cardiomyocyte damage following hypoxia/reoxygenation by modulating mitochondrial bioenergetics. The aim of the current study was to examine the effect of DHSB on isolated perfused rat heart and cardiomyocyte cell culture.
Langendorff perfused rat hearts were used to investigate the response of Left ventricular developed pressure (LVDP), end-diastolic pressure (LVEDP) and contractility (dP/dt_{max}) to exposure to 10μM DHSB, 10μM quercetin, with 100nM bradykinin as a positive control. In vitro testing of mitochondrial network and involvement of adrenergic receptors was performed on cardiomyocyte cell cultures. Activation of signaling pathways in culture models was assessed via a CREB-Luciferase reporter system, western blotting and fluorescent Ca^{2+} probes.
Quercetin treated hearts demonstrated no significant changes from controls. Bradykinin, as expected, caused vasodilation. DHSB, on the other hand, caused a prompt and dynamic elevation of LVEDP, heart rate and contractility along with enlargement and hardening of the organ and ruffling of the ventricular surface during perfusion. Contractility and heart rate were restored during washout in the DHSB group, while heart rate was reduced to half of the initial value. Pretreatment with propranolol abolished DHSB induced elevation of heart rate, LVEDP and contractility but not morphological changes. Western blots of signalosome fractions indicate that DHSB triggers signalosome formation. Morphological changes are also evident in the mitochondrial network. CREB reporter assays indicate that DHSB enhances transcription from the CRE promoter. Additionally, DHSB enhances isoproterenol, but not norepinephrine induced transcription from the CRE promoter. This effect was not inhibited by propranolol. The results suggest that DHSB interacts with the adrenergic signaling pathway, but not at the level of the adrenergic receptors.
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Inhibition of Camp-Dependent PKA Activates β2-Adrenergic Receptor Stimulation of Cytosolic Phospholipase A2 via Raf-1/Mek/Erk and Ip3-Dependent Ca^{2+} Signaling in Atrial Myocytes
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We previously reported in atrial myocytes that inhibition of PKA by laminin (LMN)-integrin signaling activates β2-adrenergic receptor (β2-AR) stimulation of cytosolic phospholipase A2 (cPLA2). Here, we determined the effects of zinterol (0.1 μM; zint-β2-AR) to stimulate ICa,L in atrial myocytes in the absence (+PKA) and presence (-PKA) of the PKA inhibitor (1 μM) KT5720 and compared these results with atrial myocytes attached to laminin (+LMN). Inhibition of Raf-1 (10 μM GW5074), phospholipase C (PLC; 0.5 μM edelfosine), PKC (4 μM chelerythrine) or IP3 receptor (IP3R) signaling (2 μM 2-APB) significantly inhibited zint-β2-AR stimulation of ICa,L in -PKA but not +PKA myocytes. Western blots showed that zint-β2-AR stimulation increased ERK1/2 phosphorylation in -PKA compared to +PKA myocytes. Adenoviral (Adv) expression of dominant negative (dn) -PKCα, dn-Raf-1 or an IP3 affinity trap, each inhibited zint-β2-AR stimulation of ICa,L in + LMN myocytes compared to control +LMN myocytes infected with Adv-βgal. In +LMN myocytes, zint-β2-AR stimulation of ICa,L was enhanced by adenoviral overexpression of wild-type cPLA2 and inhibited by double dn-cPLA2S505A/S515A mutant compared to control +LMN myocytes infected with Adv-βgal. In -PKA myocytes depletion of intracellular Ca^{2+} stores by 5 μM thapsigargin failed to inhibit zint-β2-AR stimulation of ICa,L via cPLA2. However, disruption of caveolae formation by 10 mM methyl-β-cyclodextrin inhibited zint-β2-AR stimulation of ICa,L in -PKA myocytes significantly more than in +PKA myocytes. We conclude that inhibition of PKA removes inhibition of Raf-1 and thereby allows β2-AR stimulation to act via PKCα/Raf-1/MEK/ERK1/2 and IP3-mediated Ca^{2+} signaling to stimulate cPLA2 signaling within caveolae. These findings may be relevant to the remodeling of β-AR signaling in failing and/or aging heart, both of which exhibit decreases in adenylate cyclase activity.

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Cell Proliferation and Migration Induced by Angiotensin-II is Mediated by ACE
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We have demonstrated that the Angiotensin I Converting Enzyme (ACE) acts as a membrane receptor for Angiotensin II (AngII). Upon binding to ACE with high affinity, AngII activates a signaling cascade that generates inositol 1,4,5-triphosphate (InsP3), culminating in intracellular Ca^{2+} increase. The goal of this work was to investigate cellular function that AngII plays through ACE activation. For that, we used CHO-ACE, CHO-AT1 and melanoma cell line (Tm-5), as well as, confocal microscopy, immunofluorescence, western blotting, small interfering RNA (siRNA) and BrDU assay. We found that AngII binds to ACE and this complex is internalized through clathrin, to trigger preferential nucleoplasmic Ca^{2+} increase. Since nuclear Ca^{2+} is known to regulate cell growth, we then investigated whether the proliferative response induced by AngII is mediated by ACE activation. We verified that silencing either ACE or clathrin reduced cell proliferation stimulated by AngII by 33% or 71% respectively, compared to control ($p<0.001$). In melanoma cell line (Tm-5), that was known to expresses mainly ACE instead of the classical AngII receptor, the AT1, AngII increased cell migration by 75.7 ± 2% compared to control condition ($p<0.05$). This data correlated with reduced focal adhesion formation induced by AngII in the same cell line (154 ± 23.9 a.u. for control vs, 54.9 ± 9.8 a.u. for AngII, $p<0.05$), Together, these results indicate a new role for ACE in cell proliferation and migration induced by AngII. Moreover, these findings point ACE signaling as a novel therapeutic target for cancer treatment.

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Capsaicin Causes Vasorelaxation of Rat Aorta by Activation of CB1 Receptors but not by Trpv1 or CB2 Receptors
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Blood pressure mainly depends on vascular tone, which is regulated by several mechanisms. Some substances like cannabinoids cause vasorelaxation not only by interacting on cannabinoid receptors (CB1 or CB2) but also by interacting with some other receptors (TRPV1, GPR55) or with ion channels. The main TRPV1 agonist (capsaicin) can cause vasorelaxation in several artery preparations as well. However, there is still lack of evidence to know the mechanism of action of capsaicin in modulating the vascular tone. Although there are some reports implicating the role of TRPV1 in the modulation of vascular tone, in our Lab and in a recent report, it was found that capsaicin caused vasorelaxation of rat aorta by a mechanism independent of TRPV1. Thus, the aim of this work was to determine the possible role of CB1 and CB2 receptors on the vasorelaxation caused by capsaicin in rat aorta. Our results show, by confocal microscopy, the localization of TRPV1 on endothelial and smooth muscle cells of rat aorta. However, capsaicin caused vasorelaxation of this artery through a mechanism independent of TRPV1, since the antagonist capsazepine did not block the effect of capsaicin. Moreover, as the expression of CB1 and CB2 receptors has been reported in aorta, we used antagonists for these receptors prior to the evaluation of capsaicin. In these experiments we found that using the CB1 antagonist AM281 partially blocked the vasorelaxant effect observed with capsaicin alone. On the other hand, by incubating with the CB2 antagonist AM630, we found that capsaicin caused a vasorelaxation similar to control conditions. Thereby, our results show that the vasorelaxant effect of capsaicin on rat aorta did not depend on TRPV1 or CB2 receptors, but partially depends on CB1 activation.

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Supramolecular Organization of Rod Outer Segment Membrane: New Rhodopsin Dimer Interface and Insights from the β2Ar-Gs Complex
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How the components of the G protein-coupled receptor (GPCR) ''signalosome'' assemble and function in the membrane bilayer is not known. Nevertheless the highly symmetrical organization of visual receptors (rhodopsin, rho) in rows-of-dimers suggested by AFM images of the rod cell disk membrane has a simple and direct consequence: the exposure of one unique surface of the receptor to the lipid phase of the membrane. This organization defines the encounter complex between rho and the G protein (transducin, Gt), and the orientation of rho in the dimer is therefore determinant. We have previously identified both computationally[1] and experimentally[2] a stable rho dimer involving TM1/TM2 and H8. Here we describe the investigation of alternative receptor binding modes leading to the identification of a new interface involving TM5/TM6. PMF analysis of this interface using the MARTINI coarse grain model indicates a similar order of stability as the TM1/TM2/H8 interface. However it involves a significant conformational change of a loop as compared