properties of the diseased arterial wall in addition to regression or prevention of atherosclerosis. Perhaps more uniform circumferential distribution of residual strain (vs. compliance mismatch) conferred by lipid reduction prevents high circumferential stress regions and subsequent plaque rupture.

9:15

775-4 Effects of Stretch and Pressure-induced Crush of the Arterial Wall on the Induction of Immediate Early Protooncogenes

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In coronary angioplasty, the arterial wall is subjected to very high intraluminal pressures, resulting in both stretch and crush of vascular cells. In an attempt to investigate the respective effects of pressure-induced stretch and crush on the induction of *c-fos, c-jun* and *c-myc*, an *in vitro* experimental model was developed. Segments of descending rabbit thoracic aorta with intact endothelium were canulated and excised at their *in vivo* length and maintained at physiological pressure level. Vessels were then incubated in bath containing DMEM and antibiotics. The arterial segments were connected to a mercury manometer or a coronary inflation device in order to rise the internal diameter from 4 to 6 mm (submaximal distension) for 2 minutes under various levels of intraluminal pressure: A) 90 mmHg, which mimics physiological conditions; B) 200 mmHg, the vessel diameter being maintained at 6 mm using a rigid external support; C) 2 bars, using the same external support. The intaluminal pressure was then brought back to its initial value and the vessels were examined for protooncogenes expression using Northern and slot blot analyses, 30 and 90 minutes thereafter. In addition, *in vivo* experiments were conducted to determine protooncogenes expression in thoracic aorta, 30 and 90 minutes following endothelial denudation by a Fogarty balloon. We found that *c-fos, c-jun and c–myc* were induced in the arterial wall after *in vivo* balloon denudation. In organ culture, brief and unopposed stretch of the arterial wall (condition A) was not sufficient to induce the expression of protooncogenes. In contrast, although the artery was kept at the same level of stretch, crush of vascular cells under high intaluminal pressures (200 mmHg and 2 bars) greatly increased the expression of *c-fos, c-jun* and *c-myc*. The mRNA levels of these protooncogenes were proportional to the magnitude of intraluminal pressure. Levels of *c-fos and c-jun* mRNAs peaked at 30 minutes, while those of *c-myc* peaked at 90 minutes. Since the contractility of aortic rings obtained from vessels studied under each experimental condition was not altered, the increased expression of protooncogenes observed under 200 mmHg and 2 bars cannot be viewed as a signal of cell death.

Conclusion: These data suggest that brief, pressure-induced crush of vascular cells in the arterial wall, is a potent stimulus for the induction of immediate early genes implicated in cellular proliferation and vascular remodeling.

9:30

775-5 Increased Incidence of Chlamydia Bacteria Detected within Coronary Arteries of Patients with Atherosclerotic Versus Transplant Induced Coronary Artery Disease

Joseph B. Muhlestein, Elizabeth H. Hammond, John F. Carlquist, Ellen Radicke, Matthew J. Thomson, Daniel J. Parker, Labros A. Karagounis, Jeffrey L. Anderson *University of Utah, LDS Hospital, Salt Lake City, Utah*

Previous work has suggested an association between *Chlamydia pneumoniae* infection and coronary atherosclerosis, based on the demonstration of increased serologic titers and the detection of bacteria within atherosclerotic tissue. We sought to strengthen a possible etiologic link by comparing the incidence of *Chlamydia* infection in coronary atherosclerotic tissue to that in coronary arteries of patients with non-atherosclerotic cardiovascular disease.

Coronary tissue specimens obtained from 90 consecutive patients (mean age = 57 yrs) with symptomatic coronary artery disease undergoing directional coronary atherectomy were tested for the presence of *Chlamydia* species by direct immunofluorescence using mouse monoclonal antibody. Immunofluorescence was positive in 68 (76%), equivocal in 5 (6%) and negative in 19 (21%) of specimens. Polymerase chain reaction confirmed the specific *Chlamydia* strain TWAR in 2 cases. No clinical factors except the presence of a primary non-restenotic lesion (odds ratio = 3.0, p = 0.057) predicted the detection of *Chlamydia*.

As a control, coronary tissue specimens from 11 patients with previous cardiac transplantation and subsequent transplant-induced coronary artery disease were also tested. Direct immunofluorescence performed on these diseased coronary artery segments was negative for *Chlamydia* in every case (p = 0.0001 compared with atherosclerotic specimens).

