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Ceramide Activates $I_{Cl,swell}$ in Rabbit Ventricular Myocytes via Mitochondrial ROS Production

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We previously showed that $I_{Cl,swell}$ is activated by ROS generation via NADPH oxidase (NOX) and the mitochondrial electron transport chain (ETC). Sphingolipid signaling is implicated in channel regulation. Here we examined the role of ceramide in the modulation of $I_{Cl,swell}$. Under isosmotic conditions, the addition of exogenous C_2-ceramide (C_2-Cer, 2 ∝M) increased Cl^- current density by 0.7 ± 0.1 pA/pF at +60 mV after 10 min ($n = 11$, $P<0.01$). DCPIB (10 ∝M), a highly selective $I_{Cl,swell}$ antagonist, inhibited C_2-Cer-induced $I_{Cl,swell}$ by 76 ± 8% ($n = 6$, $P<0.01$). The inactive analogue C_2-H_2Cer (2 ∝M) failed to stimulate $I_{Cl,swell}$ ($n = 6$). Bacterial sphingomyelinase (SMase, 0.03 U/mL) was used to elicit endogenous ceramide production. SMase increased $I_{Cl,swell}$ by 1.1 ± 0.1 pA/pF after 14 min ($n = 30$, $P<0.01$). This activation was inhibited by DCPIB (78 ± 6%, 10 ∝M, $n = 7$ and 81 ± 6%, 30 ∝M, $n = 4$) and tamoxifen (116 ± 16%, 10 ∝M, $n = 5$). Next we identified the source of ROS. Exposure to the NOX-specific inhibitor apocynin (500 ∝M, 10 min) failed to suppress SMase-induced $I_{Cl,swell}$ ($n = 9$), whereas we previously showed apocynin blocks activation of $I_{Cl,\ swell}$ on swelling and stretch. Diphenyleneiodonium (60 ∝M), a flavoprotein oxidase antagonist that suppresses both NOX and ETC Complex I, fully inhibited SMase-induced $I_{Cl,swell}$ after 20 min (100 ± 14%, $n = 4$, $P<0.01$). Rotenone (10 ∝M), a specific ETC Complex I inhibitor, also abrogated the SMase-induced activation of $I_{Cl,swell}$ after 20 min (110 ± 18%, $n = 5$, $P<0.05$). These data indicate that there is a ceramide-sensitive component of $I_{Cl,swell}$, and it is regulated by mitochondrial ROS. Ceramide signaling may modulate $I_{Cl,swell}$ in cardiac disease.

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The persistently beating hagfish heart

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To elucidate the evolution of autonomic cardiac reflexes in primitive chordates, we measured isometric force and trans-gap action potentials in ventricular and atrial strips (diameter 0.6-0.8 mm) from the systemic heart of the Atlantic hagfish, *Myxine glutinosa*. All parts of the heart paced spontaneously with a frequency, that at all temperatures was ~30% faster for atrial than for ventricular tissue. Active force development increased with stretch, and remained high as passive force rose concurrently. In spite of the low blood pressure of hagfish, the maximal contractile force (~60 mN/mm^2) was comparable to that of higher vertebrates. Electrical stimulation at frequencies higher than the inherent, demonstrated capture and refractoriness consistent with the atrial pacing observed in the intact heart. The isometric twitches that developed during the long lasting plateaus of the action potentials were relatively insensitive to $[Na^+]_o$, $[Ca^{2+}]_o$, epinephrine, and carbachol, but were promptly abolished by depolarization by $[K^+]_o$. Beat kinetics showed no indication of releasable intracellular Ca^{2+} stores. KCl-contractures developed extremely slowly and were insensitive to epinephrine. In addition, we report a partial sequencing (1077 bp) of the cardiac Na^+-Ca^{2+} exchanger using degenerate CODEHOP primers determined from known marine species Na^+-Ca^{2+} exchangers. It was surprising, therefore, that the sequencing of the Na^+-Ca^{2+} exchanger showed both a putative PKA site and indication of the typical ''cardiac'' splicing pattern of NCX1 (exon pattern ACDEF). The results are compared to similar measurements from tunicates, sharks, and higher vertebrates. The most surprising finding is the ability of all parts of the heart to generate pacemaker activity at a frequency close to that of the heart *in situ.*

