isolated and grown in cultures. In 12 experiments, cocaine, 10^{-5} M to 10^{-2} M or vehicle- control, in the absence or presence of metoprolol or temperature, was added to each culture on day 2 and cells were harvested on day 5. In 12 separate experiments, cocaine, 10^{-5} M, cocaine, 10^{-3} M plus the PKC inhibitor bisindolylmaleimide, 10^{-6} M, or vehicle were added to each culture for a mean of 5 min. then cells were harvested. We determined myocyte total protein and myosin heavy chain protein content, and the presence of PKC isoforms in cytosol and particulate (nuclear) fractions. PKC translocation from cytosol to particulate fractions indicates PKC activation. RESULTS: Cocaine, 10^{-5} M, increased myocyte protein content by 28% (P<0.01) and 51% myosin heavy chain protein content (P<0.01) but decreased alpha myosin heavy chain protein content. Neither beta- nor alpha-adrenergic blockade inhibited this process. We found that adult ventricular myocytes contain alpha (a), delta, epsilon, and zeta PKC isoforms. Cocaine, 10^{-5} M, increased uPKC by 37% (P<0.001) in the particulate fraction, decreased aPKC in the cytosolic fraction, and increased myocyte protein content by 22% (P<0.01). In separate experiments, aPKC stimulation mimicked heavy chain promoter and increased 51% myosin heavy chain protein transcription 3-fold. Addition of bisindolylmaleimide, 10^{-6} M, to myocyte cultures prevented the cocain-induced translocation of aPKC to the particulate fraction and cocain-induced increases in myosin 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.

Cardiac Fibroblast Actions of Cardiotrophin-1 and its Receptor Complex with CrossTalk With the Endothelin System In Vitro: Modulation by Early Dilated Cardiomyopathy


Background: The cardiac interstitium and its fibrillar collagen matrix play a critical role in determining cardiac function and structure. Cardiomyopathy (CT-1) is a potent hypertrophic stimulus 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 on its receptors, glycoprotein 130 (gp130) and leukemia inhibitory factor receptor (LIFR), and elucidate possible crosstalk with the endothelin-1 (ET-1) endothelin type A (ETA) 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 [3H]thymidine incorporation into cells. Recombinant CT-1 (P<0.01) stimulated DNA synthesis by 10^{-5} M and 10^{-6} M, with maximal effect at 10^{-6} M (186%). Administration (10 pM/L) 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} M, B2212, an ETA receptor antagonist, abolished CT-1 but was ineffective as well as ET-1 stimulated DNA synthesis. Western Blotting showed that 10^{-6} M, 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 immunohistoechemistry as compared to normal ventricular myocardium. Staining revealed that CT-1 and gp130 were increased in affen with EDCM.

Conclusion: 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/ETA receptor. In 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.

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


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 semiquantitative RT-PCR) of ox-LDL receptor (ACE) in a concentration-dependent fashion. Ox-LDL increased the expression of mRNA (determined by semiquantitative RT-PCR) of ACE in a concentration- and time-dependent fashion. Native-LDL had no effect on the expression of ACE.

Conclusion: These observations provide a novel mechanism of interaction between