GENERAL INFORMATION

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

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

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

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

Fees:
APS Member .................. $235.00
Nonmember .......................... $280.00
Retired Member .................. $75.00
Student .......................... $75.00
Guest .......................... $40.00
(Non-scientist family members of registrants)

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

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

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

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

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

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

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

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

UPPER LEVEL: American/Spanish, British/French
LOWER LEVEL: All other meeting rooms

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

**Evening Lecture**

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

5:00 PM — Hernando Ballroom

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

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**Thursday, October 6**

**Symposium**

2.0  **Musculoskeletal Responses to Mechanical Stimuli**

8:30 AM — Hernando Ballroom

Chair: Herman Vandenburgh

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>8:30</td>
<td>Skeletal muscle use and regulation of contractile protein gene expression. Radovan Zak. Univ. of Chicago.</td>
</tr>
<tr>
<td>9:00</td>
<td>Discussion.</td>
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<tr>
<td>9:35</td>
<td>Discussion.</td>
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<tr>
<td>10:45</td>
<td>Discussion.</td>
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<tr>
<td>10:50</td>
<td>Biochemical signal transduction of mechanical strain in osteoblast like cells. D.B. Jones. Univ. of Munster, Germany.</td>
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<tr>
<td>11:20</td>
<td>Discussion.</td>
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**Symposium**

3.0  **Pulmonary Responses to Mechanical Stimuli**

7:00 PM — Hernando Ballroom

Chair: D. Eugene Rannels

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>7:00</td>
<td>Physical signals in compensatory growth of the lung. D. Eugene Rannels. Penn. State Univ.</td>
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<tr>
<td>7:30</td>
<td>Discussion.</td>
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<tr>
<td>8:05</td>
<td>Discussion.</td>
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<tr>
<td>8:40</td>
<td>Discussion.</td>
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**Posters**

4.0  **Pulmonary Responses to Mechanical Stimuli**

Posters are on display: 8:30 AM—10:30 PM.

Authors are in attendance: 3:30—5:00 PM.

**Board #**

1  4.1  Reduction in wall tension in isolated pulmonary vessels stimulates matrix metalloprotease activity. C.A. Tozzi, S. Thakker-Varia, and D.J. Riley. UMDNJ-R W Johnson Med. Sch. and VA Med. Ctr., Lyons, NJ.


3  4.3  Physical responses of the trachea to transection in the living rat. M. Lorber. Georgetown Univ.


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**Poster**

5.0  **Musculoskeletal Responses to Mechanical Stimuli**

Posters are on display: 8:30 AM—10:30 PM.

Authors are in attendance: 3:30—5:00 PM.

**Board #**


8  5.4  Regulation of proliferation and platelet-derived growth factor expression in palmar fibromatosis (Dupuytren’s disease) by mechanical strain. B.A. Alman and D.A. Greet. Tufts Univ.
5.5 Mechanical stress and expression of stress protein in growth and differentiation of skeletal muscle. Y. Atomi. Univ. of Tokyo, Japan.

Poster

6.0 Cardiovascular Adaptations to Mechanical Stimuli: Peripheral Vasculature

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

Board #

17 6.8 Mechanical strain enhances expression of the PDGF-A and EGR-1 genes in vascular smooth muscle cells. G.J. Vives, E. Wilson, T. Collins, V. Sukhatme, and H.E. Ives. UCSF, Beth Israel Hosp., and Harvard Univ.
18 6.9 Mechanical strain induces expression of smooth muscle myosin in vascular smooth muscle cells. H. Papadopoulos, B.G. Dukes, T. Neely, and F. Davis. UCSF.
19 6.10 Intercellular communication via receptor-mediated mechanisms in ATP and bradykinin-induced increases in endothelial intracellular calcium. H.M. Houda, J.I. Goldhaber, L.L. Demer, and J.N. Weiss. UCLA.
24 6.15 Elevated stretch and shear stress produce distinctly different changes in capillary hydraulic conductivity. D.A. Williams and V.H. Huxley. Univ. of Missouri.

Poster

7.0 Cardiovascular Adaptations to Mechanical Stimuli: Myocardial Cells

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

Board #

34 7.3 Cardiac responses to chronic angiotensin II hypertension in sympathectomized dogs. H. McDonald, Jr. and L.N. Cothran. Howard Univ.
35 7.4 Mechanical strain stimulates extracellular matrix gene expression in adult rat cardiac fibroblasts. A.A. Lee, F.J. Villarreal, A.D. McCulloch, W.H. Dillmann, and J.W. Covell. UCSD.
36 7.5 Use of differential display technology in the identification of upregulated transcripts during acute right ventricular overload. G.T. Schleyer, A.S. Ridenour, D.R. Menick, J.D. Rozich, T.X.
O’Brien, and G. Cooper IV. Med. Univ. of South Carolina, Gazes Cardiac Res. Inst., and VA Med. Ctr., Charleston, SC.


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

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


Friday, October 7

Symposium
8.0 Cardiovascular Adaptations to Mechanical Stimuli I
8:30 AM — Hernando Ballroom
Chair: Peter Davies


9:00 Discussion.

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

9:35 Discussion.


10:10 Discussion.


10:45 Discussion.


11:20 Discussion.

Symposium
9.0 Cardiovascular Adaptations to Mechanical Stimuli II
7:00 PM — Hernando Ballroom
Chair: Howard E. Morgan

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

7:30 Discussion.

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

8:05 Discussion.

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

8:40 Discussion.

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

9:15 Discussion.


9:50 Discussion.

Poster
10.0 Mechanisms of Mechanochanical Signal Transduction
Posters are on display: 8:30 AM—10:30 PM.
Authors are in attendance: 3:30—5:00 PM.

Board #

2 10.2 Mechanical regulation of intracellular Ca²⁺ in endothelial cells. J.F. Minore and W.C. O'Neill. Emory Univ.
Vol. 37, No. 4, 1994

If mechanosensitive channels in osteoblast-like cells. J.D. Klein and W.C. O’Neill. Emory Univ.


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


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


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

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


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

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

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


23. 10.23 Mechanosensitive activation of Ca²⁺ transport pathways in HT29 human colon cancer cells. L.J. Hymel, Y. Qin, and T. Ertl. Tulane Univ.


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


Posters

11.0 Regulation of Cell Shape and Function by the Extracellular Matrix

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

Saturday, October 8

Symposium
12.0 Mechanisms of Mechanochemical Signal Transduction

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


9:00 Discussion.

9:05 Structural diversity and molecular requirements for mechanochemical transduction in the adenyl cyclase family. Peter A. Watson. Geisinger Clin., Danville, PA.

9:35 Discussion.


10:10 Discussion.

10:15 Biochemical properties of stretch-activated ion channels. Ching Kung. Univ. of Wisconsin.

10:45 Discussion.

10:50 Effects of stretch-activated ion channel activation on intracellular calcium. Owen Hamill. Univ. of Texas Med. Branch, Galveston.

11:20 Discussion.

Symposium
13.0 Regulation of Cell Shape and Function by the Extracellular Matrix

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

2:00 Control of cell growth and gene expression by cell shape. Donald Ingber. Children’s Hosp., Boston.

2:30 Discussion.


3:05 Discussion.


3:40 Discussion.

3:45 Regulation of Na+/H+ exchange and inositol lipid metabolism by integrins. Martin A. Schwartz. Scripps Res. Inst., La Jolla, CA.

4:15 Discussion.

4:20 Regulation of protease gene expression by cell shape, the cytoskeleton and integrins. Zena Werb. UCSF.

4:50 Discussion.

Banquet Lecture
14.0 Complex Mechanochemical Signal Transduction Involved in the Regulation of Development

8:30 PM — Hernando Ballroom
Speaker: David McClay, Duke Univ.
4.1 REDUCTION IN WALL TENSION IN ISOLATED PULMONARY VESSELS SIMULATES MATRIX METALLOPROTEASE ACTIVITY. C.A. Tozzi, S. Thakker-Varia, and D.J. Riley. UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903, Lyona VA Medical Center, Lyona, NJ 07039.

Reduction in wall tension in isolated segments of hypertensive PA stimulates expression of matrix metalloproteinases. We have demonstrated that mechanical tension in isolated PA segments stimulates proteolysis (Clin. Res. 1998;46:327A). Ex vivo, in situ, and in organ bath and subjected to a tangential load (blood pressure is reduced. We hypothesized that abrupt reduction in wall tension stimulates proteolytic activity because degradation of [125I]-transferrin and gelatinolysis are not released vs. 0.05±0.05 x10^5 dpm/protein in stimulated for [125I]-transferrin at 0.05, n=4) and gelatinolysis (2±0.3 in release vs. 1.9±0.2 x10^5 dpm/protein in maintained for [125I]-gelatin, p<0.05, n=4). Collagenolytic activity was not stimulated by release of tension. Western blot analysis demonstrated the presence of a doublet of 90-92kDa for stromelysin. We conclude that an abrupt reduction in wall tension stimulates the expression of stromelysin and gelatinase. Hemodynamic forces may modulate vascular remodeling by releasing factors that regulate proteolytic activity within the vessel wall and initiate breakdown of excess matrix proteins.

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

The flexibility of the rat trachea is well known. This study reveals another mechanical characteristic. It is tensed even when the neck is flexed. In eight female rats (body wt. 187±6 g) the cervical trachea was exposed and the thyroid removed. Beginning rostrally, four dots of dye were placed on alternate tracheal cartilages. With a divider and micrometer the three inter-dot lengths were measured. Width was similarly measured. Thickness was determined by a calipers. Tracheal tension of the isolated rat trachea was the total length interval of 1.76±0.7 mm to decrease by 155 (P<0.01) and the caudal one of 1.72±0.24 mm to decrease by 10% (P<0.05). In contrast, at the tracheal level between dots 2 and 3 the gape was 4.23±0.97 mm, a 24% increase over the baseline interval of 1.70±0.20 mm (P<0.001). Mural retraction alone would increase the distance between dots 2 and 3 by only 25% (155±10%). As the gape was 15% larger, the cartilages had also been acted upon by a more powerful force which in each rat visibly distracted the tracheal tracheal segment toward the chest - the negative intrathoracic pressure. As no change in its 3.0±0.11 mm width occurred, the trachea had to thicken while it shortened. Thickness was 7.7±0.06 mm rostrally and 7.0±0.08 mm caudally. It could only be measured following excision. It is concluded that both intrinsic contractile and extrinsic distractive forces tense the trachea in situ. If no tension existed the tracheal edges would have lain adjacent.

4.3 physical responses of the trachea to transection in the living rat. Martinec Peter. Georgetown University School of Medicine, Washington, DC 20007.

