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Apocynin Induces Rapid Inhibition and Slow Facilitation of $I_{Ca,L}$ and Decrease and Increase of Reactive Oxygen Species in Rat Ventricular Myocytes

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Ion channels are exposed to cellular redox change. Exogeneously applied reactive oxygen species (ROS) modulate $I_{Ca,L}$. However, whether and how $I_{Ca,L}$ is regulated by endogenous redox still remains largely unknown. Apocynin (APO), a methoxy-substituted catechol with NADPH oxidase inhibitor and antioxidant actions, reversibly inhibits $I_{Ca,L}$. We analyzed effects of APO on $I_{Ca,L}$ and dichlorofluorescein fluorescence (DCFF) which indicates cumulative activity of ROS or H_2O_2 simultaneously in rat ventricular myocytes. $I_{Ca,L}$ and DCFF were continuously recorded at 1/30 s and APO (10 mM) was applied for 5 min. Changes in $I_{Ca,L}$ and DCFF were basically in parallel. APO rapidly inhibited $I_{Ca,L}$ by 51% of control associated with 12% reduction of DCFF. Then, they increased slightly in the presence of APO and rapidly and greatly after its washout resulting $I_{Ca,L}$ to 1.3 fold and DCFF to 1.2 fold of control. N-acetylcysteine (NAC,10mM), a precursor of glutathione (GSH), slightly decreased the APO-induced inhibition of $I_{Ca,L}$ and increased following facilitation of $I_{Ca,L}$ resulting the final amplitude to 1.6 fold. In the presence of NAC, ebselen (Ebs, 10^{-5} M), a H_2O_2 scavenger, little affected APO-induced inhibiton but decreased the facilitation of $I_{Ca,L}$ to 1.2 fold increase and decreased the magnitude and duration of washout-induced increase of DCFF. Biochemical analysis revealed that APO decreased GSH-to-GSSG ratio from 1.5 to 0.5 in 3 min. APO appears to have dual redox-related actions: 1) antioxidant activity that causes rapid decrease of H_2O_2 and GSH to inhibit $I_{Ca,L}$ and prevents the slow facilitation and 2) prooxidant activity that induces slow facilitation of $I_{Ca,L}$ by increasing H_2O_2. We conclude that H_2O_2 and GSH are important regulaors of $I_{Ca,L}$ in ventriclar myocytes.

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Anti-Arrhythmic Action of an ATP-Sensitive Potassium Channel Blocker Against Atrial Fibrillation Associated with Beta-Adrenergic Stress in Rat Hearts

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Atrial fibrillation (AF) is the most common disturbance of cardiac rhythm and results in a significantly increased risk of death. Although it is widely accepted that changes to the atrial muscle associated with heart disease make prolonged episodes of AF more likely to occur, the mechanisms underlying the origins of AF remain unclear. It is thought that sympathetic innervation plays an important role in the initiation of paroxysms of AF. ATP-sensitive K^+ channels (K_{ATP} channels) have been suggested to contribute to myocardial responses to beta-adrenergic stress. To investigate the role of K_{ATP} channel opening in the genesis of AF associated with beta-adrenergic stress, we examined the susceptibility to AF of excised Langendorff-perfused hearts from adult male Wistar rats. Unipolar electrograms were recorded from the left atrial epicardial surface of perfused hearts using a multi-electrode array, allowing measurements of atrial effective refractory period (AERP) and conduction velocity (CV) through the construction of activation maps. Paroxysms of AF were induced by burst pacing and the incidence and duration of the arrhythmia noted. Beta-adrenergic stress was induced by perfusion of the hearts with isoprenaline (ISO). While it was not possible to induce paroxysms of AF in control conditions, perfusion of the hearts with ISO rendered the left atrium susceptible to pacing-induced AF; the incidence and duration of which increased in a concentration-dependent manner. The shortening of AERP and incidence of AF induced by 10^{-6} M ISO were completely blocked in the presence of the K_{ATP} channel blocker, glibenclamide (10^{-5} M). In the absence of ISO, glibenclamide had no effect on AERP or CV. Taken together, our results suggest that K_{ATP} channels can contribute to atrial arrhythmogenesis during beta-adrenergic stress.

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Functional Expression and Subcellular Localization of f-Channels in Native Human and hESC-Derived Cardiomyocytes

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The Hyperpolarization-activated Cyclic-Nucleotide gated (HCN) genes encode for the alpha subunit of f-channel present in the heart. HCN4 is the predominant isoform in the sinoatrial node cells and, in the rabbit, it is localized into membrane caveolae, where the interaction with caveolin-3 (cav3) regulates f-current properties. HCN4 is abundant in undifferentiated human embryonic stem cells (hESC) and immature hESC-derived cardiomyocytes (hESC-CMs). Maturation is associated with modifications of f-channel functional properties. To date, no information is available on i) the subcellular localization of HCN4 and cav3 in hESC- and native human CMs ii) the functional consequences of their association on f-current properties. Confocal microscopy showed that HCN4 and cav3 colocalize in native human adult CMs. In the same cells, f-current was consistently recorded (70% cells), with a voltage of half maximal activation of −102 and −101 mV in atrial and ventricular CMs, respectively. Protein and mRNA for cav3 were not detected in undifferentiated hESC, but expression increased during maturation of hESC-CMs. HCN4 was highly expressed in hESC and d30 hESC-CMs, but decreased in d60 and d110 hESC-CMs. In the d110 cells, HCN4 appeared to be associated with cav3. Activation properties of f-current recorded from d110 hESC-CMs, resembled those measured in native atrial and ventricular CMs. Current activation occurred at more positive potentials in d60 hESC-CMs and native human fetal CMs. In native atrial CMs disruption of caveolae shifted f-current activation curve to more positive potentials.

In conclusion, our data shows for the first time that HCN4 and cav3 associate in native human and hESC-derived CMs. Expression of cav3 and its association with ionic channels likely represents a crucial step of cardiac maturation, which may result in changes of cellular electrophysiological properties and modulation by endogenous signals.

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Biophysical Properties of the Kcnq1 S277l Mutation Linked to Hereditary Long Qt Syndrome with Phenotypic Variability

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Hereditary long QT syndrome (LQTS) is associated with ventricular torsade de pointes tachyarrhythmias and sudden cardiac death. Mutations in a cardiac voltage-gated potassium channel, KCNQ1, induce the most frequent form of LQTS. We identified a KCNQ1 missense mutation, KCNQ1 S277L, in a patient presenting with recurrent syncope triggered by emotional stress (QTc = 528 ms). This mutation is located in the conserved S5 transmembrane region of the KCNQ1 channel. Using *in vitro* electrophysiological testing in the *Xenopus* oocyte expression system, the S277L mutation was found to be non-functional and to suppress wild-type currents in dominant-negative fashion in the presence and absence of the regulatory ß-subunit, KCNE1. In addition, expression of S277L and wild-type KCNQ1 with KCNE1 resulted in a shift of the voltage-dependence of activation by −8.7 mV compared to wild-type I_{Ks}, indicating co-assembly of mutant and wild-type subunits. The electrophysiological phenotype of the S277L mutation corresponds well with the severe clinical phenotype of the index patient. However, investigation of family members revealed three patients that exhibit asymptomatic QT interval prolongation (QTc = 493-518 ms). In conclusion, this study emphasizes the value of biophysical testing to provide mechanistic evidence for pathogenicity of ion channel mutations identified in LQTS patients. The inconsistent association of the KCNQ1 S277L mutation with the clinical presentation suggests that additional genetic, epigenetic, or environmental factors play a role in defining the individual clinical LQTS phenotype.

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Activation of KNCQ1/KCNE1 Channel by Classic PKC is Impaired in Long QT Syndrome Type 1

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Stimulation of the cardiac K^+ channel IKs (KCNQ1/KCNE1) is crucial in mediating the QT shortening at high adrenergic stimulation states, with mutations in the KCNQ1 subunit being linked to a high risk of cardiac events triggered by adrenergic stimulation. In addition to β-adrenergic receptors (AR), α_1-AR is also activated upon adrenergic stimulation in the heart. IKs is strongly regulated by β-AR stimulation, but little is known about the role of α_1-AR-mediated regulation. In this study, we investigated the molecular mechanism underlying α_1-AR-mediated regulation of human IKs channel and mutant channels found in Long QT syndrome type 1 (LQT1). We overexpressed wild-type and mutant KCNQ1/KCNE1 subunits together with the α_{1A}-AR in HEK293T cells. α_1-AR activation strongly facilitated voltage dependence of IKs activation independently of β-AR stimulation ($V_{1/2}$ shift ≅ - 20 mV). This effect was blocked by pretreatment of cell-permeable classic PKC (cPKC) inhibitory peptide