The Role of Microdomains in Beta-Adrenoceptor Signalling

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Metoprolol induces cardiac beta-3 adrenergic receptor and Sphinogosine 1 phosphate receptor 1 signals to prevent adverse Left-ventricle remodeling and dysfunction after myocardial infarction.

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Background: β-adrenergic receptor (AR)-blockers are front-line therapies against myocardial in-function (MI)-induced and other forms of heart failure (HF). Mechanisms accounting for these benefi-cial effects remain however only partially understood. In particular, due to the difference in receptor targeting and to the great variability in human HF-patients response, the specific mechanism of action of β-blockers is still under investigation. We have recently demonstrated, in an animal model of HF, that a reciprocal down-regulation occurs between βAR and the cardioprotective sphingosine-1-phosphate (SIP) receptor-1 (S1PR1). Purpose: Hence, we hypothesize that, in addition to salutary actions due to direct βAR blockade, agents such as metoprolol improve post-MI structural and func-tional outcome via restored protective S1PR1 signal, and we sought to determine mechanisms ac-counting for this effect.

Methods and Results: In HEK293 cells and in vitro cardiomyocytes, metoprolol (Meto) pre-vented isoproterenol (βAR agonist)-dependent S1PR1 down-regulation. Treatment of infarcted mice with Meto or Sip (one week after MI for 3 weeks) markedly ameliorated cardiac function and prevented remodeling, while preserving cardiac plasma membrane S1PR1 whose levels were down-regulated in untreated MI mice. Next, we co-infused infarcted mice with SIP and Meto, and found no additional beneficial effects. Since previous evidence attests that Meto can increase cardiac βARs levels and activity, and this receptor in adipocytes is responsible for S1P secretion, we mea-sured basal and Meto-stimulated cardiac Sphingosine kinase 1 (SphK1), the enzyme responsible for S1P secretion, and circulations S1P levels in βAR KO mice. These animals displayed markedly reduced levels of both, not rescued by Meto. Importantly, the βAR blocker did not ameliorate post-MI dys-function in βAR KO mice.

Conclusions: βAR-blockers enhance βAR-signaling, promoting the secretion of S1P that, in turn, acti-vates the S1PR1 signaling. These signaling interactions represent a previously unrecognized mech-anism whereby βAR blockers prevent post-MI decompensation and adverse remodeling.

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PDE8 is a novel regulator of AMP signaling in human atrial fibrillation

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Purpose: Atrial fibrillation (AF) is associated with reduced L-type Ca2+ current (ICa,L) and altered Ca2+-dependent signal transduction. Cyclic nucleotide phosphodiesterases (PDEs) degrade cAMP and regu-late cAMP-mediated PKA-dependent phosphorylation of various proteins, including ICa,L channel subunits. PDE1 is the main PDE isozyme hydrolyzing cAMP in heart, but recent studies demon-strate the existence of a novel isoform PDE8 in ventricle. Here we assess the expression and localiza-tion of PDE8 in human atria of patients with sinus rhythm (SR), paroxysmal AF (pAF), and longstanding persistent (chronic) AF (cAF).

Methods: mRNA (RT-qPCR) and protein (Western blot) levels of PDE8A and PDE8B isoforms were assessed in right atria of SR, pAF and cAF patients. Localization of PDE8A and PDE8B in human atrial cardiomyocytes was determined by immunofluorescence. Protein-protein interaction between ICa,L channel subunit and PDE8B was studied by co-immunoprecipitation in the three rhythm groups.

Results: PDE8B mRNA is present in human atrium and increases in both types of AF (Act SR=0.84 ± 0.03 n=15 vs pAF=1.03 ± 0.03 n=18 and cAF= 1.6 ± 0.05 n, p<0.01, ANOVA). GAPDH-normalized protein expression levels of PDE8A were 2-fold higher in pAF, but unaltered in cAF patients. Conversely, PDE8B protein abundance was increased by ~77% in cAF only (p<0.05). Immunostaining confirmed the presence of PDE8A and PDE8B in human atrial cardiomyo-cytes, with PDE8A being localized in the cytosol, and PDE8B preferentially localized at the plasma membrane. Immunoprecipitation of ICa,L α1C subunit resulted in strongly enhanced co-immunoprecipitation of PDE8B in cAF, but not pAF (PDE8B/ICa,L ratio, SR=0.31 ± 0.28 n=5, pAF =0.14 ± 0.08 n=4, cAF mean= 0.47 ± 2.21 n=5, p<0.05, ANOVA), identifying PDE8B as part of the ICa,L channel complex and pointing to potential contribution of PDE8B to reduced ICa,L in cAF.

Conclusions: Our results show for the first time that PDE8B and β are expressed in human atrium. PDE8B localizes at the plasma membrane of human atrial cardiomyocytes, and upregulates and accu-mulates in the ICa,L channel complex of AF patients. PDE8B may constitute a novel regulator of atrial cCa,L, with potential implications for AF pathophysiology.

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B-blocker therapy in heart failure reduces migratory and proliferative properties of primarily cultured failing cardiac fibroblasts via reduction of g protein-coupled receptor kinase-2 expression

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Introduction: Cardiac remodeling is a cardinal process mediating the progression of heart failure (HF). Cardiac fibroblasts (CFs) play a critical role in the regulation of left ventricular remodeling fol-lowing myocardial infarction (MI), mainly through differentiation to a myofibroblast phenotype that expresses increased migratory and proliferative properties. Among several therapeutic effects of β-blocker on cardiac function, this class of drugs is known to slow the process of cardiac remodeling and to reduce cardiac fibrosis. G protein-coupled receptor kinase-2 (GRK2) is involved in the mech-anism of maladaptive ventricular remodeling and regulates CF function. Indeed, this kinase has been shown to be upregulated and to induce βAR uncoupling in CFs extracted from human failing hearts.

Purpose: The aim of the present study was to evaluate the effects of β-blocker therapy on CF ac-tivation and on GRK2 levels in HF.

Methods and Results: MI was surgically induced in mice and, 4 weeks later, mice were randomized to receive either Metoprolol (Meto) or vehicle for 4 additional weeks. Sham-operated mice were also included. Echocardiography, performed after 4 weeks of treatment, revealed that Meto was able to prevent cardiac functional deterioration and remodeling observed in HF control mice. Moreover, β-blocker therapy resulted in reduced cardiac apoptosis and fibrosis. At the end of the study period, GRK2 was markedly increased in CFs extracted from HF mice compared to those extracted from sham. Importantly, Met treatment significantly limited MI-related GRK2 upregulation in CFs. Next, we assessed proliferation and migration in primarily cultured CFs extracted from the 3 study groups. Proliferation and migration were robustly increased in CFs extracted from HF controls compared to sham. Met treatment resulted in a normalization of both proliferation and migration of failing CFs. Further-more, we extracted CF plasma membranes obtained from untreated failing hearts and we ob-served a robust βAR down-regulation compared to CFs extracted from sham. Importantly, Met treatment resulted in a complete restoration of α1AR-mediated GRK2 dysfunction observed in failing CFs. Finally, in vitro overexpression of GRK2 in Meto-treated failing CFs completely abolished the beneficial effects of β-blocker therapy on CF, recapitulating a HF phenotype with increased prolifer-ation and migration.

Conclusions: GRK2 appears to have a relevant role in regulating CF proliferation and migration and reduced expression of this kinase may be an important mechanism of the positive effects of β-blocker therapy on HF-related maladaptive remodeling mediated by CFs.