterized by DNA sequence analysis and the nucleotide binding properties were further studied in crude bacterial extracts. A number of novel mutations reducing the affinity of Goα for GTPγS or Mg^{2+} were identified. Some of the mutations substitute amino acid residues homologous to those known to interact with guanine nucleotides in p21^{ras} proteins. Other mutations show that previously unstudied residues also participate in the nucleotide binding. Several mutants lost GTPγS binding but retained the capacity to interact with the βγ subunit complex as determined by pertussis toxin mediated ADP-ribosylation. One of these, mutant S47C, was functionally expressed in *Xenopus laevis* oocytes along with the G protein coupled thyrotropin releasing hormone (TRH) receptor. Whereas wild-type Goα increased TRH-promoted chloride currents, S47C significantly decreased the hormone - induced Cl^- response suggesting that this mutation resulted in a dominant negative phenotype.

6.12

COUPLING G PROTEIN α SUBUNITS WITH SEVEN-PASS-MEMBRANE RECEPTORS IN *XENOPUS* OOCYTES. Anna M Aragay*, Mike W. Quick*, Norman Davidson*, Henry A. Lester* and Melvin I. Simon*. California Institute of Technology, Pasadena, CA, 91125.

We have studied the interaction among seven-pass-membrane receptors, G protein α subunits and effectors by co-expressing receptors with different α subunits in *Xenopus* oocytes. Oocytes were injected with receptor alone: 5HT1C (1 to 5 pg) and TRHr (0.05-0.5 ng) alone or in conjunction with increasing concentrations (0.5-5 ng) of the following Gα subunits: G_{oa}, G_{ob}, G_q, G_{11}, G_s, G_{olf} and G_t. Agonist-induced Ca^{2+}-activated Cl^- currents were measured using the two-electrode voltage-clamp method 48 to 72 h post-injection.

Oocytes injected with the 5HT1C receptor alone generated currents that were blocked an average of 80 % by pre-incubation with 4 μg/ml pertussis toxin for 24 h prior measurement. Co-expression of the receptor with G_{oa} or G_{ob} resulted in a 2 to 6 fold increase in the response that was: 1) dependent upon the amount of G protein mRNA injected and 2) blocked by PTX. Co-expression of the receptor with Gq or G11 resulted in a 2 to 9-fold increase in the response that was:1) dependent upon the amount of G protein injected and 2) not blocked by PTX. Co-expression of the receptor with G_s, G_{olf} or G_t resulted in no change in the peak currents elicited. GTPase measurements on oocyte membranes confirmed these findings.

The data indicate that in *Xenopus* oocytes, endogenous receptors can activate and increase peak Ca^{2+}-activated Cl^-currents by coupling to both PTX-sensitive and PTX-insensitive exogenous Gα subunits.

6.13

THE SPECIFICITY OF G PROTEIN γ SUBUNIT BINDING TO β IS CONFERRED BY γ1 RESIDUES 36-49. Denise J. Spring* and Eva J. Neer*. Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

β and γ subunits of heterotrimeric G proteins form dimers whose structure is poorly understood. Despite the similarity of β subunits, not all combinations of β and γ subtypes can form dimers: the γ2 subunit can form dimers with β1 and β2, but γ1 forms dimers only with β1. In order to identify the region of γ that discriminates between β1 and β2, a series of γ1/γ2 chimeras was constructed, transcribed, and translated in vitro. The interactions of these chimeras with β1 and β2 were assayed by trypsin protection and crosslinking studies. An initial series of N-terminal/C-terminal chimeras implicated the central region of the ~70 amino acid γ subunit. When amino acids 36-49 of γ1 replaced amino acids 33-46 of γ2, the chimera could only dimerize with β1; the reciprocal chimera, in which 14 residues from γ2 replaced the corresponding region of γ1, could interact with both β1 and β2. Therefore, this 14 amino acid region is sufficient for γ1 to discriminate between the β subunits. Analysis of this region will be refined by making point mutations at selected residues in this highly charged region.

This work was supported by grants from the NIH and American Heart Association.

6.14

A UNIQUE 30/32 KD GTP-BINDING PROTEIN DIMER IN MAMMALIAN BRAIN: PURIFICATION, PROPERTIES AND TISSUE DISTRIBUTION. L. Zeng* and M. M. Rasenick. University of Illinois College of Medicine, Chicago, Illinois 60612

A novel 30/32 kDa GTP-binding protein was purified from crude bovine brain membrane and its biochemical properties have been characterized. DEAE-Sephacel, Ultrogel AcA-34, Hydroxyapatite, DEAE-Toyopearl-650 and Toyopearl HW-50 chromatography were used successively to purify about 1 mg of the 30/32 kDa protein from 1.4 g of total membrane protein. The purified protein was observed as two bands on 10% SDS-PAGE with Mr of 30 and 32 kDa, whereas it has a Mr of 67 kDa on gel filtration chromatography. Peptide mapping and amino acid analysis showed that the 30K and 32K polypeptides are distinct entities. The 30K was labeled dominantly by [^{32}P]AAGTP (a non-hydrolyzable photoaffinity GTP probe), and this labeling could be blocked by excess GTP, GTPγS or GppNHp, but not by ATP, CTP or UTP; whereas the 32K was not labeled by this GTP analog. However, both 30K and 32K bound to a GTP-affinity column and were eluted together by 1 mM GTP. On SDS-polyacrylamide gels, two protein peaks were observed, whereas on native-polyacrylamide gels, only a single peak was resolved which contained both 30K and 32K. The purified 30/32 kDa protein did not hydrolyze GTP, nor did it interfere with the GTPase activity of purified G_i or G_s. A direct GTP-binding assay suggests that the purified 30/32 kDa protein contains multiple specific binding sites for GTP with positive cooperativity between the binding sites. A K_{d1} of 20.6 μM and K_{d2} of 92.6 nM (R=0.9989) were estimated by the Adair equation, and a n^H of 2.2 (R=0.9977) was estimated by the Hill equation. Specific polyclonal antibodies against this protein have been produced. Immunoblotting indicates that this protein is abundant in membranes prepared from brain and platelet, but not from liver, kidney, lung, spleen, small intestine, heart and skeletal muscle. These results, along with preliminary partial amino acid sequence data, suggest that the 30/32 kDa protein is a novel GTP-binding protein, and it is a "brain and platelet specific" dimer consisting of two different polypeptides. Studies to assess physiological roles for this protein are underway.

6.15

FLUORESCENCE RESONANCE ENERGY TRANSFER SUGGESTS THAT $G\alpha_{i1}$ BINDS TO TUBULIN αβ DIMERS. Marie E. Knight and Mark M. Rasenick. University of Illinois at Chicago, Chicago, IL, 60612

Fluorescence resonance energy transfer (FRET) was used to characterize the interaction between labeled tubulin and $G\alpha_{i1}$. Purified chicken brain tubulin was labeled with 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CM) and purified recombinant $G\alpha_{i1}$ with fluorescein-5-maleimide (FM). Donor quenching was observed due to energy transfer between CM-tubulin and FM-$G\alpha_{i1}$ upon binding of the two proteins. FRET was then used study the effect of $G\alpha_{i1}$-binding to tubulin on the concentration-dependent dissociation of CM-tubulin and FM-tubulin dimers. CM-tubulin was added to increasing concentrations of FM-tubulin and donor quenching monitored. Scatchard analysis of the FRET data gave a K_d for tubulin dimer dissociation of 195 nM, while in the presence of 200 nM $G\alpha_{i1}$, a K_d of 600 nM was determined. Total saturation of dimer association in the presence of $G\alpha_{i1}$ did not change. These data suggest that $G\alpha_{i1}$ binds to tubulin, and while this binding does decrease the affinity of tubulin monomers, it appears to be an allosteric effect. This work is consistent with previous studies in this laboratory where [^{125}I]-tubulin was hybridized to $G\alpha_{i1}$ immobilized on nitrocellulose. These studies, as well as co-immunoprecipitation, demonstrated that αβ tubulin dimers bind to $G\alpha_{i1}$, suggesting that the αβ dimerization site of tubulin is not involved in tubulin-G protein association. It has also been shown that the site of interaction between $G\alpha_{i1}$ and tubulin is not at the carboxyl terminus of tubulin, but does involve a site that is important to the polymerization of microtubules. Tubulin may modulate various G protein functions and regulation of the association between tubulin and G proteins may be important in signal transduction and may involve other second messenger systems.

6.16

CHIMERIC G PROTEINS EXPRESSED IN COS1 CELLS DEFINE DOMAINS ON Gsα WHICH INTERACT WITH TUBULIN FOR THE β-ADRENERGIC ACTIVATION OF ADENYLYL CYCLASE J.S. Popova[1]*, G.L. Johnson[2] and M.M. Rasenick[1]. U. Illinois College of Medicine[1] Chicago, IL 60612-7342 and Nat'l. Jewish Ctr. for Immunology[2], Denver CO 80206

The cytoskeletal protein, tubulin [Tub], binds to Gαs and Gαi1, but not to Gαi2, Gαi3 or Gαo. Tub with hydrolysis-resistant GTP analog bound (Tub-GppNHp) activates adenylyl cyclase [AC] in permeable C6 glioma cells, bypassing the β-adrenoceptor [β-AR]. This appears to be due to activation of Gsα by direct transfer of nucleotide from tubulin. In order to determine the role of Tub as a G protein activator, the effects of GppNHp and Tub-GppNHp on AC activity in permeable COS-1 cells after transient overexpression of wild type and chimeric Gα proteins were studied. Concomitant β-AR stimulation was assessed with (-)-isoproterenol [(-)-iso]. In naive COS-1 cells, Tub-GppNHp was significantly more potent and twice as efficacious as GppNHp in augmenting (-)-iso-stimulated AC activity. COS-1 cells overexpressing the chimeric protein Gαs/i(38) [Gαs 1-356; Gαi2 357-392] demonstrated a 13-fold increase in AC activity upon GppNHp or Tub-GppNHp addition and (-)-iso caused no further increase. The chimera Gαi/s(bam) [Gαi2 1-212; Gαs 213-292] was found to be nearly identical to naive COS-1 cells in AC activation by GppNHp or Tub-GppNHp. However, (-)-iso was found to potentiate GppNHp but not Tub-GppNHp effects. A third construct, Gαi(bam)s/i(38) [Gαi2 1-212; Gas 213-356; Gαi2 357-392] was weakly responsive to GppNHp or Tub-GppNHp and unresponsive to (-)-iso. Gαi (54)/s [Gα12 1-54; Gαs 62-394] showed equally increased AC in response to GppNHp or Tub-GppNHp, and -iso agumented this stimulation. Photoaffinity labeling studies were performed with (^{32}P)-AAGTP (azidoanilido GTP] to asses the ability of Tub to transfer nucleotide to the various G protein chimeras. The results obtained support the view that: a) the loss of the C-terminal part of Gαs inhibits β-AR potentiation of Tub-guanine nucleotide stimulation of AC activity and b) multiple regions between the 1st and 212th amino acids of Gαs are important for guanine nucleotide binding and transfer from Tub and Tub-GppNHp stimulation of AC.

6.17

ISOLATION AND CHARACTERIZATION OF A 66 kDa GTP-BINDING PROTEIN, G_{ir}, ASSOCIATED WITH THE INSULIN RECEPTOR, FROM HUMAN PLACENTA. Tushar K. Varma* and Satish K. Srivastava, University of Texas Medical Branch, Galveston, Texas 77555-0355.

GTP-binding proteins have been implicated in insulin signal transduction. After insulin binds to insulin receptor (IR) the precise mechanism of signal propagation at post-receptor level remains elusive. We have purified and characterized a binary complex of IR and a novel 66 kDa GTP-binding protein (G_{ir}) from human placenta. The binary complex (IR-G_{ir}) bound to insulin-Sepharose and anti-IR(IgG)-Sepharose columns and G_{ir} could be selectively eluted with 1 mM GDP or GTP indicating that affinity binding is accomplished through IR and that there is a strong interaction between IR and G_{ir}. Insulin (100 nM), added to IR-G_{ir} fraction, stimulated GTPγS binding to G_{ir} and its phosphorylation 3-5 fold by activated IR tyrosine kinase. Pre-incubation of this fraction with GTPγS (100 μM) significantly decreased insulin biding to and tyrosine kinase activity of IR. The G_{ir} protein has all the properties of a well characterized G-protein α-subunit: binds GTPγS with high affinity in the presence of Mg^{++}, binds 8-azido-GTP (a photoreactive analogue), can be ADP-ribosylated by pertussis toxin, has distinct GTPase activity, cross-reacts with monospecific antibodies raised against conserved GTP-binding domain of $G_{z\alpha}$ (P960) and ADP-ribosylation domain of $G_{i3\alpha}$ (UBI), and is phosphorylated at tyrosine residues by activated IR tyrosine kinase. These results indicate a physical association and functional interrelationship among IR and G_{ir}.

6.18

CHOLERA TOXIN CATALYZES ADP-RIBOSYLATION OF HETEROTRIMERIC G_s AND NOT THE FREE $G_s\alpha$-SUBUNIT. Michihiro Toyoshige, Shigeru Okuya and R.Victor Rebois, NINDS, NIH, Bethesda, MD 20892.

The stimulatory G protein (G_s) is a heterotrimer (αβγ) that mediates activation of adenylylcyclase by a ligand-receptor complex. The α-subunit of G_s ($G_s\alpha$) has a guanine nucleotide binding site. Cholera toxin (CT) catalytically transfers the ADP-ribose moiety of NAD to $G_s\alpha$. It has been reported that both free $G_s\alpha$ and the G_s heterotrimer are substrates for CT catalyzed ADP-ribosylation. However, recombinant $G_s\alpha$ was a poor substrate for CT in the absence of the βγ-subunit complex (Gβγ). Furthermore, proteolytic removal of the $G_s\alpha$ N-terminus inhibited both its ability to interact with Gβγ and to be ADP-ribosylated by CT. To investigate the nature of the CT substrate, G_s subunits were dissociated in a dose dependent manner by $MgCl_2$ (2-120 mM). The subunit composition of G_s was determined by immunoprecipitation with antiserum against $G_s\alpha$. Proteins in the immunoprecipitates were separated by SDS-PAGE and transferred to Immobilon P membranes. Membranes were probed with G_s subunit specific antisera and [^{125}I]protein A. When G_s was incubated with 2 mM $MgCl_2$, Gβ co-precipitated with $G_s\alpha$, and $G_s\alpha$ could be ADP-ribosylated by CT. GTPγS binding to the guanine nucleotide binding site of $G_s\alpha$ had no effect on subunit composition or ADP-ribosylation by CT. When G_s was incubated with 120 mM $MgCl_2$ in the presence or absence of GTPγS the amount of Gβ that co-precipitated with $G_s\alpha$ was reduced and ADP-ribosylation by CT was comparably reduced. High concentrations of $MgCl_2$ also caused subunit dissociation in the presence of fluoroaluminate. However, subunit reassociation occurred when the $MgCl_2$ concentration was reduced to 2 mM and the ability of CT to ADP-ribosylate $G_s\alpha$ was restored. Thus, the G_s heterotrimer but not free $G_s\alpha$ is a substrate for CT.

6.19

ACTIVATION OF THE STIMULATORY GUANINE NUCLEOTIDE BINDING PROTEIN CAN OCCUR WITHOUT SUBUNIT DISSOCIATION. Shigeru Okuya, Nirmal S.Basi and R.Victor Rebois, Membrane Biochemistry Section, Laboratory of Molecular and Cellular Neurobiology, NINDS, NIH, Bethesda, MD 20892.

The stimulatory G protein (G_s) mediates activation of adenylylcyclase by hormones and neurotransmitters. G_s is a heterotrimeric ($\alpha\beta\gamma$) protein. The α-subunit of G_s ($G_s\alpha$) has a guanine nucleotide binding site, and intrinsic GTPase activity. Activation of G_s requires the exchange of tightly bound GDP for GTP or a non-hydrolyzable GTP analogue (eg. GTPγS). G_s activation can be accompanied by dissociation of $G_s\alpha$ from the βγ-subunit complex but recent evidence suggests that subunit dissociation is not necessary for activation of G_s (Marbach, I., Bar-Sinai, A., Minich, M., and Lavitzki, A. (1990) *J. Biol. Chem.* 265, 9999-10004). We investigated whether subunit dissociation was required for G_s activation. G_s was prepared from bovine brain. The subunit composition of G_s was assayed by immunoprecipitating samples with $G_s\alpha$-specific antiserum and determining the amount of Gβ that was co-precipitated. G_s activation was determined by measuring the amount of ^{35}S-GTPγS bound or by assaying the ability G_s to reconstitute adenylylcyclase in G_s deficient S49 cyc$^-$ membranes. Both activation of G_s and subunit dissociation require Mg^{2+}. $MgCl_2$ (2-120 mM) caused a dose dependent dissociation of G_s subunits. Activation of G_s with GTPγS did not affect the dose dependency of $MgCl_2$-induced subunit dissociation. In the presence of 2 mM $MgCl_2$, G_s was activated by GTPγS but its subunits were not dissociated. These results suggest that dissociation of G_s can occur without activation, and that G_s activation does not require subunit dissociation.

6.20

GTP BINDING DURING G_s ACTIVATION DOES NOT PROMOTE SUBUNIT DISSOCIATION. Nirmal S Basi and R.Victor Rebois, Membrane Biochemistry Section, Laboratory of Molecular and Cellular Neurobiology, NINDS, NIH, Bethesda, Md 20892.