The flexibility of the rat trachea is well known. This study reveals another mechanical characteristic. It is tensed even when the neck is flexed. In eight female rats (body wt. 187±6 g) the cervical trachea was exposed and the thyroid removed. Beginning rostrally, four dots of dye were placed on alternate tracheal cartilages. With a divider and micrometer the three inter-dot lengths were measured. Width was similarly measured. Thickness was determined by a calipers. Tracheal tension of the isolated rat trachea was the total length interval of 1.76±0.7 mm to decrease by 155 (P<0.01) and the caudal one of 1.72±0.24 mm to decrease by 10% (P<0.05). In contrast, at the tracheal level between dots 2 and 3 the gape was 4.23±0.97 mm, a 24% increase over the baseline interval of 1.70±0.20 mm (P<0.001). Mural retraction alone would increase the distance between dots 2 and 3 by only 25% (155±10%). As the gape was 15% larger, the cartilages had also been acted upon by a more powerful force which in each rat visibly distracted the tracheal tracheal segment toward the chest - the negative intrathoracic pressure. As no change in its 3.0±0.11 mm width occurred, the trachea had to thicken while it shortened. Thickness was 7.7±0.06 mm rostrally and 7.0±0.08 mm caudally. It could only be measured following excision. It is concluded that both intrinsic contractile and extrinsic distractive forces tense the trachea in situ. If no tension existed the tracheal edges would have lain adjacent.


Force acting across the lung tissue of the developing fetus is considered to be the stimulus for lung growth. We have hypothesised that bronchomotor tone in the lower airways of the early foetus may serve this role. We sought, and then characterised, narrowing of the bronchial tree and the movement of the lung liquid in the lungs of first trimester foetal pigs. The entire bronchial tree of 3-5 piglets (30 days gestation) was freed of parenchyma and vasculature. Transmission light was used to sharply image the airway wall and lumen. Narrowing was evident in real time and quantitated using NIH Image 1.54 software. Airway narrowing was strongly developed throughout the bronchial tree to within 75µm of the epithelial buds indicating rapid onset of airway function after differentiation from the mesenchyme. Small airways from 25-30µm lumen diameter narrowed by 80% in response to a variety of stimuli. Spontaneous narrowing was seen throughout the bronchial tree in most lungs at 7µm. Constrictions varied from localised movements of the wall to strong peristaltic-like waves moving over 100µm of airway. Both spontaneous and activated narrowing produced a flow of the lung liquid along the lumen which carried with it particulate cell debris. We suggest that bronchomotor tone maintains the lung liquid as a positive pressure in the airways. Spontaneous narrowing is activated in response to small pressure fluctuations in localised regions of the airways and serves to move lung liquid along the airways preventing the accumulation of cellular debris in the lumen of the rapidly growing airways and maintaining an even positive pressure in the lumen of the bronchial tree. This may be a stimulus contributing to the growth of the lung tissue.
5.3 TRANSCRIPTIONAL REGULATION IN MECHANICALLY OVERLOADED SKELETAL MUSCLE OF TRANSGENIC MICE. Bai Riveria Rivera*, Jennifer L. Wiederman*, Lijing Guo*, S. Bharmu Vyas*, Leida T. Kwan* and Richard W. Tsika. Dept of Physiology, Univ. of Illinois, Urbana, IL

Mechanically overloaded adult skeletal muscle undergoes hypertrophic growth and demonstrative changes in the transcription (represen1ation) of both metabolic and contractile protein genes. To better understand transcriptional regulatory mechanisms which occur in response to work overload we have begun to delineate cis-acting elements in the promoters of the mouse muscle creatine kinase (MCK), and the mouse and human actin-myosin heavy chain genes in the mechanically overloaded plantaris muscle of transgenic mice. Northern blot analysis revealed a 3.5 fold repression of endogenous MCK specific transcripts after 2 days of overload whereas endogenous β-MHC specific transcripts where not significantly induced until 3 weeks post-overload. Analysis of MCK and β-MHC transgene expression levels were measured as chloromphenicol acetyltransferase activity (CAT assays). Our previous work has identified an upstream fragment (-3300 to -1256) which strongly represses (5.6 fold) MCK expression in response to 2 days of work overload. Preliminary analysis of new transcriptional changes in the promoters of the mouse actin-myosin heavy chain genes Multiple independent transgenic lines which harbor mouse M-CMH transgenes (-5600 to -600 bp) and human β-MHC transgenes (-1285 to -600) are induced (4.1 fold) after 6 to 8 weeks of work overload. These data provide the first evidence that both the MCK and β-MHC gene are transcriptionally regulated in response to a mechanical overload in adult skeletal muscle, and that the regulation of these two genes occurs over very different time courses.

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

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

CARRDOVASCULAR ADAPTATIONS TO MECHANICAL STIMULI: PERIPHERAL VASCULARITY

6.1 CALCIUM (Ca)-PERMEABLE MECHAN-TRI-actuated (MAC) ADAPTING CHANNEL IN RAT MUSCULAR CELLS (RMCs). Victor Chej, Helena Gaba, Clyde Shuman, Carlos E. Palad* (Nephrology and Endocrinology Sections, Brooklyn VA Hospital and SUNY Health Science Ctr. at. Brown, NY 12100)

Characterization of extracellular Ca+-permeable MAC prevents the increase in intracellular Ca++ that activates protein synthesis in RMCs stimulated by mechanical stretching. This has led authors to propose a Ca+-permeable MAC is RMCs. To explore this, RMCs in their 3rd to 20th culture passage were examined with patch-clamp techniques. In Ca++-stimulated patches with 100 mM NaCl, pipets, single channel inward currents with a unit conductance gCa = 21 pS (gCa = 18.9 nA; 16 cells) and a reversal potential Erev = -2.0 mV (with respect to Vmem) were elicited. Individual cell activity and membrane potential measurements were made at an estimated Erev = -2.3 mV (with respect to Vmem). In control patches, channels exhibited a cumulative open probability (N*p) of 0.03 ± 0.01 and a mean open time of approx. 100 ms. Channel kinetics were independent of holding potential and were not altered by intracellular Ca++ (500 M, n = 7 cells). In cells, a Ca++-free buffer occurred upon addition of CoCl (5 mM) to patch pipets, resulting in a reduction of gCa of 62%, with respect to control). Stopped suction (-5 to -25 mm Hg) applied to the recording pipets resulted in bursts of channel openings which showed quick adaptation. Hyperpolarizing holding voltages decreased the rate of adaptation to suction stimuli. An increase in N*p was also observed upon reduction of extracellular calcium with the response adapting in similar fashion. In conclusion, RMCs possess two types of MACs, a weakly adapting Ca++-impermeable, monovalent cation MAC (Kdatum = 33.5; 1933) and a strongly adapting Ca+-permeable MAC that responds physiologically. This pressure response is presumably necessary so as to not to override mechanisms that maintain a low cytosolic Ca++. The Ca+-permeable MAC may be involved in stretch-induced contraction of RMCs and in stretch-induced stimulation of growth promoting signals.

6.2 FEED ARTERY HEMODYNAMICS CHANGE WITH MUSCLE LENGTH IN HAMSTER RETRACTOR MUSCLE. Donald G. Welsh and Steven S. Segal. John A. Pierce laboratory, Yale University School of Medicine, New Haven CT 06510

The hypothesis that changing muscle length (LM) would alter hemodynamics and vasomotor activity of feed arteries (FAs) which supply the muscle microcirculation. Male hamsters (n=13, 100±4g; mean±SE) were anaesthetized (pentobarbital) and the retractor muscle prepared for intravital microscopy. FA diameter, red blood cell velocity and transmural pressure (servo-null) were monitored at 85% to 120% of LM in vivo. Increasing LM under control conditions reduced FA diameter (from 71±4 to 59±4; p<0.05) and blood flow (from 72±4 to 49±6 nls; p<0.05). Neither wall shear rate (WSR; 1700±180 s-1) nor pressure (60±5% of systemic arterial) changed with LM. Topical sodium nitroprusside (SNP; 10 µM) was used to eliminate active responses and reveal the direct effects of LM on FA perfusion. At LM = 85%, SNP dilated FAs (to 39±4; p<0.05), increased flow (to 209±24 nls; p<0.05) & WSR (to 2350±150 s-1; p<0.05), and reduced pressure (to 59±3%; p<0.05). Increasing LM during SNP reduced WSR (p<0.05) and increased pressure (p<0.05); diameter and flow were unchanged. These findings indicate that feed arteries actively mediate the muscle response to changes in LM, reducing blood flow while maintaining WSR and microvascular perfusion pressure.

Supported by the American Heart Association (GIA and EI).
6.3 MECHANISMS OF TRANSDUCTION OF ELEVATED VASCULAR WALL STRESS INTO INCREASED PRODUCTION AND ACCUMULATION OF ARTERIAL ELASTIN. Fred W. Keeley*, L. Bartoszewicz, and P. Robson*. Div. of Cardiovasc. Research, Hospital for Sick Children, Toronto, Canada M5G 1X8

Elevated vascular wall stress appears to be a driving force for the accumulation of arterial connective tissue proteins in both normal development and hypertension. Increased elastin production in response to elevated wall stress in in vitro aortic organ culture models is a rapid and graded response which does not require an intact vascular endothelium.

Depletion of extracellular matrix, or blocking of extracellular matrix channels with verapamil inhibits secretion and assembly of elastin but not synthesis of the protein. Gado lithium-sensitive, stretch-activated calcium channels are not involved in the stress-induced response. Stress-induced elastin production is also dependent on release of calcium from sarcoplasmic reticulum stores, although flooding of the cytoplasm with calcium through the action of ryanodine, thapsigargin or caffeine selectively blocks the stress-induced response. Inhibition of phosphodiester C and protein kinase C also selectively inhibits the response to increased wall stress. Stress-induced elastin production is correlated with a rapid increase in tyrosine phosphorylation of several cellular proteins, and both increased phosphorylation and increased elastin production are inhibited by tyrophostin-25 but not by genistein. Stress-induced increases in aortic elastin production are not correlated with increased steady state levels of mRNA for elastin, suggesting a mechanism involving increased translational efficiency.

6.4 A Model For Subjecting Vascular Wall Cells To Simultaneous Pulmonary Fluid and Mechanical Anatomic Stress. GJ L'Italien, A Benbruhim, S Dhara, CJ Kwolek, RR Milliner, TP Warmack, JP Certleri, JW Oren, WM Abbott. Vascular Surgery Department, Massachusetts General Hospital, Boston, MA 02114

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

Fourteen silicon rubber tubes 6.5 x 1 cm in diameter and 38 cm in length were transected and Anastomosed with polypropylene suture using either the interrupted (iv) or continuous (Iv) technique. Tissue was subjected to arterial levels of pressure and flow and sheared with a laminar-flow laser micrometer in the peri-anastomotic region. Measurements of diameters, wall stress and thickness were used to compute the anastomatic mechanical shear (i.e. "bending") stress.

There was no significant difference in the stress pattern for either clotting technique (Figure). The peak bending stress which occurred 1.8 mm proximal to the anastomosis (42 dyne/cm2, dyne/cm2) was significantly greater than at the distal site (19 dyne/cm2, P<.001), which was seen 3.0 mm distally from the anastomosis. The observed levels of mechanical shear stress were similar to values reported by others for host artery-graft anastomosis (2. Biondi 2.794-605, 1997). In addition, wall stress at the anastomosis was reduced by 46-65%, as we have seen in vivo.

VSD-NAHST reproduces the mechanical conditions found at the artery-graft anastomotic junction. Furthermore, we have demonstrated that tubes cell cultures subjected to these conditions can be used to examine cell proliferation and differentiation and to study the effect of these environmental variables on the anastomosis. These studies will be described in detail at the conference.

6.5 INTEGRIN EXPRESSION BY PORCINE CORONARY VASCULAR SMOOTH MUSCLE CELLS. Jon Morgan*, George E. Davies* and Gerald A. Meiningar. Department of Medical Physiology, Texas AM University Health Science Center, College Station, TX 77843

The purpose of this study is to characterize the integrin expression of porcine coronary vascular smooth muscle cells (VSMC). Cultured cells from the left anterior descending coronary artery and control samples were grown in culture and labeled with biotin. Detergent extracts from these cells were passed over five sepharose coupling columns. The results indicate that phosphorylation and increased elastin production are inhibited by tyrophostin-25 but not by genistein. Stress-induced increases in aortic elastin production are not correlated with increased steady state levels of mRNA for elastin, suggesting a mechanism involving increased translational efficiency.

The peak bending stress which occurred 1.8 mm proximal to the anastomosis (42 dyne/cm2, dyne/cm2) was significantly greater than at the distal site (19 dyne/cm2, P<.001), which was seen 3.0 mm distally from the anastomosis. The observed levels of mechanical shear stress were similar to values reported by others for host artery-graft anastomosis (2. Biondi 2.794-605, 1997). In addition, wall stress at the anastomosis was reduced by 46-65%, as we have seen in vivo.

VSD-NAHST reproduces the mechanical conditions found at the artery-graft anastomotic junction. Furthermore, we have demonstrated that tubes cell cultures subjected to these conditions can be used to examine cell proliferation and differentiation and to study the effect of these environmental variables on the anastomosis. These studies will be described in detail at the conference.


A parallel-plate, channel flow chamber and image processing system were developed to investigate the three dimensional changes of cultured human umbilical vein endothelial cell (HUCVEC) monolayers under fluid shear flow. The endothelial cell morphology was characterized by fluorescent microscopy and digital image analysis.

Three-dimensional morphologic changes were similar to values reported by others for host artery-graft anastomosis. The observed levels of mechanical shear stress were similar to values reported by others for host artery-graft anastomosis. The observed levels of mechanical shear stress were similar to values reported by others for host artery-graft anastomosis.

6.7 MECHANICAL STRAIN ENHANCES EXPRESSION OF THE PDGF-A AND EGR-1 GENES IN VSM CELLS. Glenn J. Vives*, Emily Wilson*, Tucker Collins*, Vikas Sulhiotis*, and Helen E. Ivey*. Div. of Nephrology, University of California, San Francisco and Dept. of Pathology, and Beth Israel Hospital, Boston, MA 02114

We have previously shown that calsequestrin, a calcium binding protein, is upregulated in vascular smooth muscle cells (SMCs) following mechanical strain. We have also shown that the effect of cyclic mechanical strain on the expression of the early growth response gene, egr-1, and the late growth response gene, c-myc, is mediated by the promoter-CAT constructs and transient transfection, we found a 92 base pair (bp) region proximal to the transcription start site that confers responsiveness to mechanical strain. This 92 bp minimal promoter contains consensus sequences for 3 SP1 binding sites and 2 egr-1-Wilms tumor suppressor sites. We have therefore analyzed the effect of cyclic mechanical strain on the expression of the early growth response gene, egr-1. Strain state levels of egr-1 mRNA were determined using Northern blot analysis. The mRNA levels increased 4.2 fold within 15 minutes of exposure to mechanical strain; peak expression was 5.7 fold by 30 min. Expression was reduced to baseline levels by 1 hour. These studies suggest that the strain-induced expression of PDGF-A chain gene may be mediated by enhanced expression of egr-1.

The distribution of myosin subtypes was examined in neonatal rat vascular smooth muscle cells (VSMC) in response to cyclic mechanical strain. Cells were grown on silicone elastomer plates which were subjected to strain by cyclic (1 Hz) oscillation of a vacuum. Myosin subtypes were identified by Western blots. In response to strain, smooth muscle myosin, using semiquantitative cDNA hybridization, showed a significant increase in expression, increasing over time, achieving a maximum of 3-fold after 36h. Over the same time period, non-muscle myosin decreased by 50%. We have previously shown that VSMC exposed to strain secrete both A- and B-chain of PDGF. Using the role of actin cytoskeleton and exogenous addition of PDGF or neutralizing PDGF-Ab (3ug/ml), smooth muscle myosin was decreased by approx. 50% and non-muscle myosin increased by approx. 60% in response to PDGF, opposite to the effect of strain. When cells exposed to strain were incubated with neutralizing Ab to PDGF-AB, the strain-induced increase in smooth muscle myosin was further enhanced to 4-fold and non-muscle myosin was reduced to 0.5-fold compared to cells without antibodies. Thus, unlike PDGF which causes desmoproliferation of VSMC cells, mechanical strain alters myosin subtype distribution towards that seen in differentiated VSM cells.

MECHANICAL STRAIN INDUCES MONOCYTE CHEMOTACTIC PROTEIN-1 GENE EXPRESSION IN HUMAN ENDOTHELIAL CELLS.

Chang W, B. W. Wang, A. Umemiya and V. Boyt. Cardiovascular Div., State University of Iowa, Iowa City, IA, ROC.

Monocyte chemotactic protein-1 (MCP-1), a potent monocyte chemotactic agent secreted by endothelial cells (EC) at the early stages of atherogenesis. Since VSMC are constantly subjected to mechanical stresses, the effect of cyclic strain on expression of the MCP-1 gene in human umbilical vein ECs was examined. ECs grown on a flexible membrane base were deformed by cyclic strain (16% Amplitude at 5 Hz) for 24h. ECs subjected to strains for 12 hours showed increased monocyte adhesion by about 1.8 fold as compared to unstrained control cells. By the Boyden chamber technique, cultured monocytes showed more than twice the monocyte chemotactic activity of medium from control cells. Pretreatment of the cultured medium with polyclonal anti-MCP-1 antibody suppressed this MCP-1 activity. Northern blot analysis demonstrated that MCP-1 mRNA levels in cells subjected to strain for 1 or 24 hours were double those in control cells. The induced MCP-1 level returned to its control basal level 3 days after strain had been released. Pretreatment of ECs with a protein kinase C (PKC) inhibitor, calphostin C, abolished the strain-induced MCP-1 gene expression. Pretreatment of ECs with Camp or cGMP, dependent protein kinase inhibitors (KT5720 or KT8321) only partially inhibited the strain-induced MCP-1 gene expression. The intracellular calcium chelator BAPTA/AM significantly reduced the monocyte chemotactic activity of medium from strain-treated ECs.

FLOW-MEDIATED NO RELEASE IN ENDOTHELIAL CELLS. ROLE OF CALCIUM AND K+ CHANNELS ON A7r5 VASCULAR SMOOTH MUSCLE CELLS.


Mechanical strain led to increased expression of smooth muscle myosin in ECs. Mechanical strain increases calcium and ATP, bradykinin (Bk), and acetylcholine (Ach) lead to vasodilation by the release of nitric oxide which is mediated by increases in endothelial intracellular calcium (Ca++) levels. To determine the role of receptor-mediated increases in Ca++ in the response to agonists, we examined the effects of vasoactive agents on Pura-2 AM labeled bovine aortic ECs in order to distinguish the effects of receptor-mediated increases in EC Ca++ from propagation of a Ca++ response from adjacent cells, we examined EC upstream to the point of delivery of agonist under flow conditions. Contraction induced by 0.44 and 0.54 nmoles Ca++/106 cells/min for Ach (n=2) and 10 nM Bk (n=5) or 10 uM ATP (n=9) led to the expected receptor-mediated increases in EC Ca++. Flows over these surface geometries were simulated using computational fluid dynamics. From the simulated velocity fields, shear stresses acting on endothelial surface in flow. This analysis provides the exact stress loading mechnotransduction.
6.15 ELEVATED STRETCH AND SHEAR STRESS PRODUCE DISTINCTLY DIFFERENT CHANGES IN CAPILLARY HYDRAULIC CONDUCTIVITY (Lp). Dong A Williams and Virginia H. Hudxley. Dept. of Physiol., U. Missouri, Columbia, MO 65212

In vivo, capillaries are exposed continuously to physical forces including shear stress and transmural pressure, which produces stretch or strain. We have demonstrated previously that in situ shear stress and Lp correlate positively. In this study we distinguish between responses of capillary Lp to stretch relative to shear stress here, hypothesizing that capillaries would increase with elevated shear stress. Mesonotes of pithed frogs (Rana pipiens; n=21) were exteriorized and superfused with frog finger's solution (14-16°C). Pipettes filled with 10 mg/ml bovine serum albumin in Hinkers were used to cannulate single capillaries. Volume flux (Jv) was estimated using the modified Landis technique at pressures ranging from 20 to 40 cm H2O. In 2 sets of vessels, baseline Lp (L0) was assessed. Next, pressure was raised (10 min) to 40 cm H2O plus partial block (L1.35) to elevate stretch or no block (L2.25) to increase shear. With elevated stretch, Lp strain Lp increased 4-fold (n=11; P=0.007) and remained elevated for 5% time. Altered shear stress produced modest changes (L0.25/L0 = 1 3:30.3, n=7) with an initial spike (2 ± 0.8) and decline indicating that Lp responses to stretch relative to shear stress differ. These data imply mechanistic specificity for altering filtration in capillary vessels. Supported by HL64782. DAW is an AHA-MO Affiliate Postdoctoral Fellow.


Hypertrophy of the vascular wall occurs in hypertension. To determine the capacity of pressure alone to contribute to this response, two small mesenteric arteries (250 μm) were dissected from male Wistar rats and mounted on pipettes in a dual vessel chamber. Each artery was then subjected to 1 mmHg with Dulbecco’s modified Eagles medium (MEM) nutrient mixture F12, supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). The pressure (no flow) in each artery was increased gradually to biologically values (90 mmHg) and the ability to constrict was tested with 60 mm KCl in the presence of 1 μM phenolamine. After a washout period, the pressure in one artery was increased to 140 mmHg. At set times each pair of vessels was fixed overnight in 10% formalin and embedded in paraffin for sectioning. Sense and antisense 32P-labeled riboprobes for c-fos and c-myc mRNA and 18S RNA were transcribed from the respective cDNAs and used for in situ hybridization and quantification by PhosphorImager. The hypertensive vessels showed an induction of c-fos at 30 minutes, an almost 3-fold increase in c-myc at 3 hours and a doubling of 18S RNA at 6 hours, compared with controls. These results therefore show that an increase in arterial pressure alone is sufficient to induce the early response genes, and also increase 18S RNA, and is thus likely to result in structural changes in these vessels. (This work was supported by a Grant-in-Aid from the American Heart Association.)


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


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

6.20 ELEVATED PRESSURE ALTERS MORPHOLOGY AND EXTRACELLULAR MATRIX DEPOSITION OF CULTURED ENDOTHELIAL CELLS DERIVED FROM HUMAN UTERINE ARTERIES. Qinfeng Sun, Jie Li, Giorgi Manoloulous, and Peter I. Lelkes, Univ. Wisc. Med. School, Lab of Cell Biology, Milwaukee, WI 53201.

Elevated blood pressure as can cause profound changes in morphology, proliferation and extracellular matrix production of endothelial cells. We hypothesized that we can mimic in vitro the effects of blood pressure on the endothelium by culturing endothelial cells under their normal or pathophysiological pressure regimens. Using a novel, pressurized cell culture system, we exposed for 60 hours confluent monolayers of pig pulmonary artery endothelial cells to a static transmural pressure of 165 mm Hg. (Lp=60 mm Hg). Under these pressures, the endothelial cell monolayer maintained a growth-arrested, cobblestone-like appearance. By contrast, elevated, static pressure increased cell density by 30%, reduced cell surface area by 25% and induced cell sprouting. The sprouting cells were of endothelial origin as assessed by their continued staining for specific EC markers. Elevated pressure also affected extracellular matrix production. By comparison to non-pressurized controls, we observed, by indirect immunofluorescence, a substantial down-regulation in laminin and fibronectin deposition into the matrix of the cells. By contrast, collagen IV expression was apparently not affected. We propose that these changes manifest endothelial cell participation in the remodeling of blood vessels during their exposure to pathophysiological pressures.
A-6

CARDIOVASCULAR ADAPTATIONS TO MECHANICAL STIMULI: PERIPHERAL VASCULATURE

7.1
EXTRACELLULAR ATP MODULATES STRETCH-ACTIVATED CHANNELS IN CHICK HEART CELLS. 
Abdul M. Mubarak, Hai Hu, and Fred Sachs. Dept. of Biophysical Sciences. SUNY. Buffalo, NY 14214

Adenosine 5'-triphosphate (ATP) is released during hypoxia and ischemia in heart. We tested the effects of extracellular ATP on stretch-activated channels (SACs) of chick cardiomyocytes. Using cell-attached patches, the sensitivity of the SACs to stretch was measured by using NP0 in a range of suction applied by hydrostatic pressure. We found that extracellular ATP increased sensitivity of the SACs to stretch in these cells. The pressure sensitivity curves shifted towards left showing higher sensitivity of the SACs to 500 pM ATP compared to the extracellular side of the cell. Using pipette diffusion technique, we measured the NPU in the same patch before and during exposure of SACs to 100 nM ATP. The higher pressure was 500 pM ATP was action consistent with a dose dependent mechanism. The activation of SACs was observed for a non-selective cation (50 pS) channel and for K-selective (100 pS) channel. The concentrations of ATP tested were between 1 and 100 uM and higher the concentration, the quicker was the activation. Cells treated with 4.4'-dicyanostilbene-2,2'-disulfonic acid (DIDS) or co-exposure of DIDS and ATP did not seem to affect the effects of ATP on SACs. Extracellular Ca2+ was not required for the ATP effect on SACs. Both AUP and AMP at 100 uM had similar effects on SACs but with reduced potency. ATP at 1000 uM was able to enhance the activity of SACs but required prolonged exposure. Inducing photobleaching of the SACs may be responsible for increase in sensitivity to stretch and for activation of SACs by these adenosine phosphate compounds.

Supported by American Heart Association-New York Affiliate grant #02-330.

7.2
CARDIOVASCULAR ADAPTATIONS TO MECHANICAL STIMULI: MYOCARDIAL CELLS

7.3
CARDIAC RESPONSES TO CHRONIC ANEBOGENESIS II HYPERTENSION IN SYMPATHETICIZED DOGS. HARTMANN, MEHLICH, FEI, AND L. LOWEN. Howard University College of Medicine, Washington, D.C.

Prednisolone, a corticosteroid conventionally used as a model for chronic hypertension, causes the following: a) an increase in blood pressure; and b) a decrease in plasma renin activity (PRA). ATP (20 µM) was added to the media of isolated cardiac fibroblasts from adult rat hearts at the time of isolation. The arterial systolic pressure (AP) was measured in the awake rat on the day of isolation and every other day for 14 days. The first week of treatment was characterized by a significant decrease in AP (p<0.01) and a decrease in heart weight (p<0.001) with no change in heart rate (H). The first week of treatment was followed by a significant decrease in AP (p<0.01) and a decrease in heart weight (p<0.001) with no change in heart rate (H). The first week of treatment was followed by a significant decrease in AP (p<0.01) and a decrease in heart weight (p<0.001) with no change in heart rate (H).

7.4
MECHANICAL STRAIN STIMULATES EXTRACELLULAR MATRIX GENE EXPRESSION IN ADULT RAT CARDIAC FIBROBLASTS. AA, LEF, E. J. VILLARREAL, D. A. MCCULLOCH, AND W. A. SCHNERR. Depts. of AMES-Biosciences and Medicine. UC San Diego. La Jolla CA 92039

The cardiac extracellular matrix (ECM), predominantly composed of fibrous collagen, may undergo significant remodeling during cardiac hypertrophy. We have previously shown that pressure-overload in the adult rat heart increases ECM levels for fibronectin and collagen types I and III, and that these changes are mediated by extracellular calcium (Ca2+). In this study, we examined the effects of mechanical strain on cultured adult rat cardiac fibroblasts. Cells were plated on a collagen-coated silicon membrane which was stretched 10% along the long axis of a novel culture chamber. Cell deformation was quantified by two-dimensional (2D) homogenous finite strain analysis. The 2D mechanical strain components, , , and were determined by measurements of the displacements of latex microspheres attached to the cell surface (axis in stretch direction; axis 2, perpendicular). Strain components were uniform most of the culture system except for cells located near the insertion of the chamber. In the center of the culture chamber, , = 0.11 and = 0.05, with negligible shear ( ). Near a clamped edge, , = 0.01. A polyclonal RNA was extracted from adult rat cardiac fibroblasts which were stretched or not stretched at 10% for 24 h. Levels of mRNA for collagen I and III increased 5-fold under stretch. Conclusions: (1) In a uniaxial stretch apparatus, mechanical strains are , mostly uniform, and include both tensile and compressive components. (2) The application of these strains increased ECM mRNA levels in adult rat cardiac fibroblasts. These findings suggest a role for mechanical strain in the process of ECM remodeling in the heart. Supported by NIH.
Use of Differential Display Technology in the Identification of Upregulated Transcripts During Acute Right Ventricular Overload.
Gregg T. Schuyler, Allen S. Ridenour, Donald R. Manick, John D. Rozich, Terrence X. O'Brien, George Cooper IV, Medical University of South Carolina, Gaze Cardiac Research Institute, and VA Medical Center, Charleston, SC.

Acute pressure overload has been shown to induce transient changes in gene expression of several protein-encogenes. With this, this increase in load result in cardiac hypertrophy. Acute right ventricular pressure overload (RVPO) was induced in a feline model by inflating a balloon in the pulmonary outflow tract. The balloon-induced RVPO was optimized for the detection of rare messages in cardiac tissue in response to acute RVPO. Total RNA extracted from the RV and LV free wall of a 1 hr. RVPO cat was reverse transcribed, amplified by PCR utilizing random 5' primers and 3'-oligo dT primers with cap-dependent bases; sequences were separated on PAGE, and analyzed for differences in RV vs. LV and cdNA fragments which were increased after RVPO were extracted from the gel and cloned into a suitable vector. Sequence analysis of these partial transcripts identified phosphofructokinase, transcripts with sequence identity to mitochondrial subcomplexes, and some unknown sequences. These clones are actively being characterized to determine their role in the adaptive response of the heart to load.

7.7 THE RAPID UPREGULATION OF NUCLEAR AND MITOCHONDRIAL ENCODED SUBUNITS OF THE F1-ATPase AND CYTOCHROME OXIDASE COMPLEXES IN RESPONSE TO CARDIAC LOAD. Donald R. Menick, Terrence X. O'Brien, Diane E. McDermott, John D. Rozich, George T. Schuyler, Paul J. McDermott, George Cooper IV, Medical University of SC, Gaze Cardiac Research Institute, and VAMC, Charleston, SC 29425.

Differentiation hybridization was utilized to identify transcripts upregulated during the early induction of cardiac hypertrophic growth. One clone which was greatly upregulated corresponded to the mitochondrial encoded F1-ATPase subunit Fo. Mitochondrial encoded cytochrome b and cytochrome oxidase subunit II were also upregulated at one and four hours of pressure overload as would be expected from the polyclinotic nature of mitochondrial transcription. Transcripts for nuclear encoded subunits of the F1-ATPase and cytochrome oxidase probes were observed to assess if the acute responses observed for the mitochondrial encoded subunits were also true for the nuclear encoded subunits. The nuclear encoded and subunit II of the F1-ATPase probe were both upregulated at one and four hours of pressure overload. In addition, the upregulation of the Fo transcript was examined in isolated neonatal rat ventricular cardiocytes stimulated with either phenylephrine (PE) or electrical pacing (PEf). Fo was upregulated in 1.6 fold within 15 minutes of PE stimulation and 4 hours of electrical stimulation. This upregulation persists for at least 18 hours with electrical stimulation. This is the first report in a higher eukaryote of the rapid coordinat dissolution of nuclear and mitochondrial transcripts for genes encoding oxidative phosphorylation complexes by a physiologic stimulus.

7.8 EFFECTS OF PRESSURE OVERLOAD HYPERTROPHY ON PASSIVE STIFFNESS AND VISCOUS DAMPING IN THE ISOATROPHIC CARDIAC MYOCYTE. Michael R. Zile, John M. Buckney, Ron E. Richardson, George Cooper IV, Medical University of SC, Veterans Administration, Charleston Medical Center, Gaze Cardiac Research Institute, Charleston, SC 29425.

One potential mechanism causing diastolic dysfunction during myocardial hypertrophy is an increase in the stiffness of the cardiocyte itself. We overloaded 2 components of the cardiocyte stiffness: passive spring (Kp) and viscous damping (Cvd) constant. Cardiocytes from normal cats (control) and cats with right ventricular hypertrophy (RVH), induced by pulmonary artery banding, were embedded in a 2% agarose gel. Cardiocytes were then subjected to a stepwise change in force (stress, o) and the resultant changes in cell length (strain, e) were measured. Changes in Kp were determined by examining the slope of the force strain relation during an incremental increase in o applied at a constant rate. Changes in Cvd were determined by measuring the area between the strain-velocity relation obtained during increase and decrease in force. This loop area reflects the change in mechanical energy converted to heat energy by damping. The slope and intercept of the strain-velocity relation obtained during an increase in o were similar in hypertrophied and control cardiocytes (Kp = 441 ± 1.3 ± 0.5 ± 0.99 for control and y = 1.07 ± 2.6 ± 0.09 for hypertrophied cardiocytes). However, the loop area between the strain-velocity relation obtained during an increase and decrease in force was significantly greater in hypertrophied cardiocytes (35.5 ± 5.1) compared to control cardiocytes (3.2 ± 0.4). Thus, hypertrophy did not alter the spring constant but did increase the damping constant. We hypothesized that this increased Cvd caused by an increase in microtubule density. When microtubule density was decreased by treating hypertrophied cardiocytes with colchicine, the loop area (Cvd) decreased to normal. Thus, the increased damping in hypertrophied cardiocytes was caused, at least in part, by an increase in cardiocyte microtubules.

7.9 GROWTH EFFECTS OF ELECTRICALLY STIMULATED CONTRACTION ON ADULT FELINE CARDIOTYTES IN PRIMARY CULTURE. Paul J. McDermott, Satoshi Kato, Charles T. Beyer, George Cooper IV, and Michael R. Zile, Medical University of SC & VA Med Center, Gaze Institute, Charleston, SC 29401.

Electrically stimulated cardiocytes in primary culture acutely accelerates protein synthesis rates. The purpose of the present study was to determine the effects of long term electrical stimulation of cardiocyte contraction on protein synthesis. Protein content and protein synthesis rates of adult feline cardiocytes were plated on laminin coated culture dishes and maintained in a serum-free medium consisting of M199 supplemented with ascobic, bovine serum albumin, creatine, creatinine, thymine, and 10% recombinant insulin. Cardiocytes were electronically stimulated to contract using continuous electrical pulses of alternating current at a frequency of 1 Hz and pulse duration of 5 msec. Non-stimulated cardiocytes are normally quiescent and were used as the control group. Protein synthesis rates were measured at the rate of incorporation of [3H]phenylalanine into total cell protein (nmol PHEN g protein/g). Protein synthesis rate decreased by 14% in the controls between days 1 and 4 in culture and then remained stable up to day 7. In electrically stimulated cardiocytes, protein synthesis rates were significantly increased relative to same day controls by 18% and 43% on days 1 and 7, respectively (p<0.05). Protein content/cell was determined by measuring total fluoresceinlabeled cardiocytes with calcein; results were expressed as a percentage of control. This increased rate of protein synthesis was due, at least in part, to an acceleration of steady state protein synthesis rates.

7.10 AUTOREGULATION OF TUBULIN mRNA EXPRESSION AND ORGANIZATION OF THE MICROTUBULE CYTOSKELETON IN ISCHEMIC AND DILATED CARDIOMYOPATHY. Roger D. Bies, Cardiology Division, Temple Hoyne Buell Laboratories, University of Colorado Health Science Center, Denver, CO 80262.

Monomeric tubulin is expressed in human cardiocytes and polymerizes to form microtubules. The microtubule cytoskeleton is known to play a role in cardiocyte function in heart failure. We have therefore analyzed the expression of tubulin mRNA in human cardiomyocytes by reverse transcription-polymerase chain reaction and determined that expression of tubulin is downregulated in human ischemic cardiomyopathy (ICM) and idiopathic dilated cardiomyopathy (DCM), idiopathic dilated cardiomyopathy (ICD), the cardiomyopathic hamster (CMH), and controls. Tubulin protein was separated into M1 and M2 fractions by centrifugation. Western blot analysis showed tubulin exists almost exclusively in the polymerized form in human ischemic LV, and in the LV and RV from the cardiomyopathic hamster. Controls showed an even distribution of M1 and M2 forms, and this distribution is described in normal cardiocytes. Northern blot analysis of tubulin mRNA showed markedly diminished levels of tubulin transcripts in human ischemic LV compared to RV. This decrease in tubulin expression was accompanied by a reduced cellular content of its own mRNA. The effect of microtubular inactivation on contractile function was analyzed by measuring tension after exposure of LV and RV trabeculae to ischemic (ISO) and colchicine (10 µM) which increased the tension response to ISO only when M1 was intact. These studies demonstrate that loss of MT organization and decreased tubulin mRNA stability may contribute to cardiac dysfunction in heart failure.
this study we tested the hypothesis that NO released from sensory nerves was measured from the vasculo-lysed CD in anesthetized rabbits. Capsaicin (1 mg/ml) was injected into the CS to selectively activate C-fiber NO. Efficiency of BR activation was measured by the delayed rectifier potassium current in guinea pig ventricular myocytes. Groups. James G. Maylie* and William J. Grant Oregon Health Sciences University, Portland, OR 97201.

The effect of hypertonic stretch on the slow component (Ik1) of the delayed rectifier potassium current (Ik) was studied in guinea pig ventricular myocytes with a whole cell patch clamp technique. Cells were superfused with normal Tyrode solution (296 mMNaCl) at 37 °C and dialyzed with a patch pipette solution (mM): K-AOSP, 90; KCI 40; CaCl2 10; K2HPO4 10; MgCl2 1. MgATP 5; EGTA 10; CaCl2 1; HEPES 10; NaHCO3 10; pH 7.4.

The whole cell configuration to hypotonic solution (226 mOsmol/l; Tyrode less 35 mM NaCl) induced measurable swelling within 5 min; cell length and width increased 92% and 37%, respectively (meanSEM, n=3). Within minutes after hypertonic stretch measurement was decreased by 30% and the tail current (Ik) on repolarization to -40 mV increased by 52%, p<0.05.

The Ik1 modulation of Ik1 was not affected by pretreatment of myocytes for 2 h with 10 mM cycloheximide but was reduced by pretreatment with 10 μM phallolidin. We conclude that hypertonic stretch increases Ik1 and that modulation is reduced by actin stabilization with phallolidin. Stretch modulation of Ik1 would affect the cardiac refractory period and influence the vulnerability to reentrant ventricular tachyarrhythmias.

10.1 MECHANOTRANSDUCTION ACROSS THE URUKINASE RECEPTOR.

Ning Wang, Emmanueline Pianu, Jeffrey J. Fredberg, Georgia Barlowez-Meimon Physiology Program, Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115; Laboratoire de Biologie du Microenvironnement, Université de Laval, Québec, Canada.

Past studies have focused on the enzymatic role of urukinase receptor in cell invasion. We hypothesized that the urukinase receptor may also play a mechanical role in cell migration and basal cell invasion by modulating the cytoskeleton. Using magnetic twisting cytometry with urukinase-coated ferromagnetic beads, we applied mechanical stresses directly to the urukinase receptor on cultured human myogenic cells in culture. Applied stresses of 40 dynes/cm2, the stiffness measured through the urukinase receptor was 27±3 dynes/cm2, whereas the stiffness measured through other glyceral phosphatidylcholine (IPI) linked proteins such as Thy-1 or alkaline phosphatase was only 5±1 dynes/cm2. The stiffness measured through the integrin was 5±1 dynes/cm2. In addition, the stiffening response (increase in stiffness with stress) remained unchanged in the presence of inhibitors, which are linked mechanically to the cytoskeleton. Furthermore, stiffness decreased by 70% with disruption of actin microfilaments. These results demonstrate that the urukinase receptor is coupled mechanically to the cytoskeleton. Activation of the tripartite complex formation (integrin receptor-actin microfilaments) by urukinase, antibodies, in combination with antibodies against type-1 plasminogen inhibitor (PAl), led to a 2-fold increase in cytoskeletal stiffness and a dramatic decrease in cell motility. This result cannot be explained by the conventional enzymatic function of the urukinase system; rather, it is consistent with the notion that formation of the complex facilitates cell movement independent of plasminogen activation.

10.2 MECHANICAL REGULATION OF INTRACELLULAR Ca2+ IN ENDOTHELIAL CELLS

Joseph F. Minore* and W. Charles O'Neill, Emory University, Atlanta, Ga. 30322

Mechanical stress, specifically shear stress and direct membrane deformation, raise intracellular Ca2+ concentration and may trigger intracellular Ca2+ release, which can alter intracellular signal transduction and gene transcription. By employing a variety of techniques we have examined the effect of mechanical stress on Ca2+ release in endothelial cells (EC). The underlying mechanism is unknown, due in part to the difficulty in studying cells under these conditions and in quantitating and reproducing the stresses. We have found that hypotonic cell swelling is an additional stress that increases [Ca2+] in EC and may serve as a useful model of mechanical regulation of [Ca2+] in EC. To determine the mechanism of this rise in [Ca2+] in bovine sotolic EC were grown to confluence, loaded with fura-2, trypsinized into suspension, and exposed to isotonic (osm = 290) and hypotonic (osm < 290) solutions. [Ca2+] was increased 49 ± 3 mM (mean ± SEM; n = 21) in swollen cells, and a similar rise was seen in monolayers. Increased [Ca2+] was apparent at 5% swelling, with progressively greater increases in further swelling. The changes were fully reversible. The swelling-induced [Ca2+] increase was abolished when external Ca2+ ([Ca2+]o) was removed, but was not inhibited by 10 μM La3+, 20 μM Gd3+, or by blocking the cell membrane by incubation for 1 h in 122 mM. The agonist ATP produced a typical biphasic rise in [Ca2+]i, consisting of an initial peak due to internal Ca2+ release and a prolonged phase due to influx. The latter phase, but not the initial peak, was reduced by La3+, cell depolarization, or removal of Ca2+. We conclude that the swelling-induced rise in [Ca2+]i in sotolic EC is not mediated by typical Ca2+ channels or stretch-activated channels. Swelling may increase Ca2+ influx by inducing a change in intracellular Ca2+ release from an internal store that is in rapid equilibrium with Ca2+0.
10.3 VOLUME-DEPENDENT PHOSPHORYLATION OF MYOSIN LIGHT CHAIN IN AORTIC ENDOTHELIAL CELLS Janet D. Klein* and W. Charles O'Neill, Emory University, Atlanta, GA 30322

The mechanism by which cells sense their volume and activate volume-regulatory transporters is unknown. Our previous studies suggest that volume-sensitive channels (MSC) are not expressed in smooth muscle or in other endothelial cells. We examined the effect of cell volume changes on incorporation of [32P]orthophosphate into cellular proteins and have found that cell shrinkage-induced reversible phosphorylation of a 19 kDa protein identified as myosin light chain (MLC) by immunoprecipitation with anti-myosin heavy chain antibodies. In normal saline, MLC showed one phosphopeptide, consistent with phosphorylation by MLC kinase (MLCK). Shrinkage also increased MLC phosphorylation in astrocytes and vascular smooth muscle cells. The time course of endothelial MLC phosphorylation closely matched that of Na-K-Cl cotransport activation.

10.4 CYCLIN D1 EXPRESSION IN SMC IS STIMULATED BY CYCLIC MECHANICAL LOAD IN A DOSE-DEPENDENT MANNER. E. Chikamatsu*, Y. Nimura*, J. Yamamoto*, P. Ha*, T. Fischer*, T. Lauret*, M. Tsariska*, T. Brown and A.I. Gudis. The Pennsylvania State University, University Park, PA 16802

The regulation of growth control of smooth muscle cells (SMC) is critical in the pathogenesis of vascular disease or loss of vascular patency after angioplasty. Growth factors are known to stimulate DNA synthesis in quiescent SMC. However, mechanisms by which mechanical load may regulate growth are not known. We have examined the effect of cyclic mechanical load on growth of human SMC. Cells were isolated by collagenase digestion after continuous balloon catheter injury. Cells were grown to confluence and subcultured. Cyclic mechanical load was applied by a chemiluminescence technique. RESULTS: The cloned cDNA human sequence showed a high degree of homology for the rat type 2 IP3 receptor. The regulation of cell volume and actin dynamics, message stability or protein stability. Increased CXN phosphorylation may be related to SMC hypertrophy in response to load. Upregulation of receptor expression may regulate and amplify the cell's ability to detect and respond to load. American Heart Association.