Conclusion: This high incidence of *Chlamydia* detected only in coronary arteries diseased by atherosclerosis suggests an etiologic role for *Chlamydia* infection in the development of coronary atherosclerosis, which should be further studied.

9:45

775-6 Vascular Remodeling and Endothelial Dysfunction in Coronary Disease

Charles R. Cannan, Stuart T. Higano, David R. Holmes Jr., Amir Lerman *Mayo Clinic, Rochester, MN*

Early coronary atherosclerosis (CA) is characterized by endothelial dysfunction which is manifested by coronary vasoconstriction in response to the endothelial-dependent vasodilator acetylcholine. The endothelium may be involved in the adaptive process of vascular remodeling (VR). While vascular remodeling has been demonstrated in advanced CA, it is not known if this process occurs early in the course of CA. Thus, this study was designed to test the hypothesis that VR characterized by proximal coronary artery enlargement occurs in patients with angiographically normal or mild CA and abnormal coronary endothelial function. To test this hypothesis, 20 patients found to have normal coronary angiograms or mild CA were studied. Acetylcholine was infused in incremental doses into the left anterior descending (LAD) with angiographic measurement of lumen diameter. Patients were divided into two groups based on their coronary endothelial function. Group 1 demonstrated coronary vasoconstriction in response to acetylcholine and Group 2 had a normal vasodilatory response. Intravascular ultrasound measurements of the proximal LAD diameter and area were made following nitroglycerin. Vessel diameter and area were measured at the internal elastic membrane and indexed to body surface area (BSA). Comparison between the two groups is outlined below:

	Group 1 (n = 14)	Group 2 (n = 6)
Vessel Diameter (mm)	5.1 ± 0.2*	3.9 ± 0.2
Vessel Area (mm^2)	19.1 ± 1.5*	12.4 ± 1.3
Vessel Diameter Index (mm/m^2)	2.6 ± 0.1*	2.1 ± 0.1
Vessel Area Index (mm^2/m^2)	10.0 ± 0.7*	6.6 ± 0.6

Data are mean ± SEM, *p < 0.02 vs. Group 2

Five patients in Group 1 and one in Group 2 had ultrasound evidence of mild atherosclerosis. This study demonstrates in vivo that vascular remodeling characterized by enlargement of the proximal coronary arteries occurs early in the course of coronary disease in the presence of endothelial dysfunction.

776 Hormones and Receptors: Molecular and Cellular Aspects

Wednesday, March 22, 1995, 8:30 a.m.–10:00 a.m.
Ernest N. Morial Convention Center, Room 22

8:30

776-1 Selective Down-regulation of Angiotensin II AT_1 Receptors in Failing Human Heart: Relationship to β_1-Receptor Down-regulation

Koji Asano, Wayne Minobe, Kelli D. Mitchusson, Darrin Dutcher, Robert L. Roden, J. David Port, Michael R. Bristow. *University of Colorado Health Sciences Center, Denver, Colorado*

The renin-angiotensin and adrenergic nervous systems exhibit multiple levels of cross-regulation in heart failure. These systems are bidirectionally activated in concert; i.e. activation of one system activates the other. We compared the behavior of angiotensin II AT_1 and AT_2 receptors with β_1-and β_2-adrenergic receptors in a high-yield crude membrane fraction prepared from nonfailing and failing human ventricular myocardium. Ang II receptors were measured by ^{125}I saralasin binding, with B_{max} determined by saturation binding displaceable by 1 μM cold saralasin. AT_1 and AT_2 receptor fractions were determined by the amount of specific binding displaceable by 1 μM losartan. β_1-adrenergic receptor density was determined by saturation binding of ^{125}I ICYP, with the β_1 fraction determined by binding displaceable by 0.2 μM CGP 20712A. Results in end-stage human left ventricular myocardium failing as a result of idiopathic dilated cardiomyopathy were compared to nonfailing controls taken from age- and gender-matched organ donors not used for transplant because of blood type or body size mismatch: (Receptor density is in fmol/mg ± SEM)

	β_1-AR	β_2-AR	AT_1	AT_2
Nonfailing (n = 6)	59.0 ± 9.4	20.7 ± 4.0	4.14 ± 0.62	1.52 ± 0.43
Failing (n = 6)	28.3 ± 2.8*	17.2 ± 2.6	1.53 ± 0.57*	2.68 ± 0.51

*$p < 0.05$

The down-regulation of β_1 AR and AT_1 receptors was significantly related ($r = 0.62$, $n = 12$, $p < 0.05$)

Conclusions: (1) Compared to β adrenergic receptors ang II receptors are very low density in the human heart. (2) The AT_1 receptor subtype predominates in the nonfailing human heart. (3) AT_1 but not AT_2 receptors are down-regulated in failing heart. (4) Down-regulation of Ang-II AT_1 receptor is similar in degree to down-regulation of β_1-adrenergic receptors. These data suggest that the AT_1 and β_1 receptors are respectively exposed to increased concentrations of mutually activated/induced norepinephrine and Ang-II in the failing human heart.