The stimulatory guanine nucleotide binding protein (G_s) serves a critical biological role by coupling hormone receptors to the catalytic subunit of adenylylcyclase. G_s has a guanine nucleotide binding site, and activation requires the exchange of tightly bound GDP for GTP or a non-hydrolyzable GTP analogue. The popular model for activation of G_s also requires that the G_s heterotrimer ($\alpha\beta\gamma$) dissociate into its $G_s\alpha$-subunit ($G_s\alpha$) and $G_s\beta\gamma$-subunit complex (Gβγ). We investigated the effects of guanine nucleotide binding on G_s subunit dissociation. G_s was prepared from bovine brain. Subunit dissociation was determined by immunoprecipitating G_s with $G_s\alpha$-speicific antisera and monitoring co-precipitation of the G_s ß-subunit. When G_s was incubated with 120 mM $MgCl_2$ alone it was inactivated. GTP, GDP and non-hydrolyzable GTP analogues (eg. GTPγS and Gpp[NH]p) protected G_s from inactivation by 120 mM $MgCl_2$. $MgCl_2$ (2-120 mM) caused a dose dependent dissociation of G_s subunits. $MgCl_2$-induced subunit dissociation was not affected by GTPγS (100 μM). Both GTP and GDP were equally effective at partially blocking $MgCl_2$-induced subunit dissociation. When G_s subunits were dissociated with 120 mM $MgCl_2$ in the presence of 1 mM GTP or GDP they rapidly reassociated when the $MgCl_2$ concentration was reduced to 2 mM. When G_s subunits were dissociated by 120 mM $MgCl_2$ in the presence of 100 μM GTPγS, subunit reassociation did not occur after adjusting the $MgCl_2$ concentration to 2 mM. We concluded that non-hydrolyzable GTP analogues neither promoted or inhibited $MgCl_2$-induced G_s subunit dissociation but prevented subunit reassociation. Most importantly, GTP was no more effective at promoting G_s-subunit dissociation than GDP.

6.21

G-Protein βγ Subunits are Specific for Distinct Rhodopsins. John K. Northup, Haya Tamir and David Wildman, NIMH, Bethesda MD 20892.

Signal transducing GTP-binding regulatory proteins (G-proteins) are a family of heterotrimers of approx 40 kDa GTP-binding α subunits complexed with a dimer of 37 kDa β and 8 dDa γ subunits. 16 unique α, 4 β and 7 γ subunits have been identified. We have proposed that the diversity of β and γ subunit forms increases the receptor-specificity of the αβγ trimer. To test this we have expressed and purified dimers of β1 and various γ genes in the insect epithelial cell line Sf9 using baculoviral vectors. The purified recombinant dimers (β1γ1, β1γ2 and β1ste18) were assayed for interaction with the resolved α subunit of the bovine retinal G-protein (Gtα) and with two distinct rhodopsin structures (bovine and squid). All three dimers enhanced pertussis toxin labelling of Gtα with identical affinities ($K_{0.5}$ 10 nM). The three showed considerable differences in affinities for both rhodopsins with $K_{0.5}$ values ranging from 6-700 nM. Furthermore, the rank-order affinities of the βγ dimers were distinct for the two rhodopsins (β1γ2=β1ste18>>β1γ1 for bovine rhodopsin and β1ste18>>β1γ1>β1γ2 for squid rhodopsin). These data indicate that the γ subunit can determine receptor-specificity of the βγ dimer and further suggest that the recently uncovered diversity of γ subunit structures may be related to the diversity of receptor structures with which a Gα subunit can interact.

6.22

THE G_s HETEROTRIMER ACTIVATES ADENYLYLCYCLASE IN CELL MEMBRANES. R. Victor Rebois and Shigeru Okuya Membrane Biochem. Sec., LMCN, NINDS, NIH, Bethesda, MD 20892.

The stimulatory G protein (G_s) mediates activation of adenylylcyclase (AC). G_s is a heterotrimeric ($\alpha\beta\gamma$) protein and is activated when a GTP analogue (eg. GTPγS) binds to the nucleotide binding site of the α-subunit ($G_s\alpha$). G_s activation can be accompanied by dissociation of $G_s\alpha$ from the βγ-subunit complex (Gβγ). Both activation of G_s and subunit dissociation require Mg^{2+}. $G_s\alpha$ is also ADP-ribosylated by choleragen (CT) when it is part of the G_s heterotrimer but not when it is dissociated from Gβγ. Using ADP-ribosylation to monitor G_s subunit interactions, we investigated if subunit dissociation was required for G_s mediated activation of AC in membranes. G_s prepared from bovine brain was incubated in solution with 120 mM $MgCl_2$ for 0 to 2 h and subsequently activated with GTPγS in the presence of 120 mM $MgCl_2$. These conditions caused subunit dissociation based on the inability of $G_s\alpha$ to be ADP-ribosylated by CT. $G_s\alpha$ was then used to reconstitute AC in G_s deficient S49 cyc$^-$ membranes (cyc$^-$). The amount of $G_s\alpha$ incorporated into cyc$^-$ was the same regardless of how it was treated before reconstitution. Furthermore, the amount of $G_s\alpha$ reconstituted into cyc$^-$ was not affected by detergent concentration (0.008%-0.038%). However, the ability of G_s to activate AC depended upon the length of exposure to 120 mM $MgCl_2$ and the concentration of detergent used to reconstitute cyc$^-$. The activity of G_s was maximum when it was not treated with 120 mM $MgCl_2$ prior to activation or when reconstitution was done in the presence of 0.011%-0.019% Lubrol-PX. There was good correlation between G_s activity and CT catalyzed ADP-ribosylation of $G_s\alpha$ in reconstituted cyc$^-$ suggesting that $G_s\alpha$ subunits reassociated with Gβγ in order to activate AC.

6.23

REGULATED VESICLES OF NEUROENDOCRINE AND NEURONAL CELLS CONTAIN DIFFERENT HETEROTRIMERIC G-PROTEINS. Gudrun Ahnert-Hilger[1], Karsten Spicher[2], Theo Schäfer[3], Günter Schultz[2] and Bertram Wiedenmann[1] [1]Med. Klinik u. Poliklinik, Abt. Gastroenterologie, Univ. Klinikum Steglitz, FU Berlin (FAX (+30) 7984141, [2]Institut f. Pharmakologie, FU Berlin, Germany, [3]Friedrich Miescher-Institut, Basel, Switzerland.

The association of heterotrimeric G-proteins with large dense core vesicles from bovine adrenal medulla (chromaffin granules) and small synaptic vesicles from rodent and bovine brain was analysed by immunofluorescence microscopy and Western blotting. Both vesicle types contain G-protein α-, β- and γ-subunits. In addition, two β-subunits, β_1 and β_2, as well as γ-subunits were found. Interestingly, the two vesicle types differ in the pattern of α-subunits. On purified chromaffin granules one α_o-subunit was detected by Western blotting but no α_i-subunits. By contrast, small synaptic vesicles contain two α_o-subunits (α_{o1} and α_{o2}) and two α_i-subunits (α_{i1} and α_{i2}). Thus chromaffin granules and small synaptic vesicles possess complete sets of heterotrimeric G-proteins consisting of α-, β-, and γ-subunits. Functional properties like transmitter storage and/or exocytotic membrane fusion may be modulated by the various G-protein subunits.

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6.24

TISSUE SPECIFIC DEVELOPMENTAL EXPRESSION OF $G_{\alpha s}$-ISOFORMS IN THE RAT. Norbert Fraeyman and Eric Van de Velde. Heymans Institute of Pharmacology, University of Ghent Medical School, Ghent, Belgium

$G_{\alpha s}$-proteins occur in two forms with different molecular weight, generated through alternative splicing. We addressed the question whether maturation or aging has any effect on the phenotypic expression of these isoforms in tissues from newborn, young, mature and senescent rats. Using SDS-PAGE, immunoblotting and reflectance scanning densitometry, the concentration of and the ratio between the two subtypes of α_s, further identified as α_{s1} (high MW-form) and α_{ss} (low MW-form), were assessed. We found that in rat heart and kidney, the cellular content of the α_{s1}-isoform was highest at birth and declined very rapidly till 2-3 weeks of age; further aging had no significant effect. The α_{ss}-isoform was nearly absent at birth, gained importance till 2-3 weeks of age and remained constant during the rest of the life of the animal. The ratio α_{s1}/α_{ss} shifted from 4-5 at birth to less than 1 at one month of age. In rat liver, α_{s1} and α_{ss} remained fairly constant over the whole life span and the ratio α_{s1}/α_{ss} remained slightly lower than 1. We conclude that the effects of maturation and aging on the expression of the α_s-isoforms are tissue specific. The possible consequences on the development of physiological functions are discussed.

6.25

ADDITION OF AMINO ACIDS TO THE N-TERMINUS OF THE STIMULATORY G-PROTEIN α-SUBUNIT INHIBITS ADP-RIBOSYLATION BY CHOLERAGEN. Dennis R. Warner and R. Victor Rebois. NINDS, NIH, Bethesda, MD 20892

G_s is a heterotrimeric (αβγ) protein and dissociation of the α-subunit ($G_s\alpha$) from the βγ-subunit complex (Gβγ) can accompany activation of G_s by a ligand-receptor complex. There is evidence that free $G_s\alpha$ is required for stimulation of adenylylcyclase. However, evidence also exists that the G_s heterotrimer can activate adenylylcyclase. Choleragen is a useful tool for monitoring G_s subunit interactions because heterotrimeric G_s, but not free $G_s\alpha$, is the substrate for ADP-ribosylation by choleragen [Toyoshige, M., *et al.* (1993) *FASEB J.* 7, #575]. Previous studies have shown that deletion of the $G_s\alpha$ N-terminus inhibited ADP-ribosylation by choleragen because it was unable to associate with Gβγ [Journot, L., *et al.* (1991) *J. Biol. Chem.* 266, 9009-9014). The truncated $G_s\alpha$ also failed to activate adenylylcyclase. Using recombinant DNA techniques we modified the 52 kDa form of $G_s\alpha$ by adding a 2 kDa amino acid N-terminal extension that could be removed with the protease Factor X. *In vitro* transcription and translation of the mutant GSα cDNA resulted in a 54 kDa protein that could be immunoprecipitated by anti-$G_s\alpha$ antibodies. Unlike recombinant $G_s\alpha$ without the N-terminal extension, it could not be ADP-ribosylated by choleragen after reconstitution of adenylylcyclase in G_s deficient S49 cyc membranes, indicating that it was unable to form a heterotrimer with Gβγ. $G_s\alpha$ with the N-terminal extension was also unable to activate adenylylcyclase. Removal of the N-terminal extension restored both its ability to be ADP-ribosylated by choleragen and to activate adenylyl-cyclase. Furthermore, the presence of the N-terminal extension prevented co-precipitation of Gβ by anti-$G_s\alpha$ antiserum indicating that the modified $G_s\alpha$ could not associate with Gβγ.

6.26

HETEROGENEITY OF BOVINE BRAIN G PROTEIN γ-SUBUNIT AND THEIR CARBOXYL METHYLATION REACTION. Hitoshi Sohma*, Hideki Hashimoto*, and Toyoaki Akino. Department of Biochemistry, Sapporo Medical University School of Medicine, Sapporo 060 Japan.

Heterogeneity of G protein γ-subunit has been demonstrated by cDNA cloning (γ1-γ7) and protein purification. Previously, we prepared Gi/o from bovine brain and isolated two γ-subunits, γ-I and γ-II. In the present study, we analyzed the γ-subunits. Both of them had blocked N-terminus, and the terminal amino acids were cleaved by an acylamino-acid-releasing enzyme. The amino acid sequences of them revealed that both the γ-subunits appeared to be identical with the γ-subunits deduced from cDNAs; γ-I with γ2 (Gautam et al., PNAS 87, 7973, 1990) and γ6 (Robishaw et al. JBC 264,15758, 1989); γ-II with γ7 (Cali et al., JBC 267, 24023, 1992). Each G-protein isoform (Go*, Gi1, Go, and Gi2) was shown to possess both γ-I and γ-II with one to one molar ratio. Both γ-I and γ-II were carboxyl methylated presumably at the C-terminal cysteine when they were incubated with rat brain membrane fraction and S-adenosyl-[^{3}H]methionene. However, the maximum amount of [^{3}H]CH_3- incorporated was about 0.1 mol/mol. The radioactive γ-subunit was detected only in the pellet fraction after centrifugation. The kinetical study revealed that the Km for βγ-I was five times bigger than that for βγ-II, while the Vm for βγ-II was twice bigger than that for βγ-I. These results suggest that the βγ-subunit isoform and their methylation may have role on a signal transduction process.

6.27

HYPERTROPHIC AGONISTS STIMULATE THE MITOGEN-ACTIVATED PROTEIN KINASE CASCADE IN CARDIAC MYOCYTES. Peter H. Sugden, Antigone Lazou*, Peter E. Glennon*, Angela Clerk*, Monica B. Andersson*, Christopher J. Marshall* and Marie A. Bogoyevitch*. National Heart and Lung Institute, London SW3 6LY, U.K.

Mitogen-activated protein kinases (MAPK) are activated in myocytes cultured from neonatal rat heart ventricles by hypertrophic agonists such as phorbol 12-myristate 13-acetate (PMA), endothelin-1 (ET-1) and acidic fibroblast growth factor (aFGF). We investigated whether these agonists also activated the MAPK activator, MAPK kinase. MAPK kinase was separated from MAPK by FPLC on Mono Q. In extracts of PMA or ET-1-treated myocytes, two major peaks (P1 and P2) of MAPK kinase activity were stimulated. Both MAPK kinase peaks catalyzed the incorporation of ^{32}P from [γ-^{32}P]ATP into p42 MAPK. aFGF also stimulated P1 and P2 MAPK kinase but stimulation of P2 was significantly less than P1. Antiserum raised against MAPK kinase recognized 46 kDa proteins in both P1 and P2. The upstream signalling events that lead to MAPK kinase/MAPK activation may differ for the various agonists. Whereas ET-1 stimulated phospho[^{3}H]inositide hydrolysis 10-20 fold, stimulation by aFGF was less than 30%. Protein kinase C (PKC) isotypes expressed in these myocytes include PKC-α, -δ, -ε and -ζ. Pre-exposure of cells to 1 μM PMA completely down-regulated PKC-α, -δ, and -ε but did not down-regulate PKC-ζ. This manoeuvre abolished the ability of PMA to activate the MAPK cascade when the cells were re-exposed to this agonist but did not affect the response of the cascade to aFGF. The response to ET-1 was intermediate. Activation of the MAPK cascade may be an important integrating mechanism in the hypertrophic response.

6.28

INTERLEUKIN-1 INCREASES PROTEIN KINASE A ACTIVITY BY A cAMP-INDEPENDENT MECHANISM IN AtT-20 CELLS. Andrea R. Gwosdow, Nancy A. O'Connell and Abdul B. Abou-Samra. Massachusetts General Hospital and Shriners Burns Institute, Boston, MA. 02114.

A recent study from this laboratory has shown that the inflammatory mediator, interleukin-1α (IL-1), stimulates protein kinase A (PKA) activity and adrenocorticotropic hormone (ACTH) secretion from AtT-20 cells without any detectable increase in intracellular cAMP accumulation. The present studies were conducted to determine if cAMP is involved in IL-1 activation of PKA and if PKA is responsible for IL-1-induced ACTH release from AtT-20 cells. The data are consistent with a novel mechanism of PKA activation that does not involve cAMP. Inhibition of adenylate cyclase with 2'5'-dideoxyadenosine (2'5'-DDA) did not affect IL-1-induced increases in PKA activity and ACTH secretion. In contrast, CRF-stimulated PKA activity and ACTH secretion were inhibited by 2'5'-DDA. Additional evidence was obtained using the phosphodiesterase inhibitor, IBMX. IBMX did not alter IL-1-induced PKA activity or ACTH secretion yet IBMX potentiated CRF-induced cAMP accumulation. Inhibition of PKA with the PKA inhibitor, H-8, completely blocked activation of PKA and ACTH secretion by both IL-1 and CRF in AtT-20 cells. These observations demonstrate that a) the mechanism of IL-1 activation of PKA is independent of adenylate cyclase or cAMP and b) PKA is the intracellular mediator of IL-1-induced ACTH secretion from AtT-20 cells.

6.29

GLUCOSE REGULATES ACETYL-CoA CARBOXYLASE GENE EXPRESSION IN THE PANCREATIC ß-CELL LINE INS-1.

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Acetyl-CoA carboxylase (ACC) catalyzes the production of malonyl-CoA which may act as a metabolic coupling factor in nutrient-induced insulin release. We have studied the long term regulation of ACC by nutrients using the insulin secreting cell line INS-1. Glucose, from 5 to 20 mM, elicited a 15 fold induction of ACC mRNA. The effect was detected after 4 h and was maximal at 24 h incubation. ACC protein accumulation followed that of its transcript. 2-deoxyglucose, which is phosphorylated by glucokinase but is not further metabolized, caused ACC mRNA accumulation. The glukokinase inhibitors mannoheptulose and glucosamine abolished the action of glucose. Activation of the Ca^{2+}-, cAMP-, and C-kinase pathways with high K^+, forskolin and the phorbol ester PMA, respectively, caused insulin release but not ACC mRNA induction. Several conclusions are drawn. Glucose, within its physiological range of concentrations, is a potent inducer of ACC mRNA and protein. Glucose-6-phosphate may mediate the action of glucose. The signalling systems implicated in the action of glucose on insulin release and ACC gene expression are entirely different.
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6.30

EFFECT OF HEMORRHAGIC SHOCK ON GLUCONEOGENESIS. S.Maitra, M.Edwards, W.Pan, E.Geller. Trauma Research Laboratory, Departments of Emergency Medicine and Surgery, State University of New York, Stony Brook, New York 11794.

The early critical period of hemorrhagic shock (HS) is characterized by hyperglycemia, elevation of hepatic intracellular Ca^{2+}, and a rapid increase in gluconeogenesis. In an attempt to investigate the role of Ca^{2+} on gluconeogenesis, we studied the effect of a calcium channel blocker, diltiazem, on hepatic gluconeogenic substrate pathways following HS. HS was induced in fasted (20h) and anesthetized rats (n=10) by reduction of blood pressure to 40 mm Hg for 20 min. A second group of rats (n=10) received diltiazem (1.2 mg/kg) following HS (HS+DZ). Control (C) rats (n=10) were anesthetized, cannulated and observed for 20 min. Blood samples were obtained for plasma glucose (PG) determination. The livers of HS (n=5), C (n=5), and HS+DZ (n=5) were perfused in a non-recirculating perfusion model and perfusate collected for determination of hepatic glucose production (HGP) with and without lactate as a gluconeogenic substrate. In another set of experiments, livers were freeze clamped for determination of pyruvate (PYR), phosphoenolpyruvate (PEP), Fructose-6-phosphate (F-6-P), and glucose-6-phosphate (G-6-P) levels.

	PG (mg/dl)	HGP (µmole/g/hr) before substrate	HGP (µmole/g/hr) with substrate	G-6-P µmole/g/hr	F-6-P µmole/g/hr
C	87.05±11.45	13.6	21.7	0.126±0.047	0.029±0.011
HS	153.8±32.5	15.8	14.6	0.052±0.029	0.017±0.009
HS+DZ	138.3±12.0	8.76	13.4	0.023±0.004	0.011±0.007

These results indicate that following 20 min of hemorrhagic shock, hyperglycemia is associated with an increase in HGP and decrease of G-6-P and F-6-P levels. PEP and PYR levels are not different in C and HS groups. Diltiazem treatment attenuates hyperglycemic response and the rise in HGP. The decrease of G-6-P and F-6-P in the face of an increase of PG suggests increased activity of G-6-Pase following HS.

6.31

ENDOTOXIN TOLERANCE IS ASSOCIATED WITH DECREASED MACROPHAGE CYCLOOXYGENASES-1 AND -2 Janet Geisel*, James A. Cook and Perry V. Halushka*. Med.Univ.of SC, Charleston, SC 29425

Lipopolysaccharide (LPS) induces macrophages (MØ) to synthesize proteins and eicosanoids. LPS tolerance (TOL) induced by sublethal *in vivo* injections of LPS renders rats resistant to LPS *in vivo*, and MØ refractory to LPS stimulated eicosanoid and protein synthesis *in vitro*. We examined the effect of LPS TOL on the MØ activity and content of cyclooxgenases (COX). Rat peritoneal MØ from TOL and control rats were incubated in media, with or without LPS or arachidonic acid (AA). Thromboxane $(TX)B_2$ levels were used to evaluate COX activity, and immunoblot analysis was used to evaluate the constituitive COX-1 and inducible COX-2 content.

Thromboxane Levels (ng/ml) in Control and Tolerant MØ

	BASAL	LPS (50µg/ml)	AA (16µM)
CONTROL	4.8 ± 0.1	50.1 ± 1.5	195.4 ± 13.7
TOLERANT	3.3 ± 0.6	1.9 ± 0.1*	58.1 ± 7.9*

(n=5,*p<0.05)

TXB_2 levels stimulated by LPS and AA were diminished in TOL MØ by 96% and 70%, respectively, compared to control MØ. Immunoblot analysis of COX-1 demonstrated no induction in response to LPS. COX-2 demonstrated progressive induction at 2, 6, and 12 hr in response to LPS compared with basal. Surprisingly, COX-1 was diminished in TOL MØ compared to control MØ. The data suggest that TOL decreases COX-1 and -2 activity and content. (Supported by NIH GM 27673)

6.32

DETECTION OF ESTROGEN RECEPTORS IN BONE *IN VIVO*. Kim C. Westerlind*, Gobinda Sarkar*, Mark E. Bolander* and Russell T. Turner. Department of Orthopedic Research, Mayo Clinic, Rochester, MN 55905.

Estrogen deficiency is well recognized as a cause of post-menopausal osteoporosis. Likewise, treatment with estrogen results in prevention of this loss. Initially, this effect was thought to be indirectly mediated but, more recently, estrogen receptors (ER) have been detected in osteosarcoma cells and primary cultures originating from surgical waste, suggesting a direct effect of this steroid hormone. Detection of ER *in vivo* however has remained elusive. The purpose of this investigation was to utilize the polymerase chain reaction (PCR) technique to detect ER *in vivo*. Primers were made specific to rat ER sequences. Total RNA was extracted from rat uterus, liver, spleen, and the periosteum from the calvaria using an organic solvent method. cDNA was synthesized from 2 µg total RNA. cDNA corresponding to 40ng total RNA/sample produced intense PCR products for ER. In descending order of intensity were uterus, bone, liver, and spleen. These data provide *in vivo* evidence of ER in bone. The presence of ER is further supported by studies demonstrating that estrogen regulates mRNA levels for IGF-1 within 2 hrs, a time course similar to the hormone's actions on reproductive tissues. Comparative PCR is currently underway to examine the regulation of ER in bone under various conditions known to affect bone metabolism.

G-PROTEIN-LINKED EFFECTORS

7.1

ALDOSTERONE (*A*) STIMULATES PROTEIN ACYLATION AND G-PROTEIN (GP) SYNTHESIS IN A6 CELLS. M.D.Rokaw,P.M.Palevsky,*J.P. Johnson. UPMC & VAMC. Pittsburgh,Pa. 15213.

Small GTP-binding proteins are targeted to membranes by carboxymethylation and lipidation of a conserved C-terminal cysteine residue. Since transmethylation reactions are involved in the action of *A* in A6 cells, and since the apical Na^+ channel is gated by the GP α_3-subunit (MW 41 kDa), we sought to determine if *A* stimulates acylation or isoprenylation(ISO) of GPs in A6 cells. A6 cells were labelled with either ^{3}H-myristate or ^{14}C-palmitate for 18 hours ($\pm 10^{-7}$ M *A*). Crude membrane (M) and cytosolic(C) fractions were examined by SDS-PAGE and autoradiography(AR). *A* stimulated palmitoylation(PAL) of a 40-41 kDa protein in both M and C fractions and stimulated myristoylation of 31 and 41 kDa M proteins. ^{14}C-palmitate labelled cell lysates ($\pm 10^{-8}$ *A*) were immunoprecipitated(IP) with monoclonal antibody to the GTP-binding domain of the α-subunit of GPs(Mab-GP). The specific activity of immunoprecipitates from *A* treated cells was 2X that of controls. ISO is inhibited by blocking mevalonate(MEV)synthesis with lovastatin(LO). Preincubation (18h) of A6 cells with 25µM LO inhibited *A*-stimulated short circuit current at 1, 3 and 8 hours by 30% without an effect on basal current. This effect was reversed by incubation with 100 mM exogenous MEV. To determine whether *A* regulates GP synthesis, A6 cells were labelled with ^{35}S-methionine($\pm 10^{-8}$M *A*). Cell lysates were IP with (Mab-GP) and analyzed by SDS-PAGE and AR. Bands were seen at 97 and 41 kDa only in *A*-treated cells. We conclude: 1. Inhibition of ISO inhibits *A*-stimulated Na^+ transport; 2. *A* stimulates acylation of a 40-41 kDa M-associated protein; 3. *A* stimulates PAL of G-proteins; and 4. *A* stimulates G-P synthesis. These results suggest a model in which lipidation of *A*-induced GPs targets them to the apical membrane where they gate sodium channel activity.

7.2

ROLE OF PROTEIN KINASE C IN ANGIOTENSIN II-INDUCED MITOGENESIS OF NEONATAL RAT CARDIAC FIBROBLASTS. G. W. Booz*, H. H. Singer, K. M. Baker. Weis Center for Research, Danville, PA 17822

Angiotensin II (AII)-induced growth of fibroblasts contributes to remodeling of the cardiac interstitium in various pathologies. AII stimulates cardiac fibroblast proliferation via AT_1 receptors, which are linked to increases in cellular Ca^{2+} and protein kinase C (PKC) activity. In growth-arrested cultures, 24 h exposure to 100 nM AII and 5 ng/ml PDGF-BB stimulated thymidine incorporation into DNA by 97.7 ± 17.5% and 425.1 ± 90.1%, respectively, while 100 nM phorbol 12-myristate 13-acetate (PMA) had a modest effect, 29.8 ± 13.5%. AII-induced thymidine incorporation was unaffected by PKC downregulation, while the PDGF effect was reduced by 73%. PMA, AII, and PDGF produced peak fold-increases in mitogen activated protein (MAP) kinase activity at 5 min of 9.68 ± 2.19, 7.70 ± 1.49, and 10.82 ± 1.29, and sustained increases at 120 min of 4.03 ± 1.56, 2.49 ± 0.71, and 2.38 ± 0.71. PKC downregulation eliminated PMA-induced, but did not affect AII or PDGF-induced, MAP kinase activity, although the AII-induced sustained increase tended to be reduced. The staurosporine analog, compound 3 (0.25 µM) reduced AII-induced fold increase in MAP kinase activity at 5 min by 25%. Increased cellular Ca^{2+} may in part account for AII-induced MAP kinase activity, as ionomycin (1 µM) produced comparable increases at 5 min in MAP kinase activity in control and PKC-depleted cells. Thus, cardiac fibroblasts have PKC-independent and -dependent pathways for inducing MAP kinases and thymidine incorporation. AII can activate MAP kinases by both pathways. In addition, while the full effect of PDGF on thymidine incorporation requires a phorbol ester sensitive pool of PKC, this effect of AII does not, although the possibility that a PMA-insensitive PKC isozyme is involved in the mitogenic effect of AII can not be discounted.

7.3

Na^+-SELECTIVE CHANNELS IN TYPE II PNEUMOCYTES ARE REGULATED BY G-PROTEINS AND ARACHIDONIC ACID.

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The transition to air breathing at birth requires the fetal lung to be rapidly cleared of fluid. The driving force for the fluid reabsorption is generated by Na^+ movement across the apical membrane of the alveolar epithelium via amiloride-sensitive Na^+ channels. *In vivo* evidence suggests that the regulatory pathway involves β-adrenoceptor stimulation of cAMP production. Using the patch clamp technique we have studied the regulation of ion channels in type II alveolar epithelial cells freshly isolated from late gestation fetal guinea pig lungs. Here, in agreement with earlier evidence, we demonstrate that a Na^+-selective, amiloride-sensitive ion channel in excised patches is activated by purified catalytic subunit of protein kinase A only in the presence of ATP. However, this channel is also activated and inhibited by intracellular application of GTPγS and GDPβS respectively. Interestingly, channel activity is markedly increased by addition of arachidonic acid; that the GTPγS-evoked channel activation is not attenuated in the presence of mepacrine suggests that G-protein activation of phospholipase A_2 is not involved in the response. The regulation of fluid reabsorption is clearly not as simple as was originally proposed and probably involves the interaction of at least two classes of G-protein; a basolaterally positioned β-receptor linked G_s and another which is possibly linked to the production of arachidonic acid and is spacially close to the Na^+ channel complex.

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7.4

EFFECTS OF CYCLIC GUANOSINE MONOPHOSPHATE (cGMP) ON PREGNANT RAT LONGITUDINAL SMOOTH MUSCLE. R.E. Garfield and Hidetaka Izumi*. University of Texas Medical Branch, Galveston, Tx, 77551.

The mechanism of the relaxant effects of cGMP were investigated in pregnant rat myometrium. 8-bromo-cyclic guanosine monophosphate (8-br-cGMP) inhibited 45 mM KCl-and 1 μM carbachol-induced contractions of pregnant rat myometrial strips in Krebs' solution. The relaxant effect of 8-br-cGMP was much greater on the carbachol-induced contractions than on the KCl contractions. The 8-br-cGMP inhibited carbachol-response, in 2mM EGTA containing Ca^{2+}-free solution in intact smooth muscle tissues, was antagonized by R_P-8-bromoadenosine 3',5'-cyclic monophosphorothioate (Rp-8-br-cGMPS, a specific blocker of cGMP-dependent protein kinase) and 10 nM staurosporine (potent inhibitor of protein kinase C, PKC). Cyclic guanosine monophosphate (c-GMP, 1 nM-0.1 μM) also decreased the Ca^{2+} sensitivity for contractile proteins, but did not change the amplitudes of InsP3-induced contractions at these concentrations in β-escin treated skinned myometrial strips. However, 1 nM cGMP inhibited the contraction induced by carbachol in the presence of 1μM GTPγS in skinned strips. The inhibition by cGMP in skinned strips was antagonized by (N-[2-(methylamino)-ethyl]-5-isoquinoline sulfonamide, di-HCl) (H-8, a cGMP and cAMP-dependent protein kinase inhibitor) and 10 nM staurosporine. These results indicate that cGMP inhibits the contractile proteins and the guanosine nucleotide/phosphoinositide specific-phospholipase C (PI-PLC) pathway, but, the main site of relaxant effect of cGMP is the guanosine nucleotide/PI-PLC route. It is also suggested that these inhibitory steps might be mediated by activation of cGMP dependent protein kinase and /or PKC activated by cGMP dependent protein kinase.

7.5

PURIFICATION AND RECONSTITUTION OF A G PROTEIN-ACTIVATED PHOSPHOLIPASE C FROM SQUID PHOTORECEPTORS Jane Mitchell* and John K. Northup*, University of Toronto, Toronto, Ontario M5S 1A8 and NIMH, Bethesda, Md 20892

Invertebrate visual transduction initiated by photoactivation of rhodopsin results in membrane depolarization. Intermediate signal transduction components are unknown but evidence suggests involvement of a G protein and a phospholipase C (PLC). We have identified a PLC in squid retina outer segments. The enzyme was purified by ion exchange and affinity chromatographies and identified as a 140-kDa protein on sodium dodecyl sulfate-polyacrylamide gels. The purified PLC hydrolyzed phosphatidylinositol 4,5 bisphosphate (PIP_2) at a rate of 10-15 μmol/min/mg of protein with 1 μM Ca^{2+} and showed a marked preference for PIP_2 over phosphatidylinositol (PI) at all concentrations of Ca^{2+} up to 1 mM. Squid retina also contain a 44-kDa G protein antigenically related to mammalian $G_q\alpha$. Reconstitution of PLC with Gα-44 resulted in a significant increase in PIP_2 hydrolysis over a range of Ca^{2+} concentrations, while mammalian $G_i\alpha$ or $G_i1\alpha$ had no effect on PLC activity. These results suggest that visual phototransduction in cephalopods is mediated by Gα-44 activation of a 140-kDa cytosolic PLC.

7.6

Activation of phosphatidylinositol 3-kinase (PI3-K) by the G_i-coupled acetylcholine muscarinic M_2 receptor by Marijane Russell*, Sim Winitz* and Gary Johnson National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase which phosphorylates the D3 position of phosphatidylinositol, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate. The phsiological importance of the products of PI3K remains unclear. It is interesting however that the products of PI3K accumulate in cells activated by growth factors and also in cells transformed by middle T antigen. Other agonists, for example thrombin stimulation of platelets, have also been shown to activate PI3K. To investigate the possible role of PI3K in other G protein coupled receptor signals we chose to use Rat 1a fibroblasts overexpressing the G_i coupled M_2 receptor (M_2R). Carbachol stimulation of the M_2R causes activation of PI3K and this activation is pertussis toxin sensitive indicating that a functional G_i is critical. Upon carbachol stimulation we also see an increase in tyrosine phosphorylation of the 85 kDa regulatory subunit of PI3-K. In contrast carbachol stimulation of Rat 1a's overexpressing the M_1R which primarily couples to Gq does not result in activation of PI3K. What the functional consequence of activation of PI3K in these cells is and how this response may interplay with additional more defined G protein signal transduction pathways has yet to be determined.

7.7

PROTEIN KINASE C-α MEDIATES BOTH POTENTIATION AND DESENSITIZATION OF ADENYLYLCYCLASE IN HUMAN NEUROTUMOR SK-N-MC CELLS. X.-M. Zhou, P. K. Curran, J. Baumgold, M. Pak, and P. H. Fishman, Membrane Biochemistry section, Laboratory of Molecular and Cellular Neurobiology, NINDS, NIH, Bethesda, MD 20892, and Dept. Radiology, George Washington Univ. Med. Ctr, Washington DC 20837.

Exposure of human SK-N-MC cells to 4ß-phorbol 12-myristate 13-acetate (PMA) increased the isoproterenol stimulation of cyclic AMP levels by 3-5 fold in a time- and concentration-dependent manner. This potentiation was blocked by inhibitors of protein kinase C (PKC), and did not occur in cells treated with inactive phorbol esters and in cells in which PKC had been down-regulated. PMA treatment also enhanced the adenylyl-cyclase (AC) activity stimulated by dopamine, cholera toxin, and forskolin. Thus, the effect of PMA on the AC system was post-receptor. PMA treatment did not impair the inhibition of isoproterenol-stimulated AC by neuropeptide Y, and cholate extracts from control and PMA-treated cells equally reconstituted AC activity in S49 cyc⁻ membranes which lack $G_{s\alpha}$; thus, both G_i and G_s appeared not to be the target of PMA. Membranes from PMA-treated cells exhibited increased AC activity to forskolin in the presence of Mn^{2+} and EDTA. In addition, incubation of membranes with ATP and purified PKC from rat brain could mimic the effect. This is consistent with a direct effect of PKC on the AC catalyst in SK-N-MC cells. PMA treatment also resulted in a rightward shift in the K_{act} for isoproterenol- but not for dopamine-stimulated AC activity. Thus, ß, adrenergic but not D_1 dopamine receptors were being desensitized by PKC activation. Western blotting with antibodies against different PKC isozymes revealed that both the α and ζ isozymes were present in SK-N-MC cells. Whereas PKC-α was translocated from cytosol to the membrane when activated by PMA, ζ was not. Thus, PKC-α, which has been implicated in desensitization in other cell lines, also may be involved in potentiation of AC. The ability of phorbol esters to either potentiate or desensitize the receptor-couple AC may be related to the type of AC expressed. In this regard, the type of AC in SK-N-MC cells is under investigation.

7.8

PROTEIN HISTIDINE PHOSPHORYLATION IN EUKARYOTES. Harry R. Matthews*, Karen Pesis*, Ashok N. Hegde*, Shiv Kumar Sharma* and M.R. Das.* Biological Chemistry, Univ. of Calif., Davis, CA 95616 and Centre for Cellular and Molecular Biology, Hyderabad, India.

