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

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Background: In heart failure, hyperphosphorylation of ryosodine receptor (RyR) mediated through PKA has been shown to cause dissociation of FKBP12.6 from RyR, resulting in an abnormal Ca2+ leak through RyR and possibly consequent cardiac dysfunction. Here, we assessed whether 3-blockade can restore this defective channel function of RyR and therefore improve cardiac function in heart failure. Methods and Results. Sarcolemmal reticum (SR) was isolated from dog LV muscles (normal N=5; n=3; 4-weeks RR 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% (P(+) 114±7 bpm vs P(-) 133±15 bpm, p<0.05). In hypertrophied (8% increase in left ventricular weight) dogs, propranolol-induced increase in peak +dP/dt of LV pressure. 1) As compared with pre-RV pacing, both end-diastolic (36.2 mm in P(+) versus 41.4 mm in P(-), p<0.05) and end-systolic diameter (29.4 mm in P(+) versus 41.9 mm 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 Ca2+ leak was observed and FK506 that dissociates FKBP12.6 decreased in fractional shortening [19.0% in P(+) versus 10.2% in P(-), p<0.05]. 2) In SR from P(-), was observed like normal SR. 3) RyR was labeled in a sita-directed fashion with the fluorescent conformational probe methylcytocarimcin (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 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 attenuation of intracellular Ca2+ 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


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 heart failure.

Methods: Cardiomyocytes were isolated from the LV of 14 dogs by enzymatic digestion and microdissection. 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 assayed for apoptosis by fluorescence-activated cell sorting (FACS). AIF and Bak expression were evaluated by Western blot analysis.

Results: Expression of AIF was examined with Western blots using cytosolic fraction prepared from cardiomyocytes homogenate. Expression of Bak was also examined with Western blots using cardiomyocyte homogenate. Bands were quantified in densitometric units.

Conclusions: Expression of Bak was significantly increased in cardiomyocytes isolated from untreated HF dogs compared to NL (6.3 ± 0.6 vs. 5.2 ± 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 beta3-adrenergic receptor (AR) stimulation produces direct inhibition on [Ca2+]i contraction in conscious dogs before and after pacing-induced cardiac failure (CHF).

Methods: To define the cellular mechanism, we assessed cell contraction, relaxation, [Ca2+]i transient and Ca2+ current (Ica,L) responses to BRL-37344 (BRL), a beta3-AR agonist in freshly isolated LV cardiomyocytes obtained from 7 instrumented dogs before and after pacing-induced CHF.

Results: In normal myocytes, BRL (10^{-6}M) caused significant decrease in cell contractility measured as the percent shortening (SA, -29%, 9.1 vs 12.9%), the peak velocity shortening (dL/dtmax, -13%, 184.3 vs 193.9 mm/sec) and re-lengthening (dR/dtmax, -17%, 122.1 ± 146.3 mm/sec) with parallel decreases in [Ca2+]i transient (-22%, 277.2 ± 331.3 nM) and Ica,L (-25%, 2.7 ± 3.6 pA/F). In CHF myocytes, BRL produced much greater decreases in cell contractility and relaxation (SA, -46%, 4.1 vs 7.9%; dL/dtmax, -25.3 vs 63.9 ± 84.7 mm/sec; dR/dtmax, -67.1 ± 86.4 mm/sec) with associated significantly greater reductions in peak [Ca2+]i transient (-32%, 181.3 ± 270.1 nM) and Ica,L (-37%, 2.3 ± 3.2 pA/F). These BRL-induced responses were not modulated by pretreating myocytes with nolodil (10^{-5}M), a beta1- and beta2-AR antagonist, but were prevented by bupranolol (10^{-5}M), a beta3-AR antagonist. These responses were also nearly abolished by pretreating myocytes with PTX (2 uM), a Gs inhibitor, and dibutyryl-AMP (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 [Ca2+]i transient and Ica,L were not significantly altered.

Conclusions: beta3-AR stimulation produces negative inotropic action in both normal and CHF myocytes due to decreased [Ca2+]i transient, Ica,L, and myocyte Ca2+ sensitivity. These effects are likely to be mediated through both NO-CAMP dependent and NOS-independent mechanisms that are 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, biochemical and radioligand binding studies of a novel Gs-coupled third cardiostimulatory receptor: the putative beta 4-adrenoceptor (~4AR). ~4AR effects are defined by the non-conventional partial agonist CGP 21177 (CGP) and include positive inotropy, lusitropy and chronotropy in heart. Recent evidence suggests that the ~4AR may be a novel conformation of the ~1AR protein. We have examined the effect of ~1AR overexpression in adult rat cardiomyocytes on the inotropic responses of isoproterenol (ISO) and CGP.

Methods: Myocytes were transfected with adenosine containing sequence for the human ~1AR, ~1AR density was measured by [125I]-iodocyanopindolol binding to ventricular myooyte membranes. Inotropic responses to ISO and CGP (in the presence of propranolol) were studied in 48 h after transfection by measuring cell shortening in electrically stimulated ventricular myocytes.

Results: Binding confirmed an 18-fold increase in ventricular ~1AR density. There was a parallel left shift of the concentration-response curve (CRC) to ISO (control EC50 19nM n=21, ~1AR transfected 1.7nM n=20, p<0.0005). There was also a left shift of CRC to CGP (EC50 595nM n=16 and 69.8 nM 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 ~1AR overexpression further supports the hypothesis that the ~4AR is a novel conformation of the ~1AR.