8:45

776-2 Constitutive Expression of an Intracellular Form of Fibroblast Growth Factor Receptor Triggers Myogenic Differentiation

Anna Y. Hsu, Judith L. Swain *University of Pennsylvania, Philadelphia, PA*

Fibroblast growth factors (FGFs) have been demonstrated to regulate myogenic differentiation. Recently, forms of FGF have been generated that reside in the cytoplasm and appear to be biologically active, suggesting that an intracellular form of FGF may be able to transduce its signal through signaling pathways independent of its interaction with cell surface (transmembrane) FGF receptors (FGFR). We report in this study a potential mechanism by which such signaling can be modulated by an intracellular form of the receptor (I-FGFR). To generate this form of the receptor, the cDNA encoding the 2 IgG-like domain form of the murine transmembrane FGFR was mutated to remove its signal sequence and acidic box by PCR mutagenesis. This construct was then epitope-tagged to allow it to be distinguished from the endogenous surface FGF receptor. When the I-FGFR was transiently transfected into COS cells, a diffuse and reticular pattern was observed by indirect immunofluorescence microscopy, indicating cytoplasmic localization with association to organized intracellular structures, such as cytoskeleton or organellar membrane. To test for the biologic effect of the I-FGFR, stable cell lines containing the I-FGFR were generated in Sol 8 cells. Quadruplicate samples of four cell lines expressing high levels of the I-FGFR, and four control cell lines transfected with vector alone, were grown in mitogen poor medium (5% horse serum). Their ability to differentiate was assessed by their levels of creatine kinase activity normalized to total soluble protein (nmol NADPH/min/mg protein):

Days	0	1	2	3	4
Control	53 ± 71	55 ± 30	236 ± 60	759 ± 143	1295 ± 205
I-FGFR	47 ± 12	104 ± 32	391 ± 30*	1338 ± 312*	2143 ± 677*

*$p < 0.05$ for I-FGFR versus control

The results demonstrate that the intracellular form of the FGF receptor localizes to the cytoplasm of myocytes, and the increased expression of this receptor isoform triggers myogenic differentiation. We propose that this receptor promotes differentiation by binding endogenous FGF, and thus inhibiting the activity of FGF that normally preserves the undifferentiated state. The ability to generate and express the intracellular FGF receptor, therefore, may be a generally useful method of modulating FGF dependent regulation of proliferation and differentiation of other cell types such as smooth muscle and transformed cells.

9:00

776-3 Regulation of Basic Fibroblast Growth Factor Gene Expression in Rat Cardiac Microvascular Endothelial Cells and Adult Ventricular Myocytes

Thomas A. Fischer, Krishna Singh, Alborz Alali, Min-Ae Lee, David Kaye, Albert P. Gatbut, Jean Luc Balligand, Ralph A. Kelly, Thomas W. Smith *Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*

Basic fibroblast growth factor (bFGF) has been shown to stimulate protein synthesis in adult rat ventricular myocytes (ARVM) and DNA synthesis in microvascular endothelial cells (CMEC). To determine whether Angiotensin II (AII) and Endothelin I (ET-1), known as peptides affecting myocyte growth, regulate bFGF gene expression in the myocardium we examined the effects of these two peptide autacoids on bFGF mRNA abundance in both cardiac cell types. Baseline levels for bFGF mRNA in freshly isolated ARVM were 4 to 5 fold lower than in CMEC in RNase protection assays. AII (10^{-6} – 10^{-8} M) and ET-1 (10^{-8} – 10^{-10} M) increased bFGF mRNA abundance in CMEC and in ARVM in a dose dependent manner that was maximal 4–8 hours after treatment. This effect of AII was abolished after coincubation with the angiotensin II receptor antagonist (AT-1) losartan (10^{-6} M), and the ET-1 response was inhibited by the peptide endothelin type A (ET_A) antagonist BQ-123 (10^{-6} M). To determine whether bFGF itself autoregulates bFGF mRNA abundance, ARVM and CMEC were treated with rh bFGF (20ng/ml). A ninefold increase in bFGF mRNA was detected, thus indicating positive autoregulation. A marked induction of atrial natriuretic peptide mRNA, known as a molecular marker of phenotype dedifferentiation in hypertrophy, was observed after 24 and 48 hrs incubation with rh bFGF in ARVM. Finally, the inflammatory cytokine interleukin Iβ (rh IL-1β) upregulates bFGF gene expression in ARVM, a response that could be amplified by interferon γ (rm IFN-γ).