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

Connectin (CXN) is a 44kDa protein monomer that is assembled into hexameric (17 kDa) channel complexes at the plasma membrane. Fluid shear stress or mechanical load may upregulate CXN expression to better communicate load signals among bone cells. METHODS: PURA-2-loaded MC 373 cells were challenged with an indentation load in the presence of a micropipet to elicit release of intracellular calcium. In other experiments, MC 373 cells were plated at near confluence (50 kcells/cm2) in complete medium, allowed to attach to collagen, and rinsed 30 min before 24 h load, not loaded for 16 h, then collected at 24 h for quantitation of CXN-43 mRNA and protein. mRNA was measured by semi-quantitative PCR. CXN band strength was normalized to the β-actin signal in the same sample. CXN protein expression was semi-quantified by PAGE and Western analysis by a densitometer technique. RESULTS: CXN-43 mRNA and protein expression did not increase substantially in the first 24 h post-load compared to the uninduced state, whereas CXN protein expression increased 1.5-fold in loaded cells compared to nonloaded counterparts. A load stimulated increase in CXN-43 protein could be observed as early as 4 h post-load and rose through 8, 12, 16 and 20 h. CXN-43 phosphorylation was also increased 1.4-fold in loaded cells. Expression was normalized by day 3 between load and no load groups and was similar and declined on days 5 and 7. CONCLUSIONS: MC 373 cells responded to a perturbation in their plasma membrane by releasing Ca2+ and contained functional Gj as evidenced by their ability to propagate the calcium wave. Cyclic mechanical load increased CXN-43 protein expression and phosphorylation but not CXN mRNA expression. Increased CXN-43 protein may be a result of an increased rate of transcription, message stability or protein stability. Increased CXN phosphorylation may be related to load alteration of the oxygenated state of the channel. The cytoskeleton may play a role in the upregulation of CXN expression and may require a greater ability to communicate under mechanical load conditions. NIH AR41211.

10.6 ROLE OF G-PROTEINS IN MECHANICAL SIGNAL TRANSDUCTION. Siva Prasad Gudipati, Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802

Cells have highly specialized force sensing mechanisms such as those involved in hearing and touch which may play a mechanical role in other biochemical responses. Fluid flow itself is another such stimulus that exists in the extracellular environment. Indirect evidence suggests that flow regulation of vascular tone via the endothelial cell is involved in the control of vascular smooth muscle cell signal transduction via G-proteins. Human Umbilical Vein Endothelial Cells (HUVECs) grown on glass slides were subjected to fluid flow in parallel plate flow chambers. Fluid shear-induced GTP binding in HUVECs was assayed using a chemiluminescence technique. RESULTS: The regulation of cell volume and actin dynamics, message stability or protein stability. Increased CXN phosphorylation may be related to SMC hypertrophy in response to load. Upregulation of receptor expression may regulate and amplify the cell's ability to detect and respond to load. American Heart Association.

Supported by: Am. Heart Assoc. NY Affiliate grant 930 393 to SS and HHF of SUNY/Buffalo grant 05-04 to SPS.
10.8
MECHANICAL STRAIN IS SENSED BY VSM CELLS THROUGH AN INTERACTION WITH EXTRACELLULAR MATRIX PROTEINS. Emily Wilson, Krishnananthy Sudhir and Harlan E. Ives. Div. of Nephrology and the Cardiovascular Research Institute, University of California, San Francisco.

Cyclic mechanical strain (1 Hz) increased a synthesis in vascular smooth muscle (VSM) cells via the autocrine production and release of PDE4 into the media. Cells plated on collagen, fibronectin or vitronectin showed 5-10 fold increases in DNA synthesis in response to mechanical strain; cells on laminin or elastin did not respond. To further evaluate the role of the ECM-integrin system, we have utilized an RGD containing peptide GRGDTP and an inactive control peptide GREGSP. RGD peptide completely blocked the strain-induced increase in DNA synthesis; RGD peptide had no effect. RGD could theoretically prevent the von Willebrand factor secretion of growth factor response. However, RGD did not alter the mitogenic response to either PDGF or α-thrombin. However, RGD eliminated the secretion of growth factors (mitogenicity of conditioned media) following mechanical strain. RGD also reduced by 50% the strain induced expression of a PDGF-A promoter (990 bp)-CAT construct that was transiently transfected into VSM cells. RGE did not alter the activity. Thus interaction with matrix peptides is essential for induction and secretion of growth factors in response to mechanical strain.

10.11
ACTIVATION OF CYCLIC AMP SIGNALING INHIBITS VOLUME-SENSITIVE OSMOLYTE EFFUX IN CARDIAC MYOCYTES. James L. Smith, Eric S. Moore, Dafne Leiseh and Melyso Lieberman*. Dept. of Cell Biology, Div. of Cellular Physiology and Biophysics, Duke University Medical Center, Durham, NC.

Myocardial cell swelling induced by volume-sensitive transport systems that mediate the efflux of osmolytes (ions and amino acids) and result in a regulatory volume decrease (RVD) has been associated with the onset of cardiac failure. Although CAMP production is not stimulated during cellular swelling (Fisher, NIBP, 1996) we hypothesized that volume-sensitive transport mechanism may be regulated by Ca++-dependent protein kinase C (PKC). Relative contributions of PKC-α, -β and -γ were measured by video microscopy and correlated with osmolyte efflux (assayed by release of [3H]-taurine) over 20 min. Peak volume (measured) increased as the osmolality of the osmolyte decreased (see table) followed by RVDS that coincided with increasing taurine efflux. Upon reperfusion with isosmotic medium (290 mosm), cells returned to progressively less of their original volume (depending upon severity of swelling) reflecting the increased loss of organic osmolytes. Flooding a Ca++-sensitive construct with 0.5mM isobutylmethylxanthine (IBMX) or 10μM forskolin led to a attenuation of RVDS coincident with a decrease of taurine efflux. Results show that when cardiac myocytes are swollen, activation of a Ca++-dependent pathways will prevent volume regulation in part by inhibiting osmolyte efflux. Supported in part by NHLBI grant HL72105.

10.12
CYTOSKELETAL REARRANGEMENT ACTIVATES THE SWELLING-INDUCED CHLORIDE CURRENT IN CARDIAC MYOCYTES. Jiangping Zhao, Randall L. Kamanoand Melyso Lieberman*, Department of Cell Biology, Division of Cellular Physiology and Biophysics, Duke University Medical Center, Durham, NC, 27710.

Whole-cell patch clamp experiments investigating a swelling-induced chloride current 10.11 in cultured chick heart myocytes (Zhong et al, J. Cell. Sci. 2000;113,1993. We now investigate whether this swelling-activated chloride conductance is associated with a signal transduction pathway. Concentration-dependent activation of the cytoskeletal network Pharmacological reagents that alter the dynamic assembly and disassembly of actin filaments were applied in whole-cell patch clamp experiments. When heart cells were perfused with 130mM and perfused with a F-actin depolymerizing reagent, cytoskeletal loss (10mM), Ls was markedly reduced (20±1% of control, mV, vs. control). Removal of cytoskeletal B from the hypoosmotic solution caused a gradual increase in Ls towards the control levels. Similar results were observed when cells were pretreated with 2×10mM and perfused with another F-actin depolymerizing reagent, mV, (10mM, vs. control). These results agree with the observations in which disruption of F-actin in intact heart cells attenuated the regulatory volume decrease (RVDS) by hypoosmotic swelling. An alternative approach to test the involvement of cytoskeletal deformation was to study the effect of F-actin stabilizing reagent, phalloidin, on Ls. Pretreatment of cells with phalloidin (30mM, 2×10mM) and inclusion of phalloidin (15mM) in the pipette solution substantially attenuated Ls (2×10mM, vs. control). In conclusion, cell swelling appears to invoke signal transduction mechanisms that involve rearrangement of cytoskeletal network. Supported by NHLBI grants HL72105 and The Walter P. Human Fund.

10.13
LOAD-ACCELERATED PROTEIN SYNTHESIS OF STRETCHED ADULT CPU CELLS DEPENDS UPON STRETCH-ACTIVATED CALCIUM (Ca++) INFLUX. Robert L. Kent* and George Cooper, IV. VA Medical Center & Gees Cardiovascular Research Institute, Cardiology Division, Medical University of South Carolina, Charleston, SC 29403.

The mechanisms whereby hemodynamic load accelerates protein synthesis in cardiac hypertrophy are unknown. A role for stretch-activated Ca++ influx in the signal pathway linking load to enhanced protein synthesis is examined in the isolated adult cardiac myocyte, or cardiocyte. Feline cardiocytes were plated onto a lamin-coated deformable membrane and maintained for 24 hrs in serum- and mitogen-free culture with 1.6 mM Ca++. Cardiocyte stretch was proportional to membrane deformation until 10%, which increased the myoplasmic fluorescence of the Ca++-selective dye Fura-3 by 50%. This fluorescence with cardiocytes was abolished by washing the cardiocytes in culture medium for 1 hr and then perfusing the membrane with 0.1 mM Ca and 0.1 mM C2 ethylglycol (EGTA), or by 10μM gadolinium, a blocker of stretch-activated ion channels. Acceleration of protein synthesis was also proportional to membrane stretch (r=0.94), and was enhanced by 45% after 4 hrs of 10% stretch when compared with that of non-stretched cardiocytes. Cardiocyte stretch increased by 75% Ca++ uptake when Ca++- containing solution was replaced by Ca++-free solution containing 0.4 μM unlabeled phenylalanine. This effect of stretch was reduced to the level of that in slack cardiocytes by washing them with 0.1 mM Ca for 1 hr by gadolinium, but it was unaffected by 5 μM varapamil. The low Ca medium did not affect the acceleration of protein synthesis by insulin. This anabolic effect of load was not reduced by sodium, or an angiotensin II blocker. Direct angiotensin II (10 μM) treatment of these cardiocytes had no anabolic effect. These results indicate that stretch-stimulated Ca++ influx through stretch-activated ion channels is necessary for transduction of load into accelerated myocellular protein synthesis.

10.14

The nonionic surfactant Pluronic F-68 (PLU), a block copolymer of polyoxyethylene and polyoxypropylene (avg. MW = 8,350), protects cells against damage in ethanol and/or asparagine biocatalysts. The mechanism of protection is poorly understood. We have observed that PLU reversibly blocks Ca++ uptake via the acetylcholine receptor (AchR) in FURA-loaded human embryonic kidney cells (HEK 293) transfected with mouse AchR subunits. Relative increase in intracellular Ca++ was monitored fluorometrically (ratio method) at room temperature (22±2°C) in FHIP- buffered (PH = 7.4) saline solution. The cells (on cover slips) were incubated (flow-through cuvette) in the presence of 0.2 or 3.0 PLU for 60 min prior to addition of Ach (50 μM) in the cuvette. In the absence of PLU, intracellular Ca++ increased immediately after Ach addition. In the presence of PLU, Ca++ uptake was blocked. Flushing the cuvette with 50 μM PLU-free buffer and reinserting Ach restored Ca++ uptake (until the cells no longer respond to Ach). In addition, suspensions of 2×106 intact cells subjected to titration exhibited a 30% increase in (Ca++), compared to a 13% increase in the presence of 0.2% PLU. At concentrations as high as 0.3%, PLU had no apparent effect on Ca++ uptake via stretch-activated (hypoosmotic stress) channels of GH3 cells. Supported in part by DAAAL0392G0014.

(*) this abstract of a proposed presentation does not necessarily reflect EPA policy.)
10.15 PERCOLATION AS A POSSIBLE MODEL FOR BIOLOGICAL SIGNALING. Claire Ferguson. Clarkson University, Potsdam, NY 13699.

Random filamentous networks show up at various scales (nuclear matrix, microfilament networks, intermediate filament networks, extracellular matrix) and developmental stages in a multicellular organism. They participate in a number of vital biological processes. We propose yet another role for these networks: biological signal transduction. We introduce the notion of persistence length, a concept well-known and widely used in physical and engineering sciences, and relate it to the various macromolecular assemblies, in particular the cytoskeletal meshworks. Using properties of percolating networks, we develop a possible coupled mechano-chemical signal transduction process. Similarities and differences between biological percolation networks and tensegrity structures introduced by Fuller are discussed. The results of computer simulations are presented to support the model. Specific predictions are made and experiments are suggested. This work was supported by NSF, under Grant IBN 9317633.

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

We have previously characterized a FfH-stimulated, mechanosensitive, non-selective cation channel (SA-cat) in UMR-106.01 osteoblast-like cells which we postulate may act as a mechanotransducer for the osteogenic response of bone to physical strain. Using patch clamp techniques, we studied the response of SA-cat channels to CMS applied via the Flexercell apparatus, Chronically strained cells subjected to 8,000 to 15,000 microstrain demonstrated 37% of the patches of chronically strained cells. Graded increases in negative patch pressure demonstrated that SA-cat channels in chronically strained cells were activated at significantly lower levels of mechanical strain than non-strained controls. These data suggest that chronic, cyclic strain reduces the activation threshold of the SA-cat channel and further strengthens our hypothesis that this channel may act as a mechanotransducer for activation of bone remodeling by physical strain. (Supported by NASA Grant NAG 2-791)

10.16 MECHANOSTIMULATION OF NEURITES OF NODOSE VAGAL SENSORY NEURONS IN CULTURE INDUCES A WHOLE CELL CURRENT. J.T. Cunningham, L. Tankebauer, R. Wachtel & E.M. Albow. Dept. of Int. Med., Anest., VAMC and Cardiovascular Center, Univ. of Iowa, Iowa City, Iowa 52242.

The purpose of this experiment was to study the mechanism of mechanotransduction of baroreceptor neurones. Male Harlan-Sprague rats were anesthetized and DII was injected into the adventitia of the aorta and aorta. A week later the animals were sacrificed and whole cell patch clamp experiments were performed. The recording medium was in (in mM): KCl, 14 KOH, 10 HEPES, and in some experiments nystatin. The extracellular medium was in (in mM): 140 NaCl, 2 CaCl2, 1 MgCl2 KCl, 10 HEPES, and 2 g/l glucose. The neurites were recorded using a pneumatic ejection of extracellular solution delivered via a glass pipette (7-15 μm i.d. tip) placed 25-50 μm from the neurite. In voltage clamp experiments with holding potentials of -70 mV, neurite deformation with a 50 ms injection at 5-10 psi induced an inward current (1.7 to 0.6 μA) in 5 out of 6 DII labeled putative baroreceptor neurones. In 3 experiments these currents were suppressed by gadolinium (20 μM), a putative blocker of stretch-activated channels. Similar results were obtained in perfused patch experiments. These currents were observed without significant changes in cell resistance. Our results indicate that stretch-activated conductances in baroreceptor neurones may represent the mechanism of mechano-electrical transduction associated with deformation of baroreceptor terminals. (Supported by HL 14388).

10.19 ISOLATION OF A MECHANOSENSITIVE SUBPOPULATION OF VAGAL SENSORY NEURONS IN VITRO. Cs. Hajduzseck. Department of Physiology, Univ. of Buffalo School of Medicine, Buffalo, NY 14214.

The nodose ganglion (NG) is a sensory ganglion containing a subpopulation of vagal sensory neurons and 75±5% of them are putative baroreceptor neurons. The nodose ganglion (NG) is a vagal sensory ganglion containing a subpopulation of vagal sensory neurons and 75±5% of them are putative baroreceptor neurons. The presence of label. The increase of cytosolic free calcium ([Ca2+]i) in response to mechanical stimulation in fura- loaded cells was determined by applying an external pressure pulse of normal Ringer's via a micropipette to the focal plane of the microscope, permitted continuous visualization of the large edge of these vesicles, whose total length averaged 10.3 ± 0.3 μm (n=7). The endogenous negative pipette pressure stretched the vesicles in their linear (pipette) axis by 1.2 ± 0.3 μm and increased the rise times than in either cell-attached or excised patches. Application of 10 mmHg of a 50% decrease in bath osmolarity increased 0.1 w or 13 ± 3% and increased channel open probability (Po) from 0 to 0.2 ± 0.03. (n=4). A 50% decrease in bath osmolarity increased PO of these channels on intact oocytes, a 50% decrease in bath osmolarity increased PO of these channels.

10.20 OSMOTIC ACTIVATION OF MECHANOSENSITIVE CHANNELS IN VESELES ISOLATED FROM XENOPUS OOCYTES. Henry Sackin and Wolfgang Schirtz*.

The purpose of this experiment was to study the mechanism of mechanotransduction of baroreceptor neurones. Male Harlan-Sprague rats were anesthetized and DII was injected into the adventitia of the aorta and aorta. A week later the animals were sacrificed and whole cell patch clamp experiments were performed. The recording medium was in (in mM): KCl, 14 KOH, 10 HEPES, and in some experiments nystatin. The extracellular medium was in (in mM): 140 NaCl, 2 CaCl2, 1 MgCl2 KCl, 10 HEPES, and 2 g/l glucose. The neurites were recorded using a pneumatic ejection of extracellular solution delivered via a glass pipette (7-15 μm i.d. tip) placed 25-50 μm from the neurite. In voltage clamp experiments with holding potentials of -70 mV, neurite deformation with a 50 ms injection at 5-10 psi induced an inward current (1.7 to 0.6 μA) in 5 out of 6 DII labeled putative baroreceptor neurones. In 3 experiments these currents were suppressed by gadolinium (20 μM), a putative blocker of stretch-activated channels. Similar results were obtained in perfused patch experiments. These currents were observed without significant changes in cell resistance. Our results indicate that stretch-activated conductances in baroreceptor neurones may represent the mechanism of mechano-electrical transduction associated with deformation of baroreceptor terminals. (Supported by HL 14388).
10.21
STRETCH STIMULATES PROLIFERATION OF RENAL EPITHELIAL CELLS.

It has been suggested that in renal cystic disease, fluid accumulation within cyst lumens stretches cyst walls and this may stimulate cell proliferation. To test this idea, we stretched confluent monolayers derived from a Ham-in-Darby canine kidney cell line, using collagen-coated Flexcell dishes and subjected to 0-30% stretch for 1-48 hr. The percentage of cells in S-phase (labeling index = LI) was determined by the bromodeoxyuridine incorporation method. Stretch (25%) for 12-24 hr approximately doubled the LI. After 48 hr, population density was significantly (P<0.001) increased, with 41.7 ± 2.0 to 48.2 ± 0.5 cells/10,000 µm² (± S.E.M.) (LI = 0.58 ± 0.08, n = 12). A similar increased linear cell density was observed when cells were applied from 5 ± 0.5 µm (n = 16) with no stretch to 15 ± 1.0 µm (n = 6) with 30% stretch. Stretch had to be maintained for eight or more hours to produce an increase in LI at 18 hr. No evidence was obtained for release of a soluble growth factor by stretched monolayers in co-culture experiments. The increase in LI induced by stretch was abolished by treatment with cytochalasin B, an actin microfilament disrupting agent, but was unaffected by 50 µM gadolinium, a stretch-activated cation channel blocker. We conclude that prolonged stretch stimulates renal epithelial cells to synthesize DNA, and may contribute to cell proliferation and cyst enlargement in renal cystic disease.

10.22
Ca²⁺-DEPENDENT MECHANOSensitivity IN CARDiac FIBROBLASTS.

Peter Kohl, André Kämkin, Irina Kigelya and M.J. Lab, Dept. of Physiology, University of Oxford, OX1 3PT, UK.

Electrically non-excitable fibroblasts (F) in the sino-atrial node region of amphibian and mammalian heart have been described to be mechanosensitive (Kohli et al., Exp Physiol, 1992 & 1994). Computer simulations show that these cells can increase the spontaneous depolarization rate of adjacent pacemaker cells in a stretch dependent manner, and thus, be involved in the mechanosensitive response of the heart to stretch. In this investigation we attempted to analyze the mechanism of mechanoreception in more detail. F. were studied in isolated preparations of right atrium using focusing microelectrodes. Gadolinium (20-100 µM, blocker of stretch activated channels) added to the perfusate abolished the appearance of membrane depolarizations induced by stretch. In F. treated with thapsigargin, a specific inhibitor of the SERCA ATPases, reduced Ca²⁺ efflux g-fold during the first 5 min. Thus, it appears that a cell surface Ca²⁺ influx mechanism, a complementary force balance between microtubules and actin, may mediate stretch-induced Ca²⁺ entry in F.

10.23
MECHANOSensitive ACTIVATION OF Ca²⁺ TRANSPORT PATHWAYS IN HT29 HUMAN COLOn CANcer CELLS.

Subconfluent cultures of the HT29 human colon cancer cell line grown on collagen-coated elastomer plates demonstrated that an average of 10% surface cells studied in a microscope-based photometry system. Similar manipulation of a wide variety of cell types, including the human pancreatic cancer line CFPAC-1, was without effect on resting [Ca²⁺]i levels. Pretreatment of HT29 cells with 1 µM thapsigargin, a specific inhibitor of the SERCA ATPase, eliminated the Ca²⁺ transient upon solution exchange. These results indicate that the mechanical effect of stretching cells was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was strongly inhibited by both cell monolayers, with collagen-coated elastomer plates, and cells studied in a microscope-based photometry system. Similar manipulation of a wide variety of cell types, including the human pancreatic cancer line CFPAC-1, was without effect on resting [Ca²⁺]i levels. Pretreatment of HT29 cells with 1 µM thapsigargin, a specific inhibitor of the SERCA ATPase, eliminated the Ca²⁺ transient upon solution exchange. These results indicate that the mechanical effect of stretching cells was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was strongly inhibited by both cell monolayers, with collagen-coated elastomer plates, and cells studied in a microscope-based photometry system. Similar manipulation of a wide variety of cell types, including the human pancreatic cancer line CFPAC-1, was without effect on resting [Ca²⁺]i levels. Pretreatment of HT29 cells with 1 µM thapsigargin, a specific inhibitor of the SERCA ATPase, eliminated the Ca²⁺ transient upon solution exchange. These results indicate that the mechanical effect of stretching cells was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was strongly inhibited by both cell monolayers, with collagen-coated elastomer plates, and cells studied in a microscope-based photometry system. Similar manipulation of a wide variety of cell types, including the human pancreatic cancer line CFPAC-1, was without effect on resting [Ca²⁺]i levels. Pretreatment of HT29 cells with 1 µM thapsigargin, a specific inhibitor of the SERCA ATPase, eliminated the Ca²⁺ transient upon solution exchange. These results indicate that the mechanical effect of stretching cells was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was strongly inhibited by both cell monolayers, with collagen-coated elastomer plates, and cells studied in a microscope-based photometry system.