Alkali-stable protein phosphorylation is quantitatively a major process in eukaryotic cells. At least some of this phosphorylation is due to phosphohistidine. Eukaryotes contain a protein histidine kinase that phosphorylates specifically on histidine. The gene for this kinase is being cloned using degenerate oligonucleotides and an anti-peptide antibody, in collaboration with R. Aebersold, I. Clark-Lewis, J. Marth and H. Ziltener at the Biomedical Research Centre in Vancouver, Canada. Several of the major protein phosphatases in eukaryotic cells will efficiently remove phosphate from phosphohistidine. Thus, eukaryotic cells have the capacity to regulate protein phosphorylation on histidine. The determination of the in vitro substrates for phosphorylation and dephosphorylation on histidine requires the use of neutral and alkaline conditions and methods have been developed for the study of protein histidine phosphorylation in vivo. Data will be presented showing the occurrence of phosphohistidine on two nuclear proteins and on a plasma membrane protein, p38, which is phosphorylated in response to activated *ras* protein, suggesting that histidine phosphorylation may be involved in a signaling pathway, downstream of *ras*. (supported by the Am. Cancer Soc. #BE82)

7.9

Cloning of a Novel Rhodopsin-Activated Phospholipase C from Squid Retina. Haya Tamir, Michael J. Brownstein and John K. Northup, NIMH, Bethesda MD 20892.

A growing body of evidence supports the hypothesis that invertebrate visual transduction is mediated by inositol-phosphate second messengers generated by phospholipase C (PL-C). Recently the major G-protein from squid (*Loligo forbesi*) retina was cloned and found to be homologous to mammalian Gq, the G-protein activator of PL-C. Therefore we have examined rhodopsin-activated PL-C in squid photoreceptors.

A cytosolic PL-C of Mr 140 kDa was purified to homogeneity from squid retina (Mitchell & Northup). CnBr cleavage of the 140 kDa protein yielded numerous peptide fragments on C18 HPLC of which 7 provided unambigous protein sequence. Six of these are highly homologous with the recently identified neuronal PLC21 from *Drosophila*, while the seventh sequence has no homologue in the GenBank.

We constucted a cDNA library from squid retina and employed two sets of degenerate primers based on the peptide sequences to amplify specific sequences by polymerase chain reaction. A 432 base-pair product of the PCR which encoded the peptide sequences used to design the primers and intervening sequence also highly homologous to PLC21 was used in screening the library for clones of the squid retinal PL-C.

G-PROTEIN-LINKED SIGNALING AND GENE EXPRESSION

8.1

A DIVERGENCE IN THE MAP KINASE REGULATORY NETWORK DEFINED BY MEK KINASE. Carol A. Lange-Carter* and Gary L. Johnson, Division of Basic Sciences, Natl. Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206.

Mitogen-activated protein kinases (MAPK's) are rapidly phosphorylated and activated in response to ligand binding by both tyrosine kinase growth factor receptors and G-protein coupled receptors in many different cell types. Regulation of MAPK results from sequential activation of a series of protein kinases. The kinases that phosphorylate MAPK's, the MAP kinase kinases (MEK's) are also activated by phosphorylation. Cell- and receptor-specific differences in the regulation of MAPK's suggest that multiple regulators of MEK's exist. MEK's are related in sequence to the yeast protein kinases Byr1 (*Schizosaccharomyces pombe*) and Ste7 (*Saccharomyces cerevisiae*) which function in the pheromone-induced mating pathway. Byr1 and Ste7 are in turn regulated by the protein kinases Byr2 and Ste11. The mouse homolog of Byr2 and Ste11, denoted MEKK (MEK kinase), was elucidated from a cDNA sequence encoding a protein of 672 amino acids (73 kD). MEKK was expressed in all mouse tissues tested and phosphorylated and activated MEK. Phosphorylation and activation of MEK by MEKK was independent of Raf, a growth factor-regulated protein kinase that also phosphorylates MEK, leading to activation of MAPK. Expression of the catalytic COOH-terminal domain of MEKK resulted in a high degree of MAPK activation, indicating that NH_2-terminal deletions of MEKK are constitutively active. MEKK and Raf converge at MEK in the protein kinase network mediating the activation of MAPK's by hormones, growth factors, and neurotransmitters. Defining MEKK and Raf as a divergence in the MAPK network provides a mechanism for differential regulation of this system. (Supported by GM15843)

8.2

A REQUIREMENT FOR BOTH HETEROTRIMERIC G PROTEINS AND RAS IN THROMBIN SIGNALING. Vickie J LaMorte*, Eleanor Kennedy*, Joan H. Brown*, & James R. Feramisco*, University of California, San Diego, La Jolla, CA 92093-0636.

Thrombin is a mitogenic factor for certain cell types including fibroblasts and astrocytoma cells. In fibroblasts we have shown a requirement for both the heterotrimeric G proteins, $G\alpha_{i2}$ and $G\alpha_{q,11}$. In fibroblasts, only $G\alpha_{q,11}$ is necessary for coupling thrombin to a phosphoinositide-specific phospholipase C resulting in the observed Ca^{2+} transients. Antisera specific to $G\alpha_{q,11}$ also results in an inhibition of phosphoinositiol hydrolysis in 1321N1 astrocytoma cells. Interference with endogenous Ras by microinjection of either a dominant negative interfering protein, ASN17, or the antisera 259 specific for Ras results in complete abolition of thrombin-induced DNA synthesis in astrocytoma cells. Furthermore, expression of interfering ras mutants by transient transfection reduced thrombin stimulated AP-1 mediated transcriptional activity. Biochemically, an increase in Ras-GTP complexes was seen maximally after 10 minutes of thrombin stimulation. Neither phosphoinositol hydrolyisis nor Ca^{2+} transients as assessed by single cell ratio imaging was affected by 259 antisera or ASN17 protein respectively suggesting that some effectors of thrombin activation do not require Ras. In consanance with these findings, oncogenic Val12 Ras is mitogenic in astrocytoma cells. We are presently examining the mechanisms linking thrombin receptor activation to ras function. Together these results support a functional requirement for Ras in a previously considered heterotrimeric G protein-coupled receptor. (Supported by NIH grants GM36927, HL46345, CA39811 and the Council for Tobacco Research 2423A).

8.3

HORMONAL REGULATION OF TRANSMEMBRANE SIGNALING IN NEONATAL RAT VENTRICULAR MYOCYTES. Suleiman W. Bahouth and Edwards A. Park. Department of Pharmacology, The University of Tennessee, Memphis, TN 38163.

Thyroid hormones (T_3) exert a profound permissive effect on the transmembrane signalling pathway for ß-adrenergic catecholamines in neonatal rat ventricular myocytes (VM). T_3 increased the transcriptional activity of the $ß_1$-adrenergic receptor ($ß_1$-AR) gene by 4-fold within 30 min., resulting in an increase of $ß_1$-AR mRNA by 4-fold within 4 h. These effects of T_3 required nuclear T_3 receptors (T_3R) and were exerted in a tissue specific manner. Computer assisted analysis of the promoter region of the rat $ß_1$-AR gene identified a DNA region that contains a potential T_3R binding motif. This region binds nuclear T_3R as monomers and homodimers and heterodimerizes with nuclear extracts prepared from rat heart. The effect of T_3 on steady-state levels of G protein subunits were also determined. T_3 increased steady-state levels of $G_{s\alpha}$ (M_r=48-kDa) by 2-fold and decreased by 80% the levels of $G_{i\alpha 2}$ (M_r=41-kDa). These changes in $G_{s\alpha}$, produced a marked potentiation in the stimulation of myocardial adenylyl cyclase (AC) activity by agents that activate G_s. Metabolic labeling and immunoprecipitation studies revealed that T_3 increased steady-state levels of $G_{s\alpha}$ by decreasing its rate of degradation. On the other hand, T_3 decreased steady-state levels of $G_{i\alpha 2}$, by decreasing its rate of synthesis. Thus, it appears that T_3 markedly elevate the levels of receptors and G protein subunits positively coupled to stimulation of myocardial AC, while markedly decreasing the levels of G protein oligomers that inhibit myocardial AC. Supported by NIH Grant HL48169.

8.4

INVOLVEMENT OF CALCIUM IN THE CHOLERA TOXIN STIMULATED-PROLACTIN GENE EXPRESSION IN GH_3 CELLS. Ju-Hwa Lin* and Fung-Fang Wang*. Yang-Ming Medical College, Taipei, Taiwan, 11221, ROC

Utilyzing GH_3 pituitary tumor cells as a model system, we have studied the effect of cholera toxin (CTX) on prolactin gene expression. CTX (5 ng/ml) increased prolactin mRNA levels in a time-dependent manner. A noticeable increase was found at 3 h, and maximum level was reached at 12-24 h post CTX stimulation. The effect of CTX cannot be mimicked by pertussis toxin, 1,9-dideoxyforskolin, or 8-Br-cAMP suggesting pathways other than cAMP were involved. Calcium channel blockers nifedipine and verampamil or inhibitor for intracellular calcium mobilization TMB-8 inhibited the CTX-stimulated prolactin mRNA expression, suggesting the involvement of calcium in the CTX stimulated PRL mRNA expression. Among the protein kinase inhibitors tested, only H7 was shown to abolish the CTX-stimulated PRL mRNA accumulation. Half-life of prolactin mRNA increased from 12 h to 22 h in the presence of CTX indicating that post-transcriptional regulation played a role in increased PRL mRNA levels. Transfection studies with DNA constructs composed of chloramphenicolacetyl transferase (CAT) gene expression driven by prolactin-promoter (2.5PRL-CAT) or cAMP response element (2xCRE/tk-CAT) indicate that CTX may exert its effect at the CRE sequence of PRL promoter. 8-Br-cAMP or 1,9-dideoxyforskolin had no effect on CAT activity suggesting cAMP alone cannot account for the CTX effect on PRL gene transcription. Simultaneous presence of TMB-8, verampamil or nifedipine inhibited the CTX stimulated CAT activity in both fusion gene construct, again indicating the importance of calcium in CTX signaling. In addition, protein kinase inhibitor H7 completely blocked the CTX activation on CAT activity. Our results indicate that transcriptional activation and increased PRL transcript stability both contributed to the increased PRL mRNA expression by CTX. Furthermore, calcium played a critical role in the CTX signaling leading to the transcriptional activation of PRL gene.

8.5

IMPLICATION OF CK-2 AND ERKs IN MITOGENIC AND CYTOTOXIC SIGNALING. Patrizia Agostinis, Wilfried Merlevede and Jackie R.Vandenheede. Afdeling Biochemie, K.U.Leuven, B-3000 Leuven, Belgium

The involvement of Ser/Thr kinases in the early events of the signal transduction of the neuropeptide bombesin and of the multifaceted tumor necrosis factor (TNF), was investigated in Swiss 3T3 fibroblasts. Both cytokines act through specific receptors without a tyrosine kinase domain and are "sole mitogens" for these cells. Conversely EGF and insulin, which both signal through a tyrosine kinase receptor, are "incomplete mitogens" for Swiss 3T3 fibroblasts and able to synergise in a mitogenic stimulus only in combination with other growth factors. Our results show that both bombesin and TNF activate the same set of Ser/Thr kinases (ERK and RSK enzymes) involved in the EGF or insulin signal transduction pathways but moreover cause a drastic stimulation of casein kinase 2 (CK-2). PDGF, an other "sole mitogen" for Swiss 3T3 cells, produced a similar CK-2 activation. This observation strongly suggests that the CK-2 activation is essential for the transmission of the mitogenic stimulus. TNF is also a cytotoxic cytokine for L929 cells where it elicits the same array of Ser/Thr kinase activation, including CK-2, as in the Swiss 3T3 cells. Hence, some of the early TNF-mediated cellular steps leading to cell death appear to encompass cell proliferation events.

8.6

DEVELOPMENTAL CHANGES IN CYCLIC NUCLEOTIDES, G PROTEINS AND MUSCARINIC RECEPTORS IN HEART CELLS. Ronald Joyner, Fouzia Rishi*, and Rajiv Kumar*
Department of Pediatrics, Emory University, Atlanta, GA, 30323

We have studied the changes in the levels of G-proteins, cyclic nucleotides and muscarinic receptors during postnatal development of newborn (NB) and adult (AD) rabbit heart. Quantitative immunoblot analysis of $G_{i2}\alpha$, $G_{i3}\alpha$ and $G_s\alpha$ subunits showed that $G_{i2}\alpha$ and $G_{i3}\alpha$ (41 kd) were 150% higher in NB as compared to AD. $G_s\alpha$ was resolved in 48 and 43 kd forms. The 48 kd form was higher in the NB and the 43 kd form was higher in the AD while the total $G_s\alpha$ (sum of 48 and 43 kd forms) was not different in the two stages. Basal levels of cAMP were 80% greater in NB than in AD (1.28 $\pm$ 0.07 vs 0.71 $\pm$ 0.08 pmole/mg wet wt.) while basal cGMP levels were 265% greater in NB than AD (49.82 $\pm$ 5.3 vs 13.65 $\pm$ 0.86 fmole/mg wet wt.). The affinity and number of muscarinic cholinergic receptors (mAChR) were measured by ^{3}H-QNB binding. NB ventricular membranes had 83% more binding than AD membranes (845 $\pm$ 74 vs 461 $\pm$ 98 fmol/mg protein) without any statistically significant change in the apparent K_d (141 $\pm$ 12 vs. 83 $\pm$ 57 pmol). We conclude that the smaller effects of isoproterenol on I_{Ca} and the greater inhibitory effects of cholinergic stimulation on isoproterenol-stimulated I_{Ca} in NB than in AD may be due to the higher G_i levels and the greater mAChR density in NB as compared to AD. Developmental differences in cyclic nucleotide levels may be significant in regulating phosphodiesterase and kinase enzymes.

8.7

MECHANISM OF CYCLIC STRAIN-INDUCED cAMP PRODUCTION IN ENDOTHELIAL CELLS

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The endothelial cell (EC) monolayer is strategically located at the blood vessel wall interface and operates as a sensor for hemodynamic forces. We recently reported that subjecting EC of various origin to cyclic mechanical deformation with the FLEXERCELL apparatus (24% maximum strain, 60 cycles/min) results in increased cAMP levels in cells of arterial and microvascular, but not of venous origin (BBRC *191*:1379-1385, 1993). However, in human umbilical vein EC (HUVEC), but not in the other EC, cAMP is synergistically elevated by first subjecting the cells to cyclic strain for 5 minutes followed by 5 minutes stimulation with forskolin in the continuous presence of strain. In studying the cellular basis for these phenomena, we ruled out the involvement of a number of possible mechanisms using the protein kinase C inhibitor H7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine), the cyclooxygenase inhibitor indomethacin and the calmodulin inhibitor trifluoperazine. The synergistic effect of cyclic strain and forskolin on cAMP levels in HUVEC was not altered by pertussis toxin, which inhibits the inhibitory GTP-binding protein (G_i). By contrast, cholera toxin, which stimulates the stimulatory GTP-binding protein (G_s) abrogated the synergism. Taken together these data suggest that G_s may be involved in the transduction of cyclic strain-induced signaling in HUVEC.

8.8

REGULATION OF SUBSTANCE P RECEPTOR (NK1R) GENE EXPRESSION IN ASTROCYTOMA CELLS. Tony R. Bai, Danyi Zhou* and B. Walker.* UBC Pulmonary Research Laboratory, Vancouver, B.C. V6Z 1Y6

In preliminary experiments examining the regulation of NK1R gene expression, CCF-STTG1 astrocytoma cells (ATCC) were cultured for 1 and 48h during the log phase of growth with 100 nM dexamethasone or 10 μM forskolin or vehicle in RPM1 1640 medium plus 10% FBS. Saturation radioligand binding experiments were performed using [^{3}H]-SP and NK1R and GAPDH mRNA levels determined by ribonuclease protection assay. At 1h, dexamethasone and forskolin had no effect on receptor number or NK1R mRNA levels (relative to GAPDH mRNA levels). At 48h (compared to time-controls), dexamethasone increased NK1 receptor number per cell twofold from $3.7 \pm 0.32 \times 10^4$ to $7.8 \pm 0.44 \times 10^4$ and NK1R mRNA levels fourfold from 0.95 pg/μg to 4.12 pg/μg total RNA. Dexamethasone did not affect GAPDH mRNA levels. At 48h, forskolin had no effect on NK1 mRNA levels or receptor number/cell. These results suggest that: (1) NK1R mRNA levels can be used as a reflection of receptor number, and (2) after prolonged stimulation of glucocorticoid receptors by high concentrations of agonist, NK1R synthesis is upregulated.

8.9

RAS IS REQUIRED FOR THE FULL MITOGENIC RESPONSE TO THYROTROPIN IN WISTAR RAT THYROID CELLS. Erik Kupperman, Wei Wen, and Judy Meinkoth. UCSD, Departments of Medicine and Cancer Center, La Jolla, CA. 92093.

Thyrotropin(TSH) is the primary regulator of thyroid follicular cells. TSH signaling occurs through a G protein coupled receptor which stimulates adenylyl cyclase activity, resulting in the production of the second messenger cyclic AMP(cAMP). Increases in the intracellular levels of cAMP lead to the activation of the cAMP dependent protein kinase(cAPK), and eventually to the expression of genes containing cAMP response elements(CREs) in their promoters. Injection of antibodies raised against G s significantly reduce TSH induced DNA synthesis. TSH treatment leads to the expression of a CRE regulated reporter gene in stably transfected Wistar rat thyroid(WRT) cells. Microinjection of the heat stable inhibitor(PKI) of cAPK caused a complete reduction in TSH and 8Bromo cAMP stimulated reporter gene expression, although it only caused a partial reduction in TSH stimulated DNA synthesis. Overexpression of cellular or oncogenic Ras stimulates DNA synthesis in WRT cells. To determine if Ras is a component of signaling pathways emanating from G protein coupled receptors, we examined the role of Ras in TSH stimulated DNA synthesis. Injection of a dominant negative mutant of Ras (N17 Ras) caused a partial reduction in TSH stimulated DNA synthesis. Injection of both PKI and N17 together reduced TSH stimulated DNA synthesis to background levels. Furthermore, we found treatment with TSH led to an increase in the amount of cellular Ras found in the active, or GTP bound state. These data suggest that both cAPK and Ras are required for the full mitogenic response to TSH in WRT cells.

8.10

NON-LINEAR PROPAGATION OF AGONIST-INDUCED CALCIUM WAVES IN ASTROGLIA. S. Yagodin, C.A. Sheppard, L.A. Holtzclaw, and J. T. Russell LCMN, NICHD.

We have studied calcium signals induced by neurotransmitters such as norepinephrine and glutamate in fura-2 loaded rat cortical astrocytes. Activation of these receptors is coupled to IP_3 generation. Calcium signals consistently originated from a single locus within an astrocyte and propagated as waves. Increasing the agonist concentration increased the wave velocity, frequency, amplitude and the rate of rise. The initiation locus remained invariant during subsequent challenges with agonists that are coupled to IP_3. In these loci the resting Ca^{2+} concentration was elevated compared with the rest of the cytoplasm. From the locus of origin, wave propagation is achieved by a process of diffusion and regenerative Ca^{2+} release in multiple cellular loci provoked by the advancing wave front; in this way, wave propagation is non-linear and saltatory. The regenerative loci appear as discrete focal areas of 7 μm to 15 μm in diameter. The data show that multiple sites exist within astroglia with different thresholds of activation for calcium release in a hierarchical manner, such that the low threshold centers initiate waves, and the higher threshold centers provide for regenerative amplification. During wave propagation, very high (>8μM) calcium concentrations were achieved in local areas of the cell. These findings suggest specializations in the endoplasmic reticulum where the density of calcium release channels is higher. The spatio-temporal organization of Ca^{2+} waves in astrocytes could provide a cellular mechanism for transduction of the strength of agonist action to discrete cytoplasmic sites as well as to the cell nucleus.

8.11

EXTRACELLULAR ATP ACTIVATES MULTIPLE SECOND MESSENGER SYSTEMS AND INCREASES IMMEDIATE-EARLY GENE EXPRESSION IN CARDIAC FIBROBLASTS. Jing-Sheng Zheng*, Marvin O. Boluyt*, Lijun Song*, William H. Adler, Lydia O'Neill*, Michael T. Crow*, and Edward G. Lakatta. GRC, NIA, NIH, Baltimore, MD 21224

Cardiac fibroblasts (CAFB) comprise more than 70% of the total cell population in the mammalian heart and are stimulated by norepinephrine (NE) to increase proliferation and secretion in conjunction with immediate-early gene (IEG) activation. Since ATP is co-released from sympathetic nerve endings in the heart, we examined the effects of extracellular ATP on IEG expression in CAFB isolated from neonatal rat hearts. After collagenase digestion CAFB were passaged 3 times in serum containing media, then incubated in serum-free media for 24 hr prior to experimentation. In response to micromolar quantities of ATP, levels of IEG mRNA increased by 15 min, peaked at 30 min (8 fold), and decreased to baseline by 1 hr. Whereas ATP analogues, ATPrS, ADPβS, 2-methylthio-ATP, and ADP also increased IEG expression, adenosine did not, suggesting that activation of P_2-purinergic receptors mediated the induction of IEG by ATP. To determine the intracelluar pathways responsible for IEG activation by ATP, we investigated the roles of calcium, protein kinase C (PKC) and tyrosine phosphorylation. In CAFB loaded with Indo-1 (AM form), ATP and its analogues transiently increased intracellular Ca^{2+} concentration (Ca_i) (peak at 10-30 sec) followed by a sustained increase in Ca_i, while NE had no effect on Ca_i. The order of efficacy for these agents to increase Ca_i was similar to that inducing *c-fos* expression. Pretreatment of CAFB cultures with TPA to down-regulate protein kinase C (PKC) or pretreatment with PKC inhibitors (staurosporin) for 30 min attenuated the ATP-induced increase in IEG expression. Western blot analysis showed that ATP treatment stimulated tyrosine phosphorylation of a number of proteins including one of 40-45 kDa. These data suggest that extracellular ATP induces IEG expression via P_2-purinergic receptors in CAFB, which in turn activate multiple second messenger pathways including Ca^{2+}, PKC and a tyrosine kinase.

8.12

ANGIOTENSIN II AND PHORBOL ESTERS STIMULATE *c-fos* GENE EXPRESSION IN RAT HEPATOCYTES. POSSIBLE ROLES OF TYROSINE PHOSPHORYLATION AND *c-fos* mRNA STABILIZATION. Claudia González-Espinosa and J. Adolfo García-Sáinz. Instituto de Fisiología Celular, UNAM. Ap. postal 70-248; 04510 México D. F.

Angiotensin II increase the expression of c-fos mRNA in rat hepatocytes. A similar effect was induced by phorbol esters suggesting an involvement of protein kinase C. Actinomycin D inhibited the effect of these agents only partially indicating a possible modulation at a posttranscriptional level. By blocking transcription the decrease in *c-fos* mRNA level was studied in cells treated without any agent or with angiotensin II, PMA or cycloheximide (an agent that stabilizes c-fos mRNA). Our results indicate that PMA and angiotensin II indeed reduce the rate of decay of the mRNA signal.

It has been shown in other cells that angiotensin II increases protein phosphorylation at tyrosine residues. We explore the possibility that tyrosine phosphorylation could be involved in the action of angiotensin II and PMA on *c-fos* expression. It was observed that genistein markedly decreased the effect of these agents on the expression of this proto-oncogene.

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12.1

Signal Transduction by the Type II and Type I TGFβ Receptors. Petra I. Knaus*, Merrill B. Hille*, Herbert Y. Lin*, Aris Moustakas* and Harvey F. Lodish*, Whitehead Institute for Biomedical Research, Cambridge, MA 02142

Transforming growth factor-β, a member of the TGFβ superfamily of cytokines, has profound effects on cell growth, differentiation and development.

While it is clear that the Type II TGFβ Receptor is a Serine/Threonine kinase which is biologically active, it is still uncertain whether the Type II Receptor alone is sufficient for signal transduction, or whether there exists a heteromeric receptor complex with the Type I Receptor.

Several tumor cell lines (N2A, PC12, THP-1, SW480, T-cell lymphomas) which lack the TGFβ Receptor Type II as assessed by their ability to bind radiolabeled TGFβ1, are being used to address the issue of TGFβ signalling. The following experiments are in progress:

- Functional reconstitution of the Type II Receptor in cells lacking the Receptor using different expression systems
- PCR-cloning and screening of cDNA libraries to isolate the cDNA for the Type II Receptor from tumor cells.
- Using anti-peptide antisera to the Type II Receptor we are investigating possible intracellular localisation of the receptor in these tumor cell lines.

12.2

CLONING OF COLUMBID PROLACTIN RECEPTOR, A UNIQUE MEMBER OF THE CYTOKINE/GROWTH HORMONE/PROLACTIN RECEPTOR FAMILY Xiaojuan Chen & Nelson D Horseman Dept. of Physiology & Biophysics, College of Medicine, Univ. of Cincinnati, Cincinati, OH 45267

A prolactin receptor (PRLR) mediates induction of epithelial proliferation and differentiation in the cropsac of pigeons and doves (Columbidae). Transcriptionally regulated genes, and PRL-dependent transcription factors have been identified in this system. The role of receptor-mediated event in regulating transcription induction is being examined. One of the approaches to studying PRL signaling in the cropsac is cloning and characterizing the PRLR from the pigeon. Two oligonucleotide primers corresponding to highly conserved regions of the mammalian PRLRs were used to amplify a portion of pigeon PRLR cDNA. The amplified pigeon PRLR fragment was used to screen cropsac cDNA libraries. One clone contained an ~2.6 kb cDNA insert highly similar to the PRLR cDNAs of other species. This clone, however, did not encode the complete PRLR sequence. Additional reverse transcription-PCRs were used to synthesize cDNA for the complete coding region of the receptor. The deduced 831 a.a. sequence contains a signal peptide and a single transmembrane region. The extracellular domain comprised two repeated, highly homologous units, each of which corresponded to the extracellular domains of mammalian prolactin receptors. The characteristic structural features of the cytokine/growth hormone/prolactin receptor family, namely two pairs of cysteine residues and a wsxws motif, are conserved in both repeats of the pigeon PRLR. While the intracellular region of the receptors is much less conserved, a proline rich region, shown to be critical for signal transduction for other members of this receptor family, is found in the pigeon PRLR. There is no conserved tyrosine kinase domain in the receptor. (Supported by NIH)

12.3

MAPPING THE RECEPTOR BINDING/ACTIVATION RESIDUES OF HUMAN EGF BY PROTEIN ENGINEERING. S.K. Niyogi, S.R. Campion, D.K. Tadaki, M.R. Hauser, and J.S. Cook. Protein Eng. and Molec. Mutagenesis Program and Univ. of TN-Oak Ridge Grad. Sch. Biomed. Sci., Biology Division, Oak Ridge National Lab., Oak Ridge, TN 37831-8077

Site-directed mutagenesis has been employed to examine structure/function relationships of human EGF (hEGF). Interaction of hEGF with its specific cell-surface receptor (EGFR) requires residues scattered throughout the growth factor molecule, including the specific hydrophobic residues Tyr13, Leu15, Ile23 and Leu26 in the N-terminal domain, and Leu47 in the C-terminal domain. In general, ionic residues in hEGF are not critical for receptor binding. However, the guanidinium group of Arg41 is crucial for the formation of a stable hEGF-receptor complex. Quantitative evaluation of double-site mutant hEGF analogues indicates cumulative effects of simultaneous mutations on relative receptor affinity and suggests that each of the individual sites interacts with the receptor essentially independently. The decreased binding observed with mutants of critical hydrophobic residues also translates into a decreased ability to activate the EGFR tyrosine kinase activity. These low affinity/low Vmax mutants, like the low affinity/normal Vmax mutants, are nonetheless able to stimulate, at sufficiently high concentrations the "late" event of thymidine incorporation in cultured cells. However, low Vmax mutants are deficient in the "early" event of intracellular protein-tyrosine phosphorylation. [Research supported by USDOE contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., USPHS grant CA 50735, and NCI postdoctoral training grant CA 09336.]

12.4

TRANSFECTION OF THE HER2 RECEPTOR INTO CHO AND 293 CELLS DOES NOT PRODUCE CELLS RESPONSIVE TO HEREGULIN STIMULATION. Diana Antoniucci*, Samuel Chan*, Katherine Fok*, Liisa Alajoki*, Margaret Hirst*, H. Garrett Wada*, Molecular Devices Corp., Menlo Park, CA 94025.

The HER2 receptor is a tyrosine kinase, transmembrane glycoprotein (p185) also known as the neu or Erb B2 oncogene. This receptor is expressed in some mammary carcinoma tumors, the significance of which is that high levels of expression are associated with poor prognosis. Recently a family of glycoprotein ligands for HER2 has been characterized, the Heregulins, which stimulate auto-phosphorylation of the receptor and proliferation of mammary cells expressing HER2. It is interesting to note that although HER2 is also expressed in ovarian carcinoma cells, no Heregulin activation of HER2 phosphorylation has been detected in these cells. We have observed that Heregulin activation of mammary carcinoma cells (SKBR3) results in the stimulation of extracellular acidification rate as measured by a microphysiometer instrument, the Cytosensor™. Extracellular acidification has been shown to be activated by triggering a variety of receptors. A plasmid construct encoding the HER2 receptor was transfected into CHO-K1 and 293-EBNA cells, and the clones expressing the HER2 antigen on their extracellular surface were isolated. The CHO and 293 cells expressing high levels of HER2 antigen were tested for stimulation with partially purified Heregulin and found not to respond to this ligand. When monoclonal mouse anti-HER2 was reacted with the transfected cells, no stimulation was observed. However, when transfected cells were sequentially treated with anti-HER2 and anti-mouse IgG, the cells responded by increasing their extracellular acidification rate. These results indicate that the aggregation of the HER2 receptor with antibody can activate the receptor and transduce a stimulatory signal. The results also suggest that HER2, p185, alone does not impart Heregulin responsiveness to non-mammary cells, and that perhaps another component is required for reconstitution of the functional Heregulin receptor. Supported in part by Defense Advance Research Projects Agency, Contract MDA972-92-C-0005.

12.5

MALONYL COA: A MARKER FOR HYPERINSULINEMIA -INSULIN RESISTANCE. Asish K. Saha, Theodore G. Kurowski and Neil B. Ruderman. Boston U. Med. Center, Diabetes & Metabolism Unit, Boston, MA 02118.

Non-insulin dependent diabetes mellitus (NIDDM) is characterized by insulin resistance along with decreased insulin secretion and is often associated with obesity. The best way to treat patients with NIDDM is to increase insulin sensitivity and insulin secretion. Pioglitazone (Pio), a thiazolidine derivative that has potent activity to increase insulin sensitivity, has been developed for treatment of NIDDM. This study was designed to determine whether this novel antidiabetic agent has any effect on malonyl CoA and diacylglycerol (DAG) in the muscle and liver of different insulin resistant rodents. Treatment of the KKA^y and ObOb mice for 4 days with pioglitazone (20 mg/kg/day) decreased blood glucose, plasma insulin and triglycerides (TG) levels. Compared to control, muscle DAG level was 2-fold higher in both KKA^y and obob mice. Pio treatment increased DAG by 2-fold in obob and 3-fold in KKA^y mice. In the liver, no significant differences were observed in the DAG content between control and KKA^y mice, however DAG content in the liver of obob mice was 6-fold higher with respect to lean control. Pio treatment increased the DAG content by 2-fold in the liver of KKA^y mice. Malonyl CoA levels are increased in liver and muscle of both obob and KKA^y mice. Pio treatment restored malonyl CoA and plasma TG levels to control values in these insulin resistant rodents. Based on these data, we hypothesized that an increased level of malonyl CoA might provide a marker of the hyperinsulinemic-insulin resistant state even when the hyperinsulinemia and insulin resistance are not severe. (supported by NIH grants DK-07201 and DK-19514).

12.6

EGF-INDUCED GROWTH INHIBITOR OR STIMULATION OF A431 AND SUBLINE CELLS IS DIRECTLY CORRELATED WITH RECEPTOR TYROSINE KINASE CONCENTRATION BUT NOT WITH PLC γ ACTIVITY. Jan-Kan Chen* and Song-Shu Lin*. Dept. of Physiology, Chang Gung Medical College, Kweisan, Taoyuan 333, Taiwan

EGF-induced hydrolysis of phosphatidylinositol 4, 5, biphosphate was compared in A431 cells with respect to their growth response to EGF. A431 cells which express 4- to 5-fold more EGF receptors than A431-4 cells were growth inhibited, while A431-4 cells were growth stimulated by EGF within the same dose range. Treatment of A431 cells with EGF resulted in a 2-fold increase in cellular IP_3 levels and the effect in A431-4 cells was not as obvious. In the presence of tyrosine kinase inhibitor coumaric acid (0.2~2μM), A431 cell growth was stimulated, rather than inhibited, by EGF in a dose-dependent manner. In contrast, the stimulation of A431-4 cell growth by EGF was reduced under the same conditions. Furthermore, in the presence of coumaric acid (up to 0.5 μM), EGF-induced production of inositol phosphates in A431 cells was not obviously affected. Taken together, the results suggest that EGF-induced growth inhibition of A431 cells may be due to a quantitative changes of EGF-receptor tyrosine kinase activity in areas other than the recruitment and activation of phosphatidylinositol-specific phospholipase C_γ.

12.7

HEPARIN INHIBITS SERUM STIMULATION OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) IN VASCULAR SMOOTH MUSCLE (VSM). Eugene M. Langan, III, Jerry R. Youkey, Harold A. Singer, Geisinger Clinic and Weis Center for Research, Danville, PA. 17822.

Heparin is a relatively specific inhibitor of VSM cell proliferation *in vivo* and *in vitro*. The intracellular mechanisms of action of heparin are not known, although there are some data indicating heparin inhibition of proto-oncogene induction through a protein kinase C (PKC)-dependent pathway. This study was designed to determine whether heparin inhibits growth factor-stimulated activation of a signaling pathway involving MAPK in VSM cells. MAPK activity was quantified in cytosolic extracts of cultured rat aortic VSM cells (RAVSM) using myelin basic protein (MBP) as a substrate. Two isoforms of MAPK (42 and 44 kDa) were resolved chromatographically and identified by immunoblotting with anti-peptide antibodies specific for MAPK. MAPK activity in quiescent growth-arrested RAVSM (165±37 units; 1unit=1pmol ^{32}P into MBP/min/mg protein) was markedly stimulated within 15 min by known mitogens (10% serum: 744±44 units; 40 ng/ml PDGF: 523±30 units) and partially sustained for at least 90 min. Heparin treatment of quiescent RAVSM (200 μg/ml for 6-16 hr) inhibited serum-stimulated MAPK activation by 51±8% while the PDGF response was not affected. Activation of MAPK by 0.1 μM phorbol 12,13-dibutyrate (PDB; 604±69 units), a PKC activator, was also not inhibited by heparin treatment. Down-regulation of PKC by 36 hr exposure to PDB (0.1μM) significantly attenuated serum- and PDGF-stimulated MAPK activation. In PKC down-regulated cells, heparin further inhibited only the serum-stimulated MAPK response. Serum stimulation of a pathway involving PKC-independent activation of MAPK is inhibited by heparin in RAVSM.

12.8

DEVELOPMENTAL CHANGES IN INSULIN SIGNAL TRANSDUCTION IN FETAL RAT HEPATOCYTES J.R.Smith-Hall, L.V.V.Faur, D.C.DeSante and D.E.Peavy Indiana University School of Medicine and VA Medical Center, Indianapolis, IN 47306.

We have previously demonstrated partial insulin resistance in cultured fetal rat hepatocytes, despite an increase in insulin receptor number not present in adults. We compared 15, 17 19 and 21 day partially purified fetal insulin receptors to those of adults. Studies revealed no difference between fetal and adult hepatocytes in insulin binding kinetics, apparent molecular weight of the α and ß-subunits, or in the ability of insulin to promote autophosphorylation of the ß-subunit. Although, in the autophosphorylation reaction, the addition of insulin to the partially purified insulin receptors also stimulated the phosphorylation of another protein which ran between 170-175 on SDS-PAGE. This high molecular weight substrate displayed a developmental dependent level of phosphorylation which peaked during the last 2 days of fetal development. Immunoprecipitation using an anti-IRS-1 antibody followed by SDS-PAGE and silver staining detected 3 bands with apparent molecular weights of 95, 120 and 175 kD. mRNA was isolated from all age groups followed by northern blotting using both a rat IRS-1 cDNA probe and a human insulin receptor cDNA probe. Results suggest non-coordinate developmental expression levels between the insulin receptor and IRS-1.