Thus, bFGF gene expression in ARVM and CMEC from rat heart is regulated by AII and ET-1 through the AT-1 receptor and (ET_A) receptor subtypes. The upregulation of bFGF gene transcription in response to various stimuli known to affect myocyte and endothelial growth suggest a role for bFGF as a signalling peptide in the hypertrophic response of adult ventricular myocytes and cardiac microvascular endothelial cells.

9:15

776-4 Intact cNOS-NO-cGMP Pathway in the Failing Human Heart

Chi-Ming Wei, John Lust, Zvonimir Katusic, Michihisa Jougasaki, Denise Heublein, Sharon Sandberg, Debra Lewis, Virginia Miller, Christopher McGregor, John Burnett, Jr. *Mayo Clinic, Rochester, MN*

Nitric oxide (NO) is a potent endothelium-derived relaxing factor which also may modulate cardiac myocyte inotropism and growth via increases in cGMP. While both the constitutive (cNOS) and inducible (iNOS) forms of nitric oxide synthase have been detected in mammalian hearts, the overall presence and activity of the cNOS-NO-cGMP pathway in the normal and failing human heart remains poorly defined. The present studies were designed to investigate the cNOS-NO-cGMP pathway in normal and failing human atrial and ventricular myocardium and to determine plasma NO and cGMP in the presence and absence of CHF. Myocardial tissue and plasma were obtained from five end-stage heart failure patients undergoing cardiac transplantation and five cardiac donors. Normal plasma NO and cGMP were also determined in normal humans without disease. cNOS production and localization were determined utilizing Northern blot analysis, in situ hybridization and immunohistochemistry with probes for endothelial-NOS and brain-NOS. Plasma and tissue NO were measured by nitrate determination utilizing chemiluminescence. Northern blot analysis and in situ demonstrated cNOS to be present and localized to atrial and ventricular myocytes in equal concentrations and distributions in normal and failing hearts. Tissue NO as determined by nitrate concentration was detectable and equal in normal and failing hearts while plasma NO concentration tended to be increased in CHF patients. Cardiac tissue cGMP paralleled tissue NO, although, plasma cGMP concentration was significantly increased in CHF patients compared with normal subjects. The present studies demonstrate that cNOS mRNA and cNOS protein are present in the normal human heart and this cNOS-NO-cGMP pathway is preserved in the failing human heart. These studies suggest that this paracrine and autocrine pathway may continue to function in the control of myocardial function in the failing human myocardium.

9:30

776-5 Mechanism of Decrease of Angiotensin II Receptor Message Through Dual Signal Transduction Pathways in Vascular Smooth Muscle

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We previously have shown that the angiotensin II receptor, type 1a ($AT_{1a}R$) message transiently decreases during ang II stimulation of cultured rat aortic vascular smooth muscle cells (VSMC); and, it has been previously reported by others that the mechanism of this decrease in cultured kidney cells is through cAMP dependent pathways. In this study we examined the signal transduction pathway of this regulation in quiescent VSMC. Total cellular RNA was harvested after time course exposure to reagents, Northern blotted and probed. $AT_{1a}R$ message abundance decreased beginning two hours after exposure to ang II (10 nM) and reached a minimum at 4 hours after stimulation of 25% of baseline level. The protein kinase C (PKC) stimulator, PMA (50 nM), caused a decrease in $AT_{1a}R$ message level with the same time course and to the same extent as ang II. Downregulation of PKC with 24 hour prior exposure to PDBU (500 nM) blocked the decrease in $AT_{1a}R$ message caused by PMA. However, pretreatment with either PDBU or the potent PKC inhibitor calphostin C (50 nM) did *not* block the decrease of $AT_{1a}R$ message level caused by ang II. In contrast, stimulation of cAMP with forskolin (50 mM), cholera toxin (10 ng/ml) or the soluble analog 8-bromocyclic AMP (1