10.24
OSMOTIC STIMULATION OF AXONAL ELONGATION IS MEDIATED BY AN INCREASE IN TENSION SENSITIVITY ON THE DIFFERENTIATION OF NEURONAL SUBTYPES. Steven St. Heidemann, Phillip Lamoureux and Chinju Lin. Dept. of Pharmacology, Michigan State Univ., E. Lansing, MI 48824.

We tested for a possible osmotic effect on stretch-activated neuronal subtypes (Ray et al., J. Cell. Sci. 98:507) and tensile (Zeng et al., Neurosci. 11:1117) stimulation of elongation of cultured chick sensory neurones. Neurites were tethered by their growth cone to force-calibrated glass needles and tested at a constant force, which varied between 150-300 µyd for 0-15 sec. The neurites were then allowed to elongate in a culture dish. The percentage increase in neurite length between 75-143 µyd/hr. After 1 hr of tow, the culture medium (1-3) was diluted 1:1 with a medium similar in composition to 0-15 but lacking inorganic salts. Within 15 min. in the reduced osmolarity of the medium, the percentage increase in neurite length decreased by 38% and 115% at the same constant tow force. That is, in the decrease in osmolarity of the medium, the percentage increase in neurite elongation rate of the neurites increased by 38% and 115% at the same constant tow force. Therefore, this increase in osmolarity of the medium increased the sensitivity of neurites to tension-induced elongation. We are currently studying whether this pressure osmotic effect may be due to a decrease in extracellular [Ca²⁺]. We previously proposed a tension-sensitivity mechanism, a complementary force balance between microtubules and actin, to explain tension-induced axonal elongation. The presumed osmotic effect we observe could be due to a swelling and weakening of the actin network induced by water influx.

10.25
EFFECTS OF FLUID FLOW ON OSTEOCyTES. J. Klein-Nulend, L.J. de Vries, J. Ward, F.M. Slump, J.A. van der Plas, and Titow Ett. Dept. of Physiology and Medicine, Tulane University School of Medicine, New Orleans, LA 70112.

Subconfluent cultures of the HT29 human colon cancer cell line grown on glass coverslips were loaded with 2 µM Fura-2 acetyoxymethylester for 30 min at room temperature, washed, and incubated for further 30 min at 37°C before determination of [Ca²⁺]i. Cytosolic [Ca²⁺]i was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was strongly inhibited by both cell monolayers, with collagen-coated elastomer plates, and cells studied in a microscope-based photometry system. Similar manipulation of a wide variety of cell types, including the human pancreatic cancer line CFPAC-1, was without effect on resting [Ca²⁺]i levels. Pretreatment of HT29 cells with 1 µM thapsigargin, a specific inhibitor of the SERCA ATPase, eliminated the Ca²⁺ transient upon solution exchange. These results indicate that the mechanical effect of stretching cells was transiently increased in these cells by the simple mechanical stimulus of exchanging the buffer solution. This effect was strongly inhibited by both cell monolayers, with collagen-coated elastomer plates, and cells studied in a microscope-based photometry system.
10.27

EFFECT OF ABDOMINAL VIBROACOUSTIC STIMULATION ON ACCELERATION LEVELS AT THE FETAL HEAD IN SHEEP.


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A vibroacoustic stimulator such as the electronic artificial larynx (EAL) is commonly used by obstetricians as an adjunct to a formalized test to determine human fetal activity and thus fetal health. The EAL is placed on the maternal abdomen over the fetal head and activated to provide up to five seconds. The acceleration at the fetal head may provide important information about whether or not human fetal movement occurs and what stimulates this movement. We measured acceleration levels at the fetal head with a specially designed accelerometer (Viggo Instruments, CA-125-10X, Mentor, Inc., Fairfield, NJ), and sound pressure levels near the head with a microphone (Model E103, Bruel & Kjaer, Marlowe, MA). Animals were anesthetized and several procedures and fetuses were returned to the uterus and the abdominal wall was closed. An EAL (Corometrics Medical Systems, Inc., Wallingford, CT) was positioned against the skin with a force that depressed the skin by 2 cm. At the fundamental frequency of 25 Hz, a SPL of 91 dB and 224 m/sec² (see Figure). These values were well above the noise floor which was approximately 20 dB and 3 m/sec² through the frequency regions of interest. Both mechanical receptors and auditory receptors may play roles in evoking a response to vibroacoustics.

Supplied in part by NIH grant HD-20084.

11.1

DYNAMICS OF MECHANICAL STRESS-REGULATED MODULATION OF CELL SHAPE, INTERCELLULAR INTERACTIONS, AND MACROMOLECULAR BIOSYNTHESIS IN FIBROBLASTS.


Mechanical stress factors are known to act as epigenetic regulators of connective tissue modelling and repair. Our laboratory has developed an in vitro model system that enables better understanding of mechanisms involved in the mechanical stress-induced extracellular matrix remodelling and repair. Our model consists of a tissue equivalent comprising a fibroblast-populated collagen matrix (FPCM) in which static and mechanical loads and changes in both cell shape and the length of FPCM are measured and analyzed, and failed to orient under these conditions. This cell type may indicate some of the intercellular regulatory mechanisms that mediate the response to mechanical strain. Normal rabbit corneal stromal fibroblasts (NRC) were also studied in this model.

Young's Modulus values for FKMs and neodymium (ν) were applied to normal human fetal, neonatal and aged dermal fibroblasts and the effects of mechanical strain on cell morphology were examined. The randomly oriented neonatal cells on the unstretched control substrate. Neonatal cells also required the collagen type I coating, but both observation and immunofluorescence analyses revealed a progressive change in cell shape and bioelasticity with stretching. The cell shape and size before and after dynamic loading were analyzed, and failed to orient under these conditions. This cell type may indicate some of the intercellular regulatory mechanisms that mediate the response to mechanical strain. Normal rabbit corneal stromal fibroblasts (NRC) were also studied in this model.

11.2

Human cultures-derived macrophages contain a K⁺ channel that modulates the cytoskeleton and is activated by membrane stretch and cytokines.

Samuel N. Breit, Michelle R. Boettner, Terrence J. Campbell, Victor F. French, Donald K. Martin, Center for Engineering the Molecular Basis of Disease, Departments of Clinical Pharmacology and Cardiology, St. Vincent's Hospital and University of NSW, Sydney, NSW, 2010, Australia.

Vibroacoustic stimulation (VAS) has been shown to be beneficial in the treatment of some chronic diseases. This study investigated the effects of VAS on the behavior of human macrophages.

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11.3

A STUDY OF PARAMETERS AFFECTING FIBROBLAST MORPHOLOGY IN RESPONSE TO AN APPLIED MECHANICAL FORCE.

Roslind A. Goves 1 and Christine Sawyer 2

1 Ille Science Division 1 and Rinnetics Corp. 2 NASA Ames Research Center, Moffett Field, CA 94035

A precisely controlled stretch/relaxation regimen (20% elongation at 6.6 cycles/min) was applied to normal human fetal, neonatal and aged dermal fibroblasts cultured on flexible membranes. Culture conditions included poly(ethylene) terephthalate (PET) membranes. PET membranes were grown on the same sterile polilin in the absence of applied stretch. Direct observation and immunofluorescence analysis revealed a progressive change in cell shape with cell body orientation limited to the stretched direction. A high scolding density of cells was aligned in the fetal cell cultures. This cell strain required collagen type I coating for optimal attachment to the flexible membrane, which correlated with a reversible alteration to the morphology of the cells. Mechanical stress induced cell-spread growth at times not as previously described with no effect on cell viability. These data suggest FPCM as an excellent model system for mechanobiologic studies.

11.4

PREDICTIONS OF A MODEL FOR CELL / INTERCELLULAR MATRIX INTERACTIONS IN SHORTENING SMOOTH MUSCLE.

Richard A. Meiss, Indiana University School of Medicine, Indianapolis, IN 46202.

Most smooth muscle consists of small cells embedded in an extracellular matrix (ECM) of connective tissue, and the mechanical function of both the individual cells and the whole tissue is constrained and modified by these interconnections. A model to account for some of these interactions has been developed (Meiss, R. A., A. Adv. Exp. Med. Biol. 474-475, 1999), which proposes that active shortening is limited by the tendency of the tissue to raise radial expansion, and that this resistance is manifested in the axial stiffness of the tissue. The model parameters relate to the behavior of the radial and axial direction and to the effect of tissue cross-sectional area. This paper analyzes the relative sensitivity of the model to manipulations of these parameters in order to develop experimental strategies for testing model assumptions. The mechanical end-point for the analysis is the strong length-dependence of stiffness at short and moderate strain levels; this is shown to be weakly sensitive to axial elastic components, moderately sensitive to tissue cross-sectional area, and extremely sensitive in the unloaded condition.

Supported by the Dept. of OB/GYN, Indiana University School of Medicine.
Regulation of Cell Shape and Function by the Extracellular Matrix

Modulation by reversible inhibition of β1 integrin by antisense oligonucleotides of myoblast attachment in FMC and proliferation potential.

M.L. Massimino*, E. Rapizzi*, M. Cantini*, M. Sandri*, A. Bruson*, C. Catan*, L. Dalla Libera* and U. Cattaneo, Department of Biomedical Sciences, University of Padova, I-35131 Padova, Italy.

Myoblasts gene-engineered in vitro and then injection in vivo are safe, efficient options for gene therapy. While isolation of satellite cells is routinely achieved, their proliferation potential in vitro remains a limiting factor of cell transplantation in clinical settings. We are studying the role of reversible inhibition by antisense oligonucleotides of the synthesis of integrins (surface proteins which mediate interactions between cells and extracellular matrices and are known to modulate cell motility and proliferation) on satellite cells proliferation. Addition of antisense oligonucleotides to myoblast cultures has been used to specifically inhibit expression of the β1 integrin subunit gene. Here we show that the effects of multiple pulses of a phosphorothioate oligodeoxynucleotides antisense on myoblast proliferation and detachment from substrate (gelatin or rat tail collagen) are dose-dependent. A single pulse shows not consistent effects on myoblast proliferation, while in the presence of continuously administered antisense relative and absolute numbers of myoblasts in treated muscle culture are slightly increased. On the other hand we have not evidence of inhibition of myoblast fusion in the tested conditions. Results suggests a potential role of integrin antisense strategies on modulation of proliferation potential of myoblasts.

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