	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

Stanley A. Rubin, Tim Bui, Lee Carter *VA Medical Center, Long Beach and University of California, Irvine, CA*

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