12.9

LY294002 IS A POTENT AND SPECIFIC INHIBITOR OF PHOSPHATIDYLINOSITOL 3-KINASE

Chris J. Vlahos*, William F. Matter*, and Raymond F. Brown*. Cardiovascular Research, Lilly Research Laboratories, Indianapolis, IN 46285-0403

Phosphatidylinositol (PtdIns) 3-kinase is an enzyme implicated in growth factor signal transduction by associating with receptor and nonreceptor tyrosine kinases, including the platelet-derived growth factor (PDGF) receptor. PtdIns 3-kinase is thought to play an important role in mitogenesis; mutants of the PDGF-receptor that lacks the PtdIns 3-kinase binding site fail to exhibit increased DNA synthesis and cell division while other PDGF responses are normal. Therefore, inhibitors of PtdIns 3-kinase may be useful in preventing mitogenesis associated with cellular growth stimuli such as in restenosis and atherosclerosis. Quercetin, a naturally occuring bioflavinoid was found to inhibit PtdIns 3-kinase with an IC50 of 1.3 μg/ml (3.8 μM); inhibition appears to be directed at the ATP binding site of the kinase. Analogs of quercetin were also investigated as PtdIns 3-kinase inhibitors, with the most potent ones exhibiting IC50's in the range of 1.7-8.4 μg/ml. In contrast, genistein, a potent tyrosine kinase inhibitor of the isoflavone class, did not inhibit PtdIns 3-kinase significantly (IC50 > 30 μg/ml). Since quercetin had previously been shown to inhibit other PtdIns and protein kinases, flavinoid analogs were developed that inhibit PtdIns 3-kinase without affecting PtdIns 4-kinase or protein kinases. One such compound, LY294002 (2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one), completely and specifically abolished PtdIns 3-kinase activity (IC50 = 0.43 μg/ml; 1.4 μM) but did not inhibit protein kinases or PtdIns 4-kinase. Analogs of LY294002 demonstated a very selective structure-activity relationship, with slight structural changes causing marked decreases in inhibition. Since PtdIns 3-kinase appears to be centrally involved with growth factor signal transduction, the development of specific inhibitors against the kinase could potentially be beneficial in the treatment of proliferative diseases.

12.10

EVIDENCE FOR NEUROTROPHIN RECEPTOR EXPRESSION AND MUTABILITY IN THE DORSAL COLUMN NUCLEI OF THE BRAINSTEM OF THE RAT. D.R. Foschini*, D.P. Crockett* and M.D. Egger. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med Sch., Piscataway, NJ 08854-5635.

The dorsal column nuclei, consisting of the cuneate nucleus (CN), and gracile nucleus (GN), processes somatotopically organized primary sensory afferents. Both the CN and GN possess immunoreactivity for *p75^{NGFR}*, *trk A*, and *trk B*. While *p75^{NGFR}* is the common low affinity binding site for all neurotrophins, *trk A* binds nerve growth factor with high affinity and *trk B* binds brain derived neurotrophic factor with high affinity. We have reported on a pattern of expression of *p75^{NGFR}* which correlates with the termination of primary afferents within the CN (Crockett et al., Brain Res. 603:324-327, 1992). This pattern of *p75^{NGFR}* immunostaining is eliminated following partial dorsal rhizotomy of the seventh and eighth cervical dorsal root fibers (C7-C8), which terminate in the CN, suggesting that *p75^{NGFR}* is associated with presynaptic terminals. On the other hand, immunocytochemical localization of *trk A* and *trk B* suggest that these receptors are found on cell bodies in the CN. Consistent with this, partial dorsal rhizotomy of C7-C8 failed to disrupt immunostaining for these proto-oncogene products.

12.11

OSMOTIC ENHANCEMENT OF MITOTIC STIMULATION IN RAT THYMOCYTES. Yael Zilberman, Vladimir Melnikov and Yehuda Gutman*. Dept. Pharmacology, Sch. Med., Hebrew Univ., Jerusalem, Israel.

Several early cellular events following stimulation by growth factors resemble those occurring on volume regulation after osmotic challenge. Thus, volume readjustment after initial shrinking or swelling (RVI and RVD, respectively) is effected through activation of Na+/H+ antiporter (causing cytosolic alkalinization), activation of the Na/K/Cl cotransporter. Similar changes occur following exposure to many growth factors (EGF, PDGF, IGFI etc.). While the mechanism of the effect of the growth factors is known to involve signal transduction through receptor tyrosinekinase no known mechanism at present can account for the effect of the osmotic stimulation. Therefore, we followed the interaction of the two types of stimulation. ^{3}H-Thymidine incorporation into rat thymocytes was studied on stimulation with concanavalin A alone or in combination with PDBu (phorbol di-butyrate), as well as on exposure to A23187. Each of these treatments increased ^{3}H-thymidine incorporation. Increasing medium osmolarity to 400m Osm by the addition of NaCl caused a significant increase of ^{3}H-thymidine incorporation in all three types of stimulation. In the absence of mitotic stimulation increased medium osmolarity did not affect ^{3}H-thymidine incorporation. The effect of increased osmolarity started at 350mOsm and subsided at osmolarities above 450mOsm.

TYROSINE KINASES

13.1

ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASES BY ANGIOTENSIN II AND GROWTH FACTORS IN VASCULAR SMOOTH MUSCLE FROM SPONTANEOUSLY HYPERTENSIVE (SHR) AND WISTAR-KYOTO (WKY) RATS. Pamela A. Lucchesi*, Robert A. Redden* and Bradford C. Berk, Emory University, Atlanta, GA 30322

The mitogen-activated protein kinases (MAPK) play a central role in integrating signal transduction from both hypertrophic and hyperplastic stimuli in vascular smooth muscle cells (VSMC). To investigate the role of MAPK signalling pathways in hypertension, we stimulated MAPK with angiotensin II (angII, 100 nM) and platelet-derived growth factor (PDGF, 10 ng/ml) in early passage SHR and WKY VSMC. To measure MAPK activation we used: 1) Western blot analysis with anti-MAPK antibodies; 2) Western blot analysis with anti-phosphotyrosine antibodies to identify the tyrosine phosphorylated, active MAPK; and 3) an "in the gel kinase assay" (ITGKA) to identify kinases capable of phosphorylating myelin basic protein (MBP). Both agonists activated the 42 and 44 kD MAPK differently in SHR compared to WKY with increased phosphorylation in SHR. Complete activation of MAPK in SHR occurred at 2 min in response to angII, and at 5 min in response to PDGF, compared to only partial activation in WKY VSMC. Inactivation of MAPK also differed between SHR and WKY in response to ANGII but not PDGF; e.g., MAPK phosphorylation 20 min after angII exposure completely returned to baseline in SHR but remain elevated in WKY. Two other MBP phosphotransferases (92 kD and 120 kD) that showed increased activation by both agonists in SHR compared to WKY VSMC were identified by ITGKA. These data demonstrate differential kinetics for activation and inactivation of MAPK in SHR compared to WKY. Changes in downstream events such as Na/H exchange and growth may be related to altered MAPK function in SHR. (Supported by NIH RO1 HL44721).

13.2

IDENTIFICATION OF PROLACTIN-INDUCED TYROSINE PHOSPHORYLATED 125 kDa PROTEIN AS FOCAL ADHESION KINASE. Suzanne E. Fenton* and Lewis G. Sheffield*. Endocrinology-Reproductive Physiology Program, University of Wisconsin, 1675 Observatory Drive, Madison, WI 53706.

Physiological doses of prolactin (PRL) induce tyrosine phosphorylation of protein(s) of approximately 125 kDa in mouse mammary epithelial cells. A variety of proteins near that size which are known to be tyrosine phosphorylated were examined using anti-phosphotyrosine immunoprecipitation and Western blotting with specific antibodies. Although epidermal growth factor stimulated tyrosine phosphorylation of its receptor (170 kDa), phospholipase Cγ (145 kDa), and GTPase-activating protein (124 kDa), PRL was found to have no effect on tyrosine phosphorylation of those proteins. Neither epidermal growth factor nor PRL was able to stimulate the tyrosine phosphorylation of the *src* substrate, p120. However, PRL strongly stimulated tyrosine phosphorylation of pp125^{fak} (focal adhesion kinase). This finding was confirmed by immunoprecipitation of PRL-treated cell lysate with anti-pp125^{fak} and identification of tyrosine phosphorylation with anti-phosphotyrosine. A protein of 60 kDa co-immunoprecipitated with pp125^{fak}. That protein was immunologically identified as c-*src*. The c-*src* protein is constitutively associated with pp125^{fak}, as treatments did not affect the amount of c-*src* co-immunoprecipitating with pp125^{fak}. However, PRL does increase pp60^{src} tyrosine phosphorylation. Thus, we have provided for the first time the PRL-stimulated 125 kDa tyrosine phosphorylated protein as focal adhesion kinase and suggest that it may be part of a novel PRL-mediated cell signaling system involving pp60^{src}. This work was funded by NIH grant HD24094 and Hatch grant WIS 3108.

13.3

PROLACTIN INHIBITS EPIDERMAL GROWTH FACTOR-STIMULATED 42 kDa MAP KINASE TYROSINE PHOSPHORYLATION AND ACTIVITY. Lewis G. Sheffield* and Suzanne E. Fenton*. Endocrinology-Reproductive Physiology Program, University of Wisconsin, 1675 Observatory Drive, Madison, WI 53706.

Prolactin (PRL) inhibits epidermal growth factor (EGF)-induced mitogenic activity through alterations in EGF receptor function. It has been previously demonstrated that EGF stimulates tyrosine phosphorylation of members of the mitogen-activated protein (MAP) kinase family in several cell types. We have found that EGF induces tyrosine phosphorylation of a 42 kDa MAP kinase in mammary epithelial cells. The MAP kinase identity was determined following immunoprecipitation with an antiphosphotyrosine antibody, followed by probing with a (MAPK 1)-specific antibody. To our surprise, physiological concentrations of PRL decrease basel level MAP kinase tyrosine phosphorylation. Cells were treated with DMEM, 10 ng/ml EGF, 100 ng/ml PRL, EGF and PRL simultaneously, or PRL followed by EGF, for 10 min to further examine this phosphorylation event. When cells were treated with EGF and PRL at the same time, PRL did not alter EGF effects. However, when cells were pre-treated with PRL for 10 min, PRL eliminated EGF-stimulated MAP kinase tyrosine phosphorylation. In addition, MAP kinase (42 kDa) was immunoprecipitated from cells treated as stated to examine the effect of PRL on EGF-induced MAP kinase toward the exogenous substrate, myelin basic protein. In addition to altering MAP kinase tyrosine phosphorylation, EGF increased MAP kinase activity by approximately 16 fold, but only 2 fold in the presence of prolactin. We have demonstrated here that PRL may regulate EGF function in the mammary gland through the MAP kinase pathway. This work was funded by NIH HD24094 and Hatch WIS 3108.

13.4

TYROSINE KINASE REGULATES THE CA^{2+} CURRENT ACTIVATED BY THAPSIGARGIN AND CARBACHOL IN JURKAT CELLS TRANSFECTED WITH THE HUMAN MUSCARINIC RECEPTOR 1. S. Clare Chung and Phyllis Gardner, Stanford University, Stanford, CA. 94305

During T cell activation, an increase in $[Ca^{2+}]i$ is achieved by the release of intracellular Ca^{2+} stores and by opening of Ca^{2+} channels on the plasma membrane. To elucidate the role of tyrosine kinase (TK) in the process of calcium influx, we performed whole-cell patch clamp studies on Jurkat cells transfected with human muscarinic receptor 1 (HM1). HM1, a G-protein linked receptor, generates inositol trisphosphate which then indirectly or directly activates Ca^{2+} influx (McDonald et. al., J. Bio. Chem. 268:3889-96, 1993). HM1 cells were stimulated with carbachol (CCh) to obtain the Ca^{2+} current, then perfused with the TK inhibitors Lavendustin A and Erbstatin to observe changes in the Ca^{2+} current. The TK inhibitors blocked 92% ± 5.7, n=6 of peak Ca^{2+} current activated by CCh. By contrast, the control experiments with Lavendustin B showed only 20% ± 6.8, n=4 decrease from the peak Ca^{2+} current. The effect of TK inhibitors on thapsigargin (TG)-activated Ca^{2+} currents was also investigated. TG, a specific Ca^{2+} ATPase inhibitor, activated by a stores depletion mechanism which thought to be TK- independent. TK inhibitors blocked 60% ± 6.8, n=8 peak Ca^{2+} current previously activated by TG. Unexpectedly TK may play a role in the activation of Ca^{2+} currents by HM1 receptor as well as by TG.

13.5

DIFFERENCES IN PROTEIN INTERACTIONS WITH THE INSULIN RECEPTOR. D.A. Martin*, M. McCaleb and J. Livingston. Yale U. and Miles Pharm., Inc. West Haven, CT.

Insulin (I) stimulation induces the interaction of the insulin receptor (IR) with phosphotidylinositol 3-kinase (PIK), possibly via the docking protein IRS-1. Such associations are widely studied in Chinese hamster ovary cells (CHO-H) transfected with IR (100,000/cell). Here we show that a neuroblastoma cell line SK-N-MC (20,000 IR/cell) exhibits a different protein association profile for IR than that found in CHO-H. After I treatment, lysates from both cell lines were precipitated (ppt) with antibodies against IR, IRS-1, PIK and phosphotyrosine (PY). PIK activity in PY ppt was increased by I stimulation to similar amounts for both cell types. IR in ppts for PIK and PY was markedly increased by I treatment of SK. Although a large increase in IR was found in the PY ppt with I stimulation of CHO-H, very little increase in IR was found in PIK ppt. In contrast IRS-1 ppt had much larger increases in PIK with I stimulation than found for SK. The differences between cell lines were also evident in ^{35}S-methionine labeling studies. I stimulation of SK results in the association of 45 and 210 kDa proteins with IR, which was not evident for CHO-H. Thus, these findings argue that important tissue differences exist for insulin receptor-protein interactions.

13.6

IMPLICATION OF TYROSINE KINASE IN THE COLD-MEDIATED CONTRACTION OF NEWBORN LAMB CEREBRAL ARTERIES.

L. Craig Wagerle, Giovanni Speziali, Pierantonio Russo. Departments of Surgery and Physiology, Temple University, Philadelphia, PA 19134

Hypothermia is a favored clinical modality used to improve cerebral protection of newborns undergoing cardiac surgery. We examined the effect of tissue cooling on isolated cerebral arteries of newborn lambs and explored the signal transduction pathway of cold-mediated modulation of tension. Rings from middle cerebral arteries from six newborn lambs were mounted in organ baths for one hour at 37°C. Decreasing bath temperature to 21°C reversibly increased tension by (mean±SE) 1.70±0.19 g (n=19). The cold-mediated contraction amounted to 62±3% of the maximal contractile response to 120 mM KCl (at 37°). Addition of Genistein (20 μM), a tyrosine kinase inhibitor, to the bath relaxed the cold-mediated contraction by 96±3%, 1.34±0.30 to 0.10±0.04 g (n=7), but only moderately relaxed KCl induced contraction, 28±1% (n=3). Sodium nitroprusside (30 μM), an activator of guanylate cyclase, N^G-nitro-L-arginine methyl ester (100 μM), a nitric oxide synthase inhibitor, phentolamine (35 μM), an α-adrenoceptor antagonist, or Staurosporine (9 nM), a protein kinase C inhibitor, had little or no effect on the cold-mediated contraction. Thus in this experimental model, cold-mediated contraction appears to be independent of α-adrenergic receptor activation, nitric oxide synthesis-, or protein kinase C-dependent processes. We propose that, 1) tissue cooling enhances vascular contraction via a Genistein-sensitive mechanism implicating tyrosine kinase-associated process(es); and 2) this signal transduction machinery may be a novel biochemical basis for modulation of vascular smooth muscle contraction associated with cooling.

13.7

Tyrphostins inhibit CCK8, guanosine 5'-(3-O-thio)triphosphate-induced inositol 1,4,5-trisphosphate production and amylase secretion in pancreatic acinar cells. Albrecht Piiper*, Danuta Stryjek-Kaminska*, Wolfgang F. Caspary* and Stefan Zeuzem* (SPON : J. Piiper) Univ. of Frankfurt/M., Germany, 6000 Frankfurt/M. 70

We examined the role of tyrosine kinase inhibitors (tyrphostins) in cholecystokinin-octapeptide (CCK8)-induced inositol 1,4,5-trisphosphate (IP_3(1,4,5)) production and amylase secretion in rat pancreatic acinar cells. The data show that various specific cell-permeant tyrphostins (methyl 2,5-dihydroxycinnamate, tyrphostin 25 and genistein) inhibited CCK8-induced IP_3(1,4,5)-production as well as amylase release. Li^+ which abolishes phosphatidylinositol 4,5-bisphosphate resynthesis did not influence tyrphostin-inhibition of IP_3(1,4,5)-production, indicating that the tyrphostins did not inhibit the CCK8-induced IP_3(1,4,5)-production by inhibition of phosphatidylinositol 4,5-bisphosphate resynthesis. Tyrphostins had no effect on vasoactive intestinal peptide-induced amylase secretion. In digitonin-permeabilized cells, tyrphostins decreased IP_3(1,4,5) accumulation and amylase release generated by directly stimulating G-proteins with the weakly hydrolyzable GTP analogue guanosine 5'-(3-O-thio)triphosphate. In isolated pancreatic acinar membranes CCK8 caused a rapid increase in tyrosine phosphorylation of the synthetic tyrosine kinase substrate RR-SRC. CCK8-stimulated tyrosine phosphorylation of RR-SRC reached a maximum after two min and then declined progressively. These results provide evidence that tyrosine kinase is involved in the activation of IP_3(1,4,5) production by G-protein coupled receptors.

13.8

MOUSE OSTEOPONTIN EXPRESSED IN E. COLI IS AUTOPHOSPHORYLATED ON TYROSINE RESIDUES. R.A. Saavedra*, S. Ashkar* and M.J. Glimcher*. Harvard Medical School and Children's Hospital. 300 Longwood Avenue, Boston, MA 02115 USA.

Osteopontin is a secreted glycosylated phosphoprotein found in bone, kidney, dentin, placenta and intestine. This protein is also expressed by activated lymphocytes, macrophages and neoplastic cells. It is thought that osteopontin undergoes differential post-translational modifications in the various tissues where it is expressed, and that these modifications generate distinct forms of protein. To study these post-translational modifications, we expressed mouse osteopontin fused to glutathione S-transferase (GST) in E. coli. The fusion protein can be cleaved with factor Xa to generate recombinant osteopontin and GST. The fusion protein and recombinant osteopontin can be phosphorylated in vitro by mouse kidney homogenates, by a purified fraction containing casein kinase II activity, and by the catalytic subunit of cAMP-dependent protein kinase. During these studies, we found that the fusion protein and recombinant osteopontin become autophosphorylated after incubation with ATP or GTP. The autophosphorylated residues on osteopontin were identified as tyrosines. The biological significance of the autophosphorylation of osteopontin will be discussed.

TYROSINE KINASES AND GENE EXPRESSION

14.1

THE INTRACELLULAR SIGNALLING PATHWAY FOR VASCULAR SMOOTH MUSCLE CELL MIGRATION IN RESPONSE TO PDGF INVOLVES CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II. Michael T. Crow*, Rebecca R. Pauly*, Jing-Sheng Zheng*, and Edward Lakatta. NIA-NIH, Baltimore, MD 21224.

The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. We have previously shown that VSMC migration in vitro in response to PDGF AB or BB is suppressed in differentiated VSMCs. We have observed differences in intracellular signalling between differentiated and nondifferentiated or proliferating VSMCs that may account for this behavior. When stimulated with PDGF, both proliferating and differentiated cells upregulated expression of the immediate early response genes, c-fos and MCP-1 (JE), but only proliferating cells activated calcium/calmodulin-dependent protein kinase II activity. Blocking either calcium-calmodulin interactions (50 uM W7) or the activation of CamKinase II (10 uM KN62) blocked the migration of proliferating VSMCs by more than 90%, while inhibitors of protein kinase C had no significant effect on migration. Pretreatment of differentiated cells with the calcium ionophore, ionomycin (1 uM) or endothelin (10-100 nM) (which also increase intracellular calcium) resulted in an 84 ± 6% return to the migration rate of proliferating VSMCs. This return was also blocked by CamKinase inhibitors and unaffected by inhibitors of PKC. These results suggest that CamKinase II activation is required for VSMC migration and that differences in its activation by PDGF (possibly via phospholipase Cγ) between proliferating and differentiated VSMCs accounts for the suppression of migration in the differentiated phenotype.

14.2

DIFFERENTIAL DISPLAY IDENTIFIES CHANGES IN TRANSCRIPTION PATTERN OF PC12 CELLS IN RESPONSE TO NGF STIMULATION AND K-RAS TRANSDUCTION

R. Somogyi, M.G. Alessandri, S. Almeida, J. Strohkorb, X. Wen, J. Barker, D.L. Simpson. NIH, Bethesda, MD 20892

PC12 (rat pheochromocytoma) cells respond to NGF (nerve growth factor) by adopting a neuronal phenotype, as evidenced by extensive neurite formation, increased synthesis of neurotransmitters, expression of neuronal ion channels and surface markers. Most of the effects of NGF can be mimicked by transduction with a K-ras expressing murine sarcoma virus, suggesting that ras activation plays an important role in the NGF signal transduction pathway. Nevertheless, major questions persist as to which elements are critical for NGF-induced differention, and which genes are induced in this process. We have used the technique of differential display of reverse-transcribed mRNA from PC12 cultures to identify changes in mRNA expression. By using an oligo dT primer paired with a decamer oligonucleotide in a PCR (polymerase chain reaction) at a low annealing temperature (40°C), a set of randomly chosen mRNAs can be amplified. We have used a non-radioactive method for the detection of these PCR products electroblotted from polyacrylamide gels onto nylon membranes. Since the DNA is visualized directly, bands showing the largest variation in intensity between the different samples can be cut right from the membrane and used for reamplification and sequencing. Using two different combinations of forward and reverse primers, we were able to obtain two fingerprints comprising 70 different PCR products. Of these, eight were increased significantly due to NGF and ras stimulation. In addition, one transcript was abundantly expressed in the ras-transduced culture, but not in the control or NGF-treated cells. This method offers the advantages of speed in experimentation, unbiased identification of highly regulated transcripts, and the opportunity to discover new genes which cannot be identified using known primer sequences in conventional PCRs.

14.3

SIGNAL INDUCTION AND COORDINATION OF CELLULAR ENZYMATIC ACTIVITIES: COORDINATION OF PROTEIN PHOSPHORYLATION AND GLYCOSYLATION. Anwar A. MAKIM. Cell. Mol. Biology. 180 Longwood Dr. Kankakee. Ill. 60901.

The binding of the extracellular ligands, estrogens(E) and epithelial growth factor(EGF) activate protein tyrosine kinase (PTKs) endogenous to the E-receptor(ER) and EGF-R. Receptors involved in antigen recognition by cells of the hematopoietic lineage, mediate a cascade of cytoplasmic enzyme interactions with cytoplasmic PTKs of the Src and non-Src types. This report focusses on mechanisms involved in PTK-activation by malignant melanoma T-cell antigen recetor (MMTA-R)(Makim Fed.Amer.Soc. Exp. Biol. 35:514,1976). These receptors are involved in the initiation of cellular activation and/or differentiation.These receptors are responsible for cellular recognition, initiation of cellular activation by regulating the function of cytoplasmic PTKs that modulate the activation of intracellular signaling molecules such as phospholipase C. Human MM is made up of a morphologically/Immunologically heterogenous cell population (Makin,Neoplasma 24:81-99,1977). Cellular (PTK) and PT-phosphatase(PTP) have been determined by the Western blotting (Makim, J.Surg.Oncology 40:21-31,1989) and were correlated with the functional/structural heterogeneity of MM acid soluble glycoproteins (Makim,Proc.Soc.Exp.Biol.Med. 185:158-176, 1987). Normally theres coordination between PTK,PTP and glycosyltranferase activities. Mitogenic signal induction in MMM-cells cause uncoordination of these enzyme activities with incomplete glycosylation and MMM cell immunological heterogeneity and malignancy and invasiveness.

MECHANISMS OF GENE REGULATION

15.1

REGULATION OF CELLULAR RETINOIC ACID-BINDING PROTEINS -I AND -II (CRABP-I, -II) GENE EXPRESSION BY RETINOIC ACID (RA) AND TRANSFORMING GROWTH FACTOR-BETA (TGF-ß) IN PRIMARY CULTURES OF DEVELOPING PALATE CELLS. Paul Nugent, Kruti Shah-Ouazi and Robert M. Greene. Thomas Jefferson University, Philadelphia, PA 19107

Retinoic acid and TGF-ß are important regulators of cell proliferation and differentiation and have been implicated in the development of the mammalian secondary palate. We have examined their effects on the expression of the genes for the cellular retinoic acid-binding proteins-I and -II in primary cultures of murine embryonic palate mesenchymal (MEPM) cells. Northern blot hybridization revealed that both RA and TGF-ß down-regulated the expression of CRABP-I mRNA and up-regulated that of CRABP-II mRNA. Other growth factors, such as epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α) and basic fibroblast growth factor (bFGF), known to be important in embryonic development, were without effect, though bFGF elicited a slight inhibition of CRABP-II expression. Simultaneous treatment with RA and TGF-ß exhibited synergistic effects at lower concentrations of the two agents. Pretreatment of MEPM cells with TGF-ß altered their responsiveness to subsequent treatment with RA. We have also shown by Western blot analysis that MEPM cells secrete several size forms of TGF-ß into the culture medium, including the 25 kDa mature form and the biologically inactive 100 kDa small latent TGF-ß. Extracts of the pericellular matrix of MEPM cells contain the 25 kDa mature form. Thus, TGF-ß secreted by embryonic palatal cells may act in an autocrine/paracrine fashion to modulate CRABP gene expression. Supported in part by NIH grant DE09540 to RMG. PN is the recipient of NRSA grant DE05633.

15.2

DIFFERENTIAL REGULATION OF IMMEDIATE EARLY GENES IN RAT UTERINE EPITHELIUM BY ESTROGEN AND PROGESTERONE. Robert M. Bigsby* and Li Aixin*. Dept. of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN 46202

It has been suggested that regulation of epithelial cell proliferation in the rat uterus is related to the steroidal control of "immediate early" genes. This was examined further by analyzing mRNA levels of c-fos, c-jun, and jun-B in extracts from the whole uterus and the uterine epithelium of hormone-treated immature rats. Tissues were harvested at 3 h after estradiol (E); progesterone (P) was administered 1/2 h prior to E. RNA was extracted from the luminal epithelium of one uterine horn and from the entire contralateral horn. Northern blot analysis showed that when whole uterine RNA was analyzed, E increased mRNA levels for c-fos, c-jun and jun-B (3- to 40-fold control levels). P pretreatment reduced the E-induced increase in c-fos and c-jun. In contrast, when epithelial RNA was analyzed, E increased c-fos and jun-B mRNA levels while it repressed c-jun mRNA levels. P did not block the E-induced increase in c-fos mRNA levels in the epithelium while it did override the repressive effect of E on epithelial c-jun. Loss of c-jun mRNA from the epithelium had a half-life of 50 min., similar to the half-life of c-jun mRNA measured in cultured cells. Thus, in the uterine epithelium, E regulates c-fos and c-jun in opposite directions, thereby causing a dramatic increase in the c-fos:c-jun ratio. P blocks the effect of E on c-jun but does not affect E stimulation of c-fos, producing a more balanced ratio of expression. These changes would affect cell signalling mechanisms working through AP-1 and CREB. (supported by NIH HD23244)

15.3

REGULATION OF EXPRESSION: CLASSICAL AND NONCLASSICAL HLA ANTIGEN EXPRESSION BY TROPHOBLASTS. Mimi H. Chiang, Gail T. Colbern,Elliott K. Main. CPMC Research Institute, San Francisco, CA 94115.

HLA-G is a tissue-specific nonclassical histocompatibility antigen expressed only on placenta (no classical class I expression). The 5' promoter region of HLA-G has recently been described as highly G-C rich, suggesting a potential for methylation and regulation of mRNA expression. Half the interferon consensus sequence (ICS) as well as NF/κB domain (region I) which are present in classical HLA genes is deleted in HLA-G. We have compared 5' enhancer region of HLA-G to homologous regions of HLA (A2) and to murine class I MHC (H-2Ld). Conspicuous deletions of HLA-G occur in both positive (region I) and negative (NRE) regulatory sequences while a great deal of homology exists in the remainder of the promoter region. Recently, differential expression of enhancer A DNA-binding proteins in human first trimester trophoblast cells was identified and suggested to be related to the p50/KBF1/NF-κB proteins. This is the first line of evidence that transcriptional regulation of class I gene expression in human trophoblast cells occurs via DNA-protein interactions. This nucleoprotein complex was present in purified cytotrophoblast (HLA-G expressing) and not syncytiotrophoblast (class I negative) cells. We are investigating specific nuclear factors capable of negatively regulating classical class I gene expression in trophoblast cells. A 217bp fragment from the 5' promoter region of HLA-G (class I response element to ICS) was used in a gel shift assay to identify nuclear binding factors present in HLA class I expressing cell lines. Nuclear extract (NE) from JEG-3 (expresses HLA-G) or PBL (expresses classical class I HLA) contains a factor(s) which binds to this region. This DNA binding factor was not present in NE from Jar or BeWo (class I negative cell lines). We are currently investigating the specificity and function of these factors.

15.4

HYPOXIA INCREASES BETA$_2$-ADRENERGIC RECEPTOR mRNA IN MAMMALIAN CELLS. John F. Schmedtje, Jr. and W. L. Liu.* Department of Medicine, University of Texas Medical Branch, Galveston, TX., 77555-1064.

We reported earlier that ambient hypoxia (2% O_2) increased β_2-AR membrane density (B_{max}) in the hamster smooth muscle cell line DDT$_1$MF-2. This effect of hypoxia was abolished by incubation with either 1.0 μg/ml actinomycin D (ActD, an inhibitor of gene transcription) or 500 ng/ml cycloheximide (an inhibitor of protein synthesis) with a concomitant decrease in B_{max} using either drug. In this study DDT$_1$MF-2 cells were exposed to a 24 hour pre-treatment under normoxic conditions (95% Air, 5% CO_2 at 37°C with 3 nM epinephrine) followed by 48 hours of exposure to either continued normoxia (as above) or hypoxia (2% O_2, 5% CO_2 at 37°C with 3 nM epinephrine) with/without 1.0 μg/ml ActD. Total RNA was then extracted and a semi-quantitative reverse transcriptase polymerase chain reaction (SQRT-PCR) assay was performed to measure the amount of β_2-AR mRNA. An α-tubulin cDNA segment flanked by a β_2-AR cDNA primer pair was transcribed *in vitro* to make a control template RNA. SQRT-PCR was completed by competitive amplification of serial dilutions of the control template RNA with the mRNA for the β_2-AR. An amplification equivalent (ampeq.) was defined as the picograms of template RNA that produced an equal cDNA product when compared to the β_2-AR cDNA band obtained from 500 nanograms total cellular RNA after gel electrophoresis, ethidium bromide staining and laser densitometry. We found that normoxic cells had 1.4 ampeq., whereas hypoxic cells had 3.1 ampeq. β_2-AR mRNA. The effect of hypoxia was blunted when ActD was present; normoxic ActD cells had 2.1 ampeq., and hypoxic ActD cells had 2.5 ampeq. The increase in β_2-AR membrane density found during hypoxia is therefore associated with a relative increase in β_2-AR mRNA compared to total RNA in this cell culture model. (Supported by the Council for Tobacco Research.)

15.5

REGULATION OF GASTRIC INHIBITORY PEPTIDE (GIP) GENE EXPRESSION BY A GLUCOSE MEAL. M. Michael Wolfe, Linda A. Jarboe*, and Chi-Chuan Tseng*. Divisions of Gastroenterology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

GIP is a 42-amino acid peptide that was originally named on the basis of its ability to inhibit gastric acid secretion. We have recently cloned a rat intestinal GIP cDNA and have used this cDNA as our probe for measuring duodenal GIP mRNA concentrations following nutrient administration. Male Sprague-Dawley rats were fasted overnight, after which they were permitted to drink water or 10% glucose. The duodenum was removed at 0, 30, 60, 120, and 240 min and cut longitudinally into two parts for RNA and peptide extraction, respectively. GIP mRNA was measured by Northern analysis, and duodenal mucosal and serum GIP levels were determined by RIA. In response to oral glucose, duodenal mucosal GIP mRNA concentrations increased over the 4-h period, at which time a 4-fold increase was detected. Although mucosal GIP levels remained unchanged, serum GIP concentrations increased significantly at 60 and 120 min. In separate studies, nuclear run-on assays were performed to compare the effects of glucose and water ingestion on transcription of the GIP gene. Whereas no increase in GIP gene transcription was detected in rats ingesting water, the rate of transcription doubled in glucose-fed rats. In contrast, actin gene transcription was nearly identical in both groups, indicating genomic specificity. The results of these studies indicate that duodenal GIP gene expression is stimulated at both the pre- and posttranslational levels by glucose- and lipid-containing meals. Furthermore, these studies suggest that the release of GIP from cellular storage granules might account for the initial increase in serum GIP levels.

15.6

MULTIPLE LEVELS OF BASIC FIBROBLAST GROWTH FACTOR REGULATION IN CATECHOLAMINERGIC CELLS. Michal K. Stachowiak*, John Moffett*, Anna Joy*, Robert Florkiewicz*, Ewa K. Stachowiak*, Barrow Neurological Institute, Phoenix Az 85013, The Whittier Institute, La Jolla CA 92037.

Basic FGF is expressed in central and peripheral catecholaminergic neurons in a developmentally-regulated manner. The present study was undertaken to examine molecular mechanisms controlling expression of bFGF in bovine adrenal medullary cells (BAMC). Western blot analysis revealed 18, 23, and 24 kDa bFGF isoforms in nuclear extracts and 18 kDa bFGF in the cytosolic fraction. Stimulation of neurotransmitter (acetylcholine) or hormonal (angiotensin II) receptors in cultured BAMC increased the levels of all 3 bFGF isoforms. Direct stimulation of receptor signaling pathways (PKC or cAMP) increased levels of either 18 kDa bFGF (PMA) or all three bFGF isoforms (forskolin). PMA-induced increases in 18 KDa bFGF occurred in the cytosol. In contrast, forskolin produced a dramatic increase in nuclear bFGF-immunoreactivity. This increase reflected the induction of all three isoforoms of bFGF. Receptor or second messenger stimulation increased bFGF mRNA levels. To determine the underlying mechanisms we constructed a luciferase reporter gene containing a -2000/+314 bp fragment of the human bFGF gene. In BAMC transfected with the pbFGFLUC plasmid, stimulation of angiotensin II or acetylcholine receptors increased expression of luciferase. Forskolin and PMA both produced 5-20-fold increases in bFGF promoter activity that were additive. Promoter regions mediating those regulations were identified. We conclude that activation of bFGF expression in neural cells during development may be mediated by afferent trans-synaptic and hormonal stimuli. This activation involves: (i) transcriptional stimulation by cAMP and PKC pathways mediated by the upstream region of bFGF gene and (ii) differential translation and subcellular distribution of bFGF isoforms regulated by cAMP and PKC. Supported by Am. Parkinson's Disease Assoc.

15.7

THE ROLES OF DYAD SYMMETRY ELEMENTS IN BASAL AND ACETYLCHOLINE-STIMULATED EXPRESSION OF THE TYROSINE HYDROXYLASE GENE. E. Kim*, S. Maltchenko*, M.K. Stachowiak*, Barrow Neurol. Inst., Phoenix, AZ, 85013

Computer analysis has revealed several dyad symmetry elements (DSEs) within the proximal 430 bp promoter region of the bovine, human, and rat TH genes. Those DSEs do not share sequence homology with known protein binding sites. Their length and localization are conserved in the TH genes from different species. TH gene promoters also contain a GC-rich sequence of similar length and relative position. To examine the roles of DSEs and GC-elements, we constructed a set of bovine TH promoter mutants in which individual elements were deleted. The wild type TH promoter (-430/+25) and its deletion mutants were linked to the luciferase gene in pGL2 plasmid. pGL2TH plasmids were transiently expressed in cultured neuron-like cells (TE671 and IMR32) and in bovine adrenal medullary cells. Deletions of DSE_0, DSE_1, or both elements in the distal promoter region had the same (60-70%) reducing effect on basal promoter activity. They also reduced stimulation of luciferase expression by carbachol. This suggests that DSE_0 and DSE_1 form a regulatory unit in which both elements contribute to basal promoter activity and stimulation by acetylcholine receptors. Deletion of the GC element had no effect on TH promoter activity. Deletion of the longest DSE_4, positioned close to the TATA box (-105/-75 bp), reduced luciferase expression and abolished stimulation by carbachol. Point mutations that disrupted the symmetry of DSE_4 but did not change its putative protein binding sequences also reduced basal promoter activity and carbachol stimulation. Thus, the symmetry of DSE_4 is important for its regulatory function. We conclude that the novel dyad symmetry elements found in the TH gene play a role in the regulation of basal promoter activity and its stimulation by acetylcholine receptors. Together with classical elements (AP1, CRE) they could confer regulation specific for the TH gene. Supported by NSF, NIH, Am. Heart Assoc. and NPF.

15.8

THE POTENTIAL INVOLVEMENT OF AN INDUCIBLE ORPHAN NUCLEAR RECEPTOR, TIS1, IN TRANSCRIPTIONAL REGULATION OF MUSCLE CREATINE KINASE GENE IN SKELETAL MUSCLE CELLS. Wan-lin Yang and Robert W. Lim. Department. of Pharmacology, University of Missouri-Columbia, Columbia, MO 65212

TIS1, an inducible orphan nuclear receptor, was originally isolated as a tumor promoter-inducible gene in mouse 3T3 cells and later shown to be induced by growth factors and other extracellular stimuli. Interestingly, TIS1 mRNA was expressed at a detectable level in "unstimulated" proliferating C2C12 mouse skeletal muscle cells, and the level of TIS1 appeared to increase during muscle differentiation. In order to test if TIS1 might participate in the transcriptional activation of muscle-specific genes during the differentiation process, the effect of TIS1 expression on the activity of reporter genes directed by the 5'-flanking sequences of the muscle creatine kinase (MCK) gene was examined. Expression of TIS1 was found to transactivate in a dose-dependent manner MCK reporter genes containing as little as 80 bp of the proximal 5' flanking region. The involvement of TIS1 protein in the transactivation of the MCK proximal promoter was confirmed by showing that neither the promoterless nor the frameshifted TIS1 was able to transactivate the MCK reporter gene. The effect exerted by TIS1 may be selective for MCK since comparable activation was not observed with an SV40 promoter-driven reporter plasmid. Moreover, transactivation of the minimal (-80) MCK promoter-driven reporter plasmid was observed in mouse embryonic 10T1/2 cells and in proliferating C2C12 cells, suggesting that muscle-specific factors were not required for the transactivation by TIS1. Collectively, these results suggest that TIS1, acting in conjunction with other regulatory factors, may be involved in activating the expression of MCK gene in differentiated muscle cells and raise the possibility that changing levels of TIS1 may help modulate the expression of muscle specific genes in response to physiological alterations.

15.9

EVIDENCE FOR THE INVOLVEMENT OF AN Id-LIKE PROTEIN, HLH462, IN THE REGULATION OF SKELETAL MUSCLE SPECIFIC GENE EXPRESSION. Binbin Chen, Byung Hee Han, and Robert W. Lim. Department of Pharmacology, University of Missouri-Columbia, Columbia, MO 65212

We have examined the role of an Id-like protein, HLH462, in the regulation of muscle cell differentiation. Like the other Id proteins, HLH462 contains a helix-loop-helix (HLH) domain but lacks the basic region which is involved in DNA binding. There is little homology between HLH462 and other Id proteins outside the HLH domain. Expression of HLH462 mRNA can be detected in proliferating skeletal muscle cells and can be induced by growth factors such as fibroblast growth factor (FGF). When HLH462 was overexpressed from an Moloney sarcoma virus (MSV) LTR in C2C12 cells under differentiation-permissive conditions, it can inhibit the expression of a reporter gene containing the 3.3kb upstream regulatory region of muscle creatine kinase gene (MCK) linked to the chloramphenicol acetyl transferase (CAT) transcribed region. Antisense construct of HLH462 had no effect on the expression of MCKCAT gene when overexpressed under the same conditions. We are making HLH462 deletion mutants that are truncated at either the N-terminal, the C-terminal or both to test if these regions play some role in HLH462 functioning. Additionally, we have made a construct that has a seven amino acid epitope (FLAG) fused to the N-terminal of HLH462. We showed that overexpression of this fusion protein in C2C12 cells behaved as the wild type to inhibit MCKCAT reporter gene expression. Antibody against the FLAG epitope will be used to study in vivo processing of the FLAG-HLH462 protein and its interaction with other cellular components. The FLAGHLH462 fusion protein is also being expressed in E. coli and purified by anti-FLAG monoclonal antibody affinity column. Purified FLAG-HLH462 proteins will be used to generate antibodies and to further characterize HLH462 protein functions in vitro.

15.10

INVOLVEMENT OF A LABILE REPRESSOR ACTIVITY IN MODULATION OF ENDOTOXIN TOLERANCE. K.E.A.LaRue* and C.E.McCall*. Dept. of Medicine, Bowman Gray School of Medicine, Winston-Salem, N.C. 27157-1042

Cells can adapt to stimulation by bacterial lipopolysaccharide endotoxin (LPS) and thereby protect animals from the lethal effects of LPS. LPS causes lethality by inducing inflammatory genes such as interleukin 1 (IL-1) and tumor necrosis factor (TNF). The cellular mechanisms responsible for tolerance to LPS are unknown, but we have found that in vivo adaptation of blood neutrophils (PMN) to LPS in humans with septic shock involves repression of LPS-induced increases in IL-1β mRNA and IL-1ß protein synthesis (McCall et al. (1993) J. Clin Invest. 91,853-861). We have developed an in vitro model to study the molecular basis of LPS tolerance, using the human acute monocytic leukemia cell line, THP-1. A primary dose of LPS renders the cells refractory to subsequent doses, as measured by IL-1β mRNA and protein synthesis. This tolerant phenotype, as well as that observed in human PMN, is stimulus-specific as the cellular response to other stimuli is normal or enhanced. We now present evidence that inhibition of protein synthesis by four different agents overcomes or reverses downregulation of the IL-1β gene, in both the in vivo and in vitro models of endotoxin tolerance. We conclude that a labile repressor activity is involved in regulation of IL-1β gene expression and may play a role in the mechanism responsible for LPS tolerance.

15.11

EXTRACELLULAR Ca^{2+} MODULATES THE PREREPLICATIVE INTRACELLULAR CYCLIC AMP SURGES IN EGF-STIMULATED PRIMARY NEONATAL RAT HEPATOCYTES. U. Armato, M. Ribecco, C. Guerriero, and I.F. Whitfield*, University of Verona, I-37134, Italy and *NRC, Ottawa, K1A0N6, Ont., Canada.

Two prereplicative cAMP surges occur in hepatocytes after 70% partial hepatectomy or infusion of TAGH mixture and are involved in initiating DNA replication. The present studies explored the relationship(s) between these cAMP surges and extracellular Ca^{2+} deprivation, which is known to block the flow of cells into the S phase. Neonatal rat hepatocytes were enzymatically isolated, purified to 98% on a Percoll gradient, and seeded into collagen-covered flasks containing the synthetic $HiWo^5Ba^{2000}$ medium. Inactivated FBS (10-5% v/v) was given only during the first few hours *in vitro*. EGF (1 ng/ml) was added to ether high (1 m*M*) or a low (0.01 m*M*)-Ca^{2+} medium on day 4. Unstimulated controls received a change of either medium. Cultures were sampled during the next 10 hr. EGF elicited two pre-DNA-synthetic intracellular cAMP surges in the hepatocytes incubated in the high-Ca^{2+} medium. cAMP was released into the medium only after the peaking of the 2nd surge (6 hr). By contrast, a sharp first cAMP surge but a dramatically diminished second surge were detected in EGF-stimulated hepatocytes in Ca^{2+}-free medium. The extracellular cAMP level in this Ca^{2+}-free medium cAMP levels rose within the first hour and then remained high. Thus, cAMP synthesis stimulated by EGF was not affected by the external Ca^{2+} level, but the ability of the hepatocytes to retain the newly synthesized cAMP intracellularly depended on extracellular [Ca^{2+}]. Hence, the G1/S block caused by external Ca^{2+} deprivation is due to the inability of hepatocytes to retain cAMP needed to activate proliferation-related events. (This work was supported by the Italian Association for Cancer Research [AIRC], Milan).

15.12

ACTIVATION OF THE TYPE -B NATRIURETIC FACTOR GENE IN MOUSE P19 AND D3 STEM CELL CULTURES INDUCED FOR CARDIAC MYOGENESIS. Poppo H. Boer*, Jenny Phipps and Zahra Rassi*. University of Ottawa Heart Institute*, 40 Ruskin Street, Ottawa, Ontario, Canada, K1Y 4E9 and National Research Council of Canada, M54, Montreal Road, Ottawa, Ontario, Canada K1A 0S2.

We examined the temporal transcriptional activity profiles of the gene for type-B natriuretic factor, BNF, and other cardiac muscle specific genes in cultured multipotential mouse cell lines. We used P19 embryonal carcinoma cells and D3 embryonic stem cells which were induced for in vitro cardiac myogenesis and displayed spontaneous beating activity. RNA was isolated at regular intervals throughout the differentiation programs and on Northern blots it revealed abundant cardiac α-actin transcripts beginning at Day 6, reaching maximum levels during Days 7 to 8 and declining to low levels by Days 11 - 15. The transcriptional activation profile of the BNF gene, like that established for the type-A natriuretic factor gene, was similar to that of the induced α-actin gene, but there were quantitative differences that were best assayed by reverse transcriptase-mediated polymerase chain reactions. The BNF transcript levels in the P19 developmental model system may reach up to 15 - 20% that of adult mouse ventricular muscle tissue. This opens up the possibility of defining regulatory control elements for BNF gene expression in stably transfected cell-lines. Supported by the Medical Research Council of Canada and the Heart and Stroke Foundation of Ontario.

15.13

POSITIVE REGULATION OF THE CDC2 PROMOTER BY C-MYC OVEREXPRESSION. Teresa L. Born*, Jeffrey A. Frost* and James R. Feramisco*. University of California, San Diego. La Jolla, CA 92093.

The c-myc oncogene is known to be involved in many human cancers, yet its precise molecular function has remained elusive. The myc protein has a high degree of sequence homology with known B-HLH-transcriptional activators, and it is able to upregulate synthetic reporters containing consensus c-myc binding sites. However, few target genes for c-myc transactivation have been described as of yet. Our lab has been interested in examining the transcriptional regulation of $p34^{cdc2}$,since it is a key regulator of cell cycle progression. Since c-myc has been implicated in cell cycle regulation, we decided to investigate the effect of c-myc overexpression on cdc2 transcription. We have microinjected various c-myc constructs along with a cdc2-promoter-luciferase reporter (cdc2-LUC; kindly provided by Stephen Dalton) in order to address this question. Coinjection of a wild-type c-myc expression construct with cdc2-LUC into quiescent REF52 cells leads to an accelerated accumulation of cdc2-driven luciferase protein. This effect is serum-dependent, and is enhanced if limiting amounts of myn are co-expressed. Mutants lacking the leucine-zipper dimerization motif, or with a mutated Ser62 phosphorylation site (kindly provided by George C. Prendergast) are inactive with respect to the cdc2 promoter. We are currently investigating the precise molecular mechanism of this activation by c-myc.
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15.14

AN IN VITRO MODEL TO STUDY SIGNALING MECHANISMS OF PROGRAMMED CELL DEATH. Reginald Halaby[1]*, Richard A. Lockshin[2], and Zahra F. Zakeri[1]*. Dept. of Biol., Queens College/CUNY, Flushing, NY 11367 and [2]Dept. of Biol. Sci., St. John's Univ., Jamaica, NY 11439.

We have established an in vitro culture system for the labial glands of *Manduca sexta* to isolate early markers of programmed cell death (PCD). PCD involves cells dying under their own control, and is clearly evident in embryos and metamorphosing animals. In *Manduca*, cells of the labial gland die synchronously over five days during the larval to pupal molt. Hormonal signals provoke PCD; however, the molecular mechanisms of PCD are poorly understood. To study these mechanisms, we have used in vitro culturing of labial glands. Measurement of the level of energy metabolism of the glands in culture was via a colorimetric assay based on the ability of living cells to reduce (3-[4,5-Dimethythiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). MTT activity reaches a peak at Day -1 (counted from the beginning of metamorphosis) and progressively declines throughout metamorphosis. Labial glands were incubated in Grace's medium with or without ecdysone for 1, 2, 4, 8, 12, and 24 hours. The effects of ecdysone (molting hormone) on the pattern of protein synthesis and ^{35}S-methionine incorporation into protein were examined. Trichloroacetic (TCA) precipitable counts of Day -2 glands (presumably the most sensitive to ecdysone) plotted versus time of in vitro incubation revealed that from 4 to 24 hours of incubation with ecdysone there is a general increase in total incorporation. Control glands showed an overall increase in total incorporation over 24 hours. One dimensional gel electrophoresis of Day -2 glands incubated with or without ecdysone, show the appearance of an ecdysone-induced protein band approximately 70 kD after 4 hours of incubation. These results suggest that ecdysone plays a role in signaling during PCD of the labial gland and that the in vitro model will allow us to decipher the molecular mechanisms of PCD.

15.15

ALTERATIONS IN α_{1B}-ADRENOCEPTOR mRNA EXPRESSION DURING CYCLIC STRETCH IS CELL-TYPE SPECIFIC IN CULTURED VASCULAR SMOOTH MUSCLE. Martha S. Lundberg, Devaki N. Sadhu, Kenneth S. Ramos and William M. Chilian. Texas A&M University, College Station, Texas 77843

The circulation is organized into a series of vascular microdomains each subjected to different dynamic physical forces (i.e., flow, pressure, stretch). Furthermore, smooth muscle cells (SMCs) making up the wall of these distinct vessel segments display unique functional characteristics, e.g., distribution of α-adrenergic receptors. Thus, we hypothesized that these particular characteristics may be attributable to mechanosensitive gene regulation. To further understand the process of smooth muscle specialization, we examined the role of cyclical stretch (120 cycles/min) for 72 h on collagen on the expression of specific genes [α1b-adrenergic receptor (α1b-AR), the contractile-specific α-smooth muscle (α-SM) actin and nonmuscle β-actin] using Northern analysis from different vessel types: rat heart coronary artery (P5) and thoracic aorta (P4). Coronary SMCs displayed a 1.6-fold increase in α1b-AR mRNA and aortic SMCs showed a 1.4-fold decrease over unstretched controls. α-SM actin expression was significantly reduced in both coronary and aortic SMCs after 72 hrs of stretch ($P \leq 0.05$), 1.8- and 3.1-fold, respectively. Steady-state levels of the α-actin mRNA also decreased 1.7-fold in stretched aortic cells, however, it was relatively unchanged in coronary SMCs. These data show that rat SMCs derived from the smaller, more muscular coronary artery respond to *in vitro* mechanical stimulation in a manner different from thoracic aortic cells indicating that a dynamic environment is important for SMC phenotypic modulation in culture. The results here, along with the fact that different sized vessels have unique vasoactive properties to physiological stimuli *in vivo*, suggests that physical forces participate in normal vascular cell specialization.

15.16

MM-LDL INDUCED cAMP LEVELS INHIBIT ELAM EXPRESSION AND NEUTROPHIL BINDING TO ENDOTHELIAL CELLS.
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We have previously reported that minimally oxidized low density lipoprotein (MM-LDL) and other agents which increase cAMP levels induce the adhesion of monocytes but not neutrophils to cultured endothelial cells. We have also shown that this effect of MM-LDL as well as the induction of monocyte chemotactic protein 1 (MCP-1) mRNA and protein, and the activation of the transcription factor NFkB are highly associated with the elevation of cAMP levels in endothelial cells following MM-LDL treatment. In this abstract we report that pretreatment of endothelial cells with MM-LDL or cholera toxin caused a 60-85% ± 5% inhibition of the induction of neutrophil binding by LPS. This decrease in binding was associated with 60% inhibition of ELAM mRNA accumulation and 40-60% ± 4% inhibition of ELAM expression detected by northern analysis and ELISA, respectively. However MM-LDL pretreatment did not significantly inhibit the induction of monocyte binding, accumulation of MCP-1 mRNA, or ICAM expression induced by LPS. These data suggest that agents which increase cAMP levels including MM-LDL and cholera toxin inhibit the induction of neutrophil binding by causing the inhibition of ELAM expression. The finding that elevated cAMP activates NFkB and differentially regulates the expression of ELAM and MCP-1, both of which contain NFkB binding sequences in their promoter region, is consistent with the multiplicity of factors necessary for regulation of gene expression. Differential regulation of leukocyte binding to endothelium may regulate the monocytic and not neutrophilic infiltration of atherosclerotic as well as other chronic inflammatory lesions.