1133-139 **Low Dose of Propranolol Prevents the Development of Heart Failure by Restoring the Defective Interaction of FKBP12.6 With Cardiac Ryanodine Receptor**

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Background. In heart failure, hyperphosphorylation of ryanodine receptor (RyR) mediated through PKA has been shown to cause dissociation of FKBP12.6 from RyR, resulting in an abnormal Ca^{2+} leak through RyR and possibly consequent cardiac dysfunction. Here, we assessed whether β-blockade can restore this defective channel function of RyR and therefore improve cardiac function in heart failure. ***Methods and Results.*** Sarcoplasmic reticulum (SR) was isolated from dog LV muscles {normal (N), n=5; 4-weeks RV pacing with or without propranolol [P(+): n=4, P(-): n=5, respectively]}. In normal dogs, the dose of propranolol (0.05 mg/kg/day, iv) decreased heart rate at baseline by 14 %, but did not attenuate the isoproterenol (0.8 μg/kg/min)-induced increase in peak +dP/dt of LV pressure. 1) As compared with pre-RV pacing, both end-diastolic [36.2mm in P(+) versus 41.9mm in P(-), p<0.05] and end-systolic diameter [29.4mm in P(+) versus 37.5mm in P(-), p<0.05] were less increased in P(+) than P(-), associated with lesser decrease in fractional shortening [19.0% in P(+) versus 10.2% in P(-), p<0.05]. 2) In SR from P(-), a prominent Ca^{2+} leak was observed and FK506 that dissociates FKBP12.6 from RyR did not induce further Ca^{2+} leak because of a partial loss of FKBP12.6 from RyR. However, there was no appreciable Ca^{2+} leak in SR from P(+), and FK506-induced Ca^{2+} leak was elicited like normal SR. 3) RyR was labeled in a site-directed fashion with the fluorescent conformational probe methylcoumarin acetate (MCA). In SR from P(+), the FK506-induced increase in MCA fluorescence, which was virtually absent in SR from P(-), was observed like in normal SR. 4) Indeed, both stoichiometry of FKBP12.6 versus RyR assessed by [^{3}H]FK506 and [^{3}H]ryanodine-binding assays [3.6 : 1 in N, 1.1 : 1 in P(-), 2.4 : 1 in P(+); p<0.05] and protein expression of FKBP12.6 assessed by Western Blot analysis were restored towards those in normal SR.
Conclusions. Low dose of propranolol attenuated LV remodeling presumably by ameliorating the defective interaction of FKBP12.6 with RyR, presumably resulting in an inhibition of intracellular Ca^{2+} overload and hence a prevention of the development of heart failure.

1133-140 **Chronic Therapy With Metoprolol CR/XL Prevents Apoptosis Inducing Factor and Downregulates the Pro-Apoptotic Protein Bak in Cardiomyocytes of Dogs With Heart Failure**

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Background: Chronic therapy with beta-adrenergic receptor antagonists in heart failure (HF) has been shown to attenuate cardiomyocyte apoptosis. We previously showed that beta-blockers also downregulate the expression of active caspase-3, a key enzyme that promotes nuclear DNA fragmentation, a hallmark of programmed cell death. Activation of caspase-3 is regulated, in part, by apoptosis inducing factor (AIF), a mitochondrial protein, which is activated by pro-apoptotic members of the Bcl-2 family that include Bak. In the present study, we examined the effects of chronic therapy with metoprolol CR/XL (Toprol-XL) on the expression of AIF and Bak in cardiomyocytes of dogs with chronic HF.
Methods: HF was produced in 14 dogs by intracoronary microembolizations. Dogs were randomized to 3 months of monotherapy with Toprol-XL (100 mg once daily, n=7) or to no therapy at all (control, n=7). At the end of 3 months of therapy, dogs were sacrificed, and cardiomyocytes were enzymatically isolated from the LV free wall. Cardiomyocytes were also isolated from the LV free wall of 5 normal (NL) dogs and were used for comparisons. Expression of AIF was examined with Western blots using cytosolic fraction prepared from cardiomyocyte homogenate. Expression of Bak was also examined with Western blots using cardiomyocyte homogenate. Bands were quantified in densitometric units.
Results: Expression of Bak was significantly increased in cardiomyocytes isolated from untreated HF dogs compared to NL (6.3 ± 0.6 vs. 0.5 ± 0.1, P<0.05). Treatment with Toprol-XL significantly decreased the expression of Bak (1.7 ± 0.24) compared to untreated controls (P<0.05). Expression of AIF was significantly increased in cardiomyocytes isolated from untreated HF dogs compared to NL (6.3 ± 0.4 vs. 4.3 ± 0.2, P<0.05). Treatment with Toprol-XL significantly decreased the expression of AIF (3.1 ± 0.5) compared to untreated control (P<0.05).
Conclusions: AIF and Bak are upregulated in cardiomyocytes of dogs with HF. Chronic therapy with Toprol-XL attenuates the upregulation of both AIF and Bak. These data provide further support that long-term therapy with beta-blockers limits apoptosis-mediated ongoing cardiomyocyte loss in HF.

1133-161 **Negative Modulation of Beta3-Adrenergic Stimulation on Cardiomyocyte Contractile Performance and [Ca2+]i Regulation Before and After Heart Failure: Insights Into the Underlying Cellular Mechanisms**

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Background: We have previously reported that β_3-adrenergic receptor (AR) stimulation produces direct inhibition on LV contraction in conscious dogs before and after pacing-induced heart failure (CHF).
Methods: To define the cellular mechanism, we assessed cell contraction, relaxation, $[Ca^{2+}]_i$ transient and Ca^{2+} current ($I_{Ca,L}$) responses to BRL-37344 (BRL), a β_3-AR agonist in freshly isolated LV cardiomyocytes obtained from 7 instrumented dogs before and after pacing-induced CHF.
Results: In normal myocytes, BRL (10^{-8}M) caused significant decrease in cell contraction measured as the percent shortening (SA, -29%, 9.1 vs 12.9%), the peak velocity shortening (dL/dt_{max}, -13%, 164.3 vs 189.3 μm/sec) and relengthening (dR/dt_{max}, -17%, 122.1 vs 146.3 μm/sec) with parallel decreases in $[Ca^{2+}]_i$ transient (-22%, 277.2 vs 331.3 nM) and $I_{Ca,L}$ (-25%, 2.7 vs 3.6 pA/pF). In CHF myocytes, BRL produced much greater decreases in cell contraction and relaxation (SA, -46%, 4.1 vs 7.6%; dL/dt_{max}, -25%, 63.3 vs 84.7 μm/sec; dR/dt_{max}, -24%, 67.1 vs 88.4 μm/sec) with associated significantly greater reductions in peak $[Ca^{2+}]_i$ transient (-32%, 181.3 vs 270.1 nM) and $I_{Ca,L}$ (-37%, 2.0 vs 3.2 pA/pF). These BRL-induced responses were not modified by pretreating myocytes with nadolol (10^{-5}M), a β_1- and β_2-AR antagonist, but were prevented by bupranolol (10^{-6}M), a β_3-antagonist. These responses were also nearly abolished by pretreating myocytes with PTX (2 μg/ml, 6 hrs, 36°C) and dibutyryl-cAMP ($5x10^{-4}$M). In contrast, BRL-induced decrease in SA (-12% vs -29%) was significantly attenuated by pretreatment with NOS inhibitor, L-NAME (10^{-4}M, 30 min), but the BRL caused decreases in $[Ca^{2+}]_i$ transient and $I_{Ca,L}$ were not significantly altered.
Conclusion: β_3-AR stimulation produces negative inotropic action in both normal and CHF myocytes due to decreased $[Ca^{2+}]_i$ transient, $I_{Ca,L}$, and myocyte Ca^{2+} sensitivity. These effects are likely to be mediated through both NO-cAMP dependent and NOS-independent mechanisms that is coupled with PTX-sensitive G protein pathway and may involve a decreased level of cAMP.

1133-162 **The Putative Beta Four-Adrenergic Receptor Is a Novel State of the Beta One-Adrenergic Receptor**

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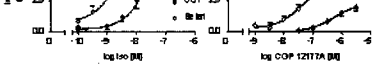
Background: In human cardiac tissue, there is evidence from functional, second messenger and radioligand binding studies of a novel Gs-coupled third cardiostimulatory receptor: the putative beta 4-adrenoceptor (β4AR). β4AR effects are defined by the non-conventional partial agonist CGP 12177 (CGP) and include positive inotropy, lusitropy and chronotropy in heart. Recent evidence suggests that the β4AR may be a novel conformation of the β1AR protein. We have examined the effect of β1AR overexpression in adult rat cardiomyocytes on the inotropic responses of isoprenaline (ISO) and CGP.
Methods: Myocytes were transfected with adenovirus containing sequence for the human β1AR. β1AR density was measured by [125I]-iodocyanopindolol binding to ventricular myocyte membranes. Inotropic responses to ISO and CGP (in the presence of propranolol) were studied 48 h after transfection by measuring cell shortening in electrically stimulated ventricular myocytes.
Results: Binding confirmed an 18-fold increase in ventricular β1AR density. There was a parallel left shift of the concentration-response curve (CRC) to ISO (control EC50 19nM (n=21), β1AR transfected 1.7nM (n=20), p<0.0005). There was also a left shift of CRC to CGP (EC50 595nM (n=16) and 69.8nM (n=22) respectively, p<0.005) as well as an increase in maximum response.



Conclusions: The similar magnitude of the decreases in EC50 to ISO and CGP following β1AR overexpression further supports the hypothesis that the β4AR is a novel conformation of the β1AR.

POSTER SESSION

1134 Heart Failure Trials and Outcomes

Monday, March 18, 2002, 3:00 p.m.-5:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: 3:00 p.m.-4:00 p.m.

1134-146 **Bone Loss and Prevention of Osteoporosis in Congestive Heart Failure**

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Background: Cross sectional studies have shown that more than 50 % of the patients (pts.) with congestive heart failure (CHF) have decreased bone mineral density (BMD). There is limited knowledge about the longitudinal changes of BMD and no prevention studies of osteoporosis in pts. with CHF. Methods: The present study was a prospective, longitudinal, randomized trial in which 34 pts. with CHF were assigned to 1000mg calcium supplementation (17m; 51,1±9,1 years; EF 32±12%) or no osteoporosis prevention therapy (15m,2w; 48,6±11,9 years; EF 29±9%). BMD (g/cm2; t- and z-score) was measured at lumbar spine (LS) and femoral neck (FN) by dual-energy-X-ray-absorptiometry (Lunar Expert) at baseline and after 12 months. Fractures were assessed by X-ray of chest, thoracic and lumbar spine. Biochemical analysis included parameters of calcium metabolism, renal function, gonadal hormones and intact parathyroid hormone (iPTH). Results: Osteopenia (41% in the LS, 35% in the FN) and osteoporosis (15 % in the LS, 6 % in the FN) were frequently seen in these pts.. 47% of the pts. showed an impaired renal function, 30% a secondary hyperparathyroidism and 32% a hypogonadism. Pts.