isolated and grown in cultures. In 12 experiments, cocaine, 10^{-8} to 10^{-6} M or vehicle-control, in the absence or presence of metoprolol or phentolamine, was added to each culture on day 2 and cells harvested on day 5. In 12 separate experiments, cocaine 10^{-6} M, cocaine 10^{-6} M plus the PKC inhibitor bisindolylmaleimide, 10^{-6} M, or vehicle were added to each culture for a mean of 5 mins. then cells were harvested. We determined myocyte total protein and myosin heavy chain protein content, and the presence of PKC isozymes in cytosol and particulate (nuclear) fractions. PKC translocation from cytosol to particulate fractions indicates PKC activation. **RESULTS**: Cocaine, 10^{-6} M, increased myocyte protein content by 28% ($p<0.001$) and fetal β-myosin heavy chain protein content/cell by 82% ($p<0.001$) but decreased adult α-myosin heavy chain protein content. Neither beta- nor alpha-adrenergic blockade inhibited this process. We found that adult ventricular myocytes contain alpha (α), delta, epsilon, and zeta PKC isozymes. Cocaine, 10^{-6} M, increased αPKC by 37% ($p<0.001$) in the particulate fraction, decreased αPKC in the cytosol fraction, and increased myocyte protein content by 22% ($p<0.01$) . In separate experiments, αPKC stimulated rat myocyte β-myosin heavy chain promoter and increased β-myosin heavy chain protein transcription 3-fold. Addition of bisindolylmaleimide, 10^{-6} M, to myocyte cultures, prevented the cocaine-induced translocation of αPKC to the particulate fraction and cocaine-induced increase in myocyte protein content. **CONCLUSIONS**: Cocaine increases myocyte protein content by PKC mechanisms. Protein kinase C translocation and nuclear transcription factor phosphorylation are important in the cardiac hypertrophy and myopathy resulting from chronic cocaine use.

1178-84 Cardiac Fibroblast Actions of Cardiotrophin-1 and Its Receptor Complex with Crosstalk With the Endothelin System In Vitro: Modulation by Early Dilated Cardiomyopathy

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Background: The cardiac interstitium and its fibrillar collagen matrix play a critical role in determining cardiac function and structure. Cardiotrophin-1 (CT-1) is a potent hypertrophic factor in cardiomyocytes, and its gene expression is up-regulated in severe heart failure. To date, the role of CT-1 on cardiac fibroblast function is unknown. This study was designed to clarify the actions of CT-1 with its receptors, glycoprotein 130 (gp130) and leukemia inhibitory factor receptor (LIFR), and elucidate possible crosstalk with the endothelin-1 (ET-1) /endothelin type A (ET_A) receptor axis in adult canine cardiac fibroblasts (CCF). In addition, we investigated the modulation of these proteins in ventricular myocardium from normal dogs and those with tachycardia-induced early dilated cardiomyopathy (EDCM).

Methods and Results: We assessed DNA synthesis of cardiac fibroblasts by [^{3}H]thymidine incorporation into cells. Recombinant CT-1 ($P<0.01$) stimulated DNA synthesis between 10^{-11} and 10^{-8} mol/L, with maximal effect at 10^{-8} mol/L (186%). Administration (10 μ/mL) of gp130 or LIF receptor antibody ($P<0.01$) completely inhibited not only CT-1 stimulated DNA synthesis in CCF but also ET-1 stimulated DNA synthesis. By contrast, 10^{-5} mol/L BQ123, an ET_A receptor antagonist, abolished CT-1 as well as ET-1 stimulated DNA synthesis. Western Blotting showed that 10^{-7} mol/L ET-1 stimulated the translocation of LIFR from cytosol to the cell membrane. CT-1 and gp130/LIFR in a canine model of EDCM produced by progressively increasing pacing rates (180 to 200 bpm for approximately 14-20 days) were characterized by immunohistochemistry as compared to normal ventricular myocardium. Staining revealed that CT-1 and gp130 were decreased and LIFR were enhanced.

Conclusions: This study demonstrates that CT-1 and its receptor complex are functionally important and contribute to cardiac fibroblast activation. In addition, CT-1 stimulation in CCF involves crosstalk with the gp130/LIF receptor complex and the ET-1/ ET_A receptor. The in vivo findings of decreased CT-1 protein and gp130 in a model of EDCM also suggest that an impairment of this axis could contribute to progressive cardiac dilatation.

1178-85 Oxidized LDL Through LOX-1 Increases the Expression of Angiotensin Converting Enzyme in Human Coronary Artery Endothelial Cells

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Background and objectives: Abnormalities of both renin-angiotensin system (RAS) and lipids play a critical role in the pathogenesis of cardiovascular diseases. Our previous studies demonstrate that angiotensin II (Ang II) and oxidized low-density lipoprotein (ox-LDL) in a synergistic fashion induce vascular endothelial injury. This study was conducted to examine the modulation of ACE gene expression by ox-LDL in human coronary artery endothelial cells (HCAECs).

Methods and results: HCAECs were cultured and incubated with ox-LDL (10 to 80 mg/ml) for 3-24 hours. Ox-LDL increased the expression of mRNA (determined by semi-quantitative RT-PCR) and protein (determined by Western blot) of ACE in a concentration- and time-dependent fashion. Native-LDL had no effect on the expression of ACE gene. These effects of ox-LDL was mediated by its endothelial receptor LOX-1, since pretreatment of HCAECs with the blocking antibody to LOX-1 (10 mg/ml) prevented the expression of ACE ($P<0.01$ vs. control) in response to ox-LDL. In parallel experiments, HCAECs were treated with the mitogen-activated protein kinase (MAPK) inhibitor (PD98059, 10 mM) for 30 min and then exposed to ox-LDL. MAPK inhibitor blocked the effects of ox-LDL on the expression of ACE ($P<0.01$ vs. ox-LDL group). In other experiments, we observed that pretreatment of HCAECs with simvastatin (10 mM) attenuated ox-LDL-induced ACE gene expression ($P<0.01$ vs. ox-LDL alone).

Conclusions: These observations provide a novel mechanism of interaction between RAS and ox-LDL (upregulation of ACE via LOX-1 activation). MAPK activation plays a critical signal transduction role in this process. Lastly, statins can modulate ACE expression by ox-LDL.

1178-86 Activation of the Tissue Endothelin System in Severe Polyglobulia

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We generated a novel erythropoietin overexpressing mouse, which reaches adulthood despite hematocrit levels of about 80%. The role of endothelin in severe polyglobulia, however, remains still to be determined. In this novel transgenic model of severe polyglobulia, we analyzed endothelin-1 (ET-1) promoter activity, ET-1 immuno-histochemistry, ET-1-protein tissue levels, ETA/B-receptor subtype mRNA expression and vascular reactivity to ET-1.

Transgenic polyglobulic animals xhibited increased ET-1 promoter activity ($P<0.05$ vs. wildtype controls) and ET-1 tissue levels in heart (265±36 vs. 165±25 pg/mg tissue; $P<0.05$ for left ventricle and 320±38 vs.170±31 pg/mg tissue; $P<0.05$ for right ventricle, respectively), renal cortex (783±67 vs. 395±32 pg/mg tissue; $P<0.01$), and aorta (2.2±0.3 vs. 0.5±0.1 pg/mg tissue; $P<0.01$). Aortic ET receptor subtype mRNA expression was enhanced in transgenic animals (1.5±0.2 vs.1.0±0.2 RU for ETA; 1.2±0.1 vs. 0.7±0.1 RU ($P<0.05$) for ETB receptor mRNA, respectively). Vascular reactivity to ET-1 was reduced (35±5% vs. 78±7% of KCl; $P<0.05$), but increased by preincubation with the nitric oxide (NO) synthase inhibitor L-NAME ($P<0.05$), thus indicating that the vascular effects of ET-1 are offset by NO, the endothelial counterpart of ET-1, in vivo. Correspondingly, treatment with the ETA receptor antagonist darusentan prolonged survival of transgenic mice exposed to the NO synthase inhibitor L-NAME ($p<0.01$). We here for the first time demonstrate that polyglobulia induces a marked activation of the tissue endothelin system. Since treatment with darusentan prolonged survival after acute NO blockade, activation of the tissue endothelin system may thus advance as a new target in the treatment of cardiovascular disease associated with severe polyglobulia.

POSTER SESSION

1179 Improving Screening and Lipid Treatment to Prevent Coronary Heart Disease

Tuesday, March 19, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: Noon-1:00 p.m.

1179-75 Calculated LDL Cholesterols Frequently Underestimate Directly Measured LDL Determinations in Patients With Triglyceride Levels ≤400 mg/dL

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Elevated low density lipoprotein (LDL) cholesterol is a risk factor for development of coronary artery disease (CAD). Recent guidelines detail specific LDL cutpoints for risk assessment. In clinical practice LDL concentration is usually calculated (C-LDL) from the Friedewald formula (FF). **Methods:** To assess the accuracy of C-LDL by the FF compared to directly measured LDL (D-LDL), we analyzed 1618 fasting lipid samples obtained in 661 patients (pts) [mean age 46±12 yr, 52% female] without overt CAD. Pts with triglycerides (TG) >400 mg/dL were excluded. **Results:** Mean total cholesterol was 246±41, HDL 52±15, and TG 147±77 mg/dL. C-LDL and D-LDL were significantly different (165±34 vs 187±41, $p<0.0001$) and D-LDL exceeded C-LDL in 93% of measurements. Although C-LDL and D-LDL were related ($r=0.90$), the discrepancy between C-LDL and D-LDL (LDL-difference) increased linearly with TG ($r=0.67$, figure) and clinically important differences existed at normal or slightly elevated TG levels. Regression analysis indicated that this LDL difference increased by 17 mg/dL for every 100 mg/dL TG increase. **Conclusions:** (1) Significant differences between C-LDL and D-LDL existed in pts with TG ≤400 mg/dl. (2) These differences were related to TG. (3) C-LDL by the FF may be unreliable to accurately categorize cardiac risk.