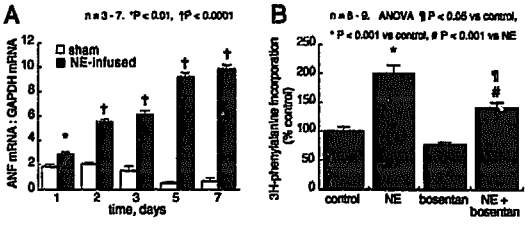
and molecular markers of hypertrophy, ventricular and non-cardiac expression of ET-1 mRNA and the effects of bosentan, an orally-active competitive ET_A and ET_B receptor antagonist without adrenoceptor antagonism. *Methods and Results* Initially, a rat model of ventricular hypertrophy due to continuous infusion of NE (600 μg/kg/h up to 7 days) by subcutaneous osmotic pumps was used. Male Sprague-Dawley rats (175–200 g, n = 70) were divided into 4 groups: 1. Sham-operated, 2. NE-infused, 3. Sham-operated given bosentan (100 mg/kg/day by gavage), 4. NE-infused given bosentan. NE caused a 35-fold increase in ventricular ET-1 mRNA within 1 day (sham – ET-1:GAPDH mRNA, 0.01 ± 0.01 at 1 day, n = 6 vs NE – ET-1:GAPDH mRNA, 0.35 ± 0.08, n = 8, P < 0.01), an effect not seen in lung, kidney or skeletal muscle. NE also caused significant increases in ventricular weight, RNA:protein, and expression of mRNAs for atrial natriuretic factor (ANF), beta-myosin heavy chain and skeletal alpha-actin, which in adult ventricle are indicators of hypertrophy (fig. A). Bosentan blocked NE-induced hypertrophy at 5 days. *In vitro*, NE increased expression of ET-1 mRNA by cultured ventricular myocytes and led to hypertrophy with increased cell size, sarcomerization and expression of ANF mRNA. Myocyte protein content and [3H]-phenylalanine incorporation also increased, effects blocked by 10 μM bosentan (fig. B). *Conclusion* ET-1 plays a direct role in mediating NE-induced hypertrophy with 'cross-talk' between these systems *in vivo* and *in vitro*.



2:30

408-3 Cis/Trans Regulation of Vascular Endothelial Growth Factor mRNA Stability by Hypoxia

Andrew P. Levy, Nina S. Levy, Mark A. Goldberg. *Boston University School of Medicine, Boston, MA*

Hypoxia has been shown to be an important stimulus for the formation of new blood vessels in the coronary collateral circulation in coronary artery disease. Vascular endothelial growth factor, a potent angiogenic factor, is regulated by hypoxia in vitro and in vivo. The major control point for the hypoxic induction of the VEGF gene is the regulation of the steady-state level of the mRNA which is determined by the relative rates of mRNA synthesis and decay. We previously demonstrated a discrepancy between the transcription rate and the steady-state mRNA level induced by hypoxia. This lead us to examine the post-transcriptional regulation of VEGF expression. Actinomycin D experiments revealed that hypoxia increased VEGF mRNA half-life from 43 ± 6 minutes to 106 ± 9 minutes. Using an in vitro mRNA degradation assay the half-life of VEGF mRNA 3′ untranslated region (UTR) transcripts were also found to be increased when incubated with hypoxic versus normoxic extracts. Both cis-regulatory elements involved in VEGF mRNA degradation under normoxic conditions and in increased stabilization under hypoxic conditions were mapped using this degradation assay. Hypoxia-induced proteins were identified by RNA electromobility shift assay that bound to the sequences in the VEGF 3′ UTR which mediated increased stability in the degradation assay. The binding site of one of these proteins was localized in the VEGF 3′ UTR within 20 bases of the nonameric sequence, UUAUUUAUU, previously shown to mediate the rapid degradation of multiple cytokine and oncogene mRNAs. This suggests a novel mechanism whereby these hypoxia-inducible proteins stabilize VEGF mRNA by interfering with the nonameric site binding to its cognate binding protein(s). Furthermore, genistein, a tyrosine kinase inhibitor which was recently shown to inhibit the hypoxic induction of VEGF mRNA through its action on src, blocked the hypoxia-induced stabilization of VEGF 3′ UTR transcripts and inhibited hypoxia-induced protein binding to the VEGF 3′ UTR while having no effect on the hypoxia-induced increase in transcription of the VEGF gene. This suggests a signal cascade through src/raf and MAP kinase that has as its terminal event a protein that binds to VEGF mRNA and mediates its hypoxic stabilization. An understanding of the molecular basis of the regulation of VEGF by hypoxia forms the essential groundwork for the rational design of pharmacological agents to increase VEGF expression and thereby augment neovascularization in regions of ischemic myocardium.

2:45

408-4 A Novel Atherogenic Activity of Lipoprotein(a): Induction of Monocyte Chemotactic Activity in Human Umbilical Vein Endothelial Cells

Michael Poon, Xiaoxia Zhang, Mark B. Taubman, Peter C. Harpel. *Mount Sinai School of Medicine, New York, NY*

Elevated levels of lipoprotein(a) (Lp(a)) are associated with increased risk of cardiovascular mortality and morbidity. Recruitment of monocytes to the blood vessel wall is an early event in the pathogenesis of atherosclerosis. We now report that Lp(a) induces the secretion of monocyte chemotactic activity (MCA) by human umbilical vein endothelial cells (HUVEC). MCA was assayed using a modified Boyden Chamber. Minimal MCA was produced by HUVEC incubated in serum-free medium. Lp(a) induced MCA in HUVEC beginning at 30 min and peaking at 2–3 hr. Peak levels were ≈80% of those seen with f-met-leu-phe, a potent monocyte chemoattractant. The induction of MCA was concentration dependent, with maximum MCA seen at 100 μg/ml of Lp(a). LDL from the same donor failed to induce MCA. In addition, Lp(a) had no direct chemotactic activity for monocytes. Polymyxin B failed to inhibit the stimulatory effect of Lp(a), suggesting that lipopolysaccharide was not the active agent. Checkerboard analysis documented that the activity presents in Lp(a)-conditioned medium was primarily chemotactic rather than chemokinetic. Concomitant treatment of HUVEC with Lp(a) and either 10 μM actinomycin D or cycloheximide completely inhibited the secretion of MCA into the culture medium, indicating that protein and RNA synthesis were required. It has been previously shown that oxidized LDL induces monocyte chemotactic protein-1 (MCP-1) mRNA and activity in human aortic endothelial cells. In the current study, Northern blot analysis showed that Lp(a) did not induce MCP-1 mRNA in HUVEC, indicating that Lp(a) may induce a chemoattractant other than MCP-1. These studies suggest that Lp(a) may be involved in the recruitment of monocytes to the vessel wall and provide a novel mechanism for the participation of Lp(a) in the atherogenic process.

3:00

408-5 Genetic Expression of Endothelial and Inducible Forms of Nitric Oxide Synthase in the Normal and Failing Human Hearts

Chi-Ming Wei, Shi-Wen Jiang, Richard C. Daly, Christopher G. A. McGregor. *Mayo Clinic, Rochester, MN*

Nitric oxide (NO) is a potent endothelium-derived relaxing factor which also may modulate cardiomyocyte inotropism and growth via increasing the cGMP level. While both the endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) isoforms have been detected in non-human mammalian tissues, expression and localization of the eNOS and iNOS in the normal and failing human heart are poorly defined. The present study was designed to investigate the eNOS and iNOS in human cardiac tissues in the presence and absence of congestive heart failure (CHF). Normal and failing atrial tissue were obtained from twelve cardiac donors and twelve end-stage heart failure patients undergoing cardiac transplantation. eNOS and iNOS expression and localization were investigated utilizing Northern blot analysis, in situ hybridization and immunohistochemistry. Northern blot analysis and in situ hybridization demonstrated similar levels of the eNOS and iNOS mRNA are present in cardiomyocytes from normal and failing hearts. Positive immunohistochemical staining was observed within the cytoplasm of cardiomyocytes. No significant different eNOS and iNOS immunoreactivities between normal and failing human myocardium were detected. The present studies demonstrated for the first time the genetic expression and distribution of eNOS and iNOS in the normal and failing human hearts. These studies suggest that the NOS mediated paracrine and autocrine pathway may continue to control the myocardial function in the failing human hearts.

Discussion 3:15