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The Role Of Depolarizing And Repolarizing Currents In The Induction Of Early Afterdepolarizations During Acute Hypoxia In Ventricular Myocytes

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Coronary occlusion is associated with acute hypoxia and increased catecholamine levels. Acute hypoxia (decreasing PO_2 from 150 to 17mmHg) is not energy limiting but can alter the function of ion channels. Hypoxia decreases the transient Na^+-current ($I_{Na\text{-}T}$), the basal L-type Ca^{2+} channel current ($I_{Ca\text{-}L}$) and the slow component of the delayed rectifier K^+-current (I_{Ks}), without effecting the rapid component (I_{Kr}). Hypoxia also increases the persistent Na^+-current ($I_{Na\text{-}P}$) and the sensitivity of $I_{Ca\text{-}L}$ and I_{Ks} to the β-adrenergic receptor agonist isoproterenol (Iso). The net effects of acute hypoxia and catecholamines on the cardiac action potential are not known.

We incorporated all published data reporting the effects of acute hypoxia on cardiac ion channels into the Luo-Rudy model. Results from the model were compared with experimental data we obtained from myocytes.

Our modelled data predicts acute hypoxia, in the absence of Iso, has little effect on resting membrane potential (RMP), action potential peak (APP) or action potential duration (APD). Hypoxia alone did not trigger EADs. When we added 0.6nM Iso to $I_{Ca\text{-}L}$ during hypoxic conditions, APD was prolonged and EADs were generated. Repeating this for I_{Ks} did not alter APD or generate EADs. When we modelled the effects of hypoxia on $I_{Ca\text{-}L}$ and I_{Ks}, APD was prolonged and EADs were generated, suggesting any anti-arrhythmic effect of I_{Ks} is small and that $I_{Ca\text{-}L}$ effects predominate.

Experimental data confirmed that acute hypoxia alone did not markedly alter RMP, APP or APD. In the presence of 3nM Iso, hypoxia significantly increased APD by 20% and induced EADs and spontaneous tachycardia. We conclude that during acute hypoxia, EADs are induced predominantly as a result of increased sensitivity of the L-type Ca^{2+} channel to β-adrenergic receptor stimulation.

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Increasing Cardiac Contractility after Myocardial Infarction Exacerbates Cardiac Injury and Pump Dysfunction

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Myocardial infarction (MI) induces cardiac remodeling and leads to poor cardiac pump function. Increasing the contractility of the surviving myocytes is one therapy thought to improve the function of failing heart. However, the excess sympathetic activity in heart failure coupled with increased inotropic therapy could induce cell death and exacerbate cardiac dysfunction. In this study we tested the effects of increasing Ca^{2+} influx through the L-type Ca^{2+} channel (LTCC) in the post MI heart on myocyte contractility, cardiac function and remodeling.

Methods: Double transgenic mouse lines with inducible (Tet-off) and cardiac myocyte specific (αMHC promoter) expression of the β2a subunit of the L-type Ca^{2+} channel were used. MI was produced by permanent ligation of the left anterior descending coronary artery. In-vivo cardiac function was measured with the Visual Sonics Velvo 770 system. Myocytes were isolated and LTCC Current ($I_{Ca\text{-}L}$) and fractional shortening (FS) were measured in wild type (WT) and β2a hearts before, and 3 weeks after MI. **Results:** Echo measurements showed decreased heart function in both groups after MI, but β2a mice had significantly lower ejection fraction (EF) and larger left ventricular internal diameter (LVID) than WT mice (EF: 24.7% vs.42.6%; LVID: 5.2 mm vs. 4.2 mm). $I_{Ca\text{-}L}$ and FS were greater in uninfarcted myocytes from β2a vs WT mice ($I_{Ca\text{-}L}$ 24.5 ± 1.7 vs.13.7 ± 1.8 pA/pF FS: 12.31 ± 1.15% vs. 9.0 ± 0.5%). 3 weeks after MI, $I_{Ca\text{-}L}$ and FS in myocytes from both groups were decreased, but β2a myocytes still had significantly higher $I_{Ca\text{-}L}$ and FS than in WT myocytes (β2a vs. Control: 18.37 ± 1.90 vs.11.57 ± 0.77 pA/pF and 12.05 ± 0.74% vs. 7.16 ± 0.99%). **Conclusions:** Increasing Ca^{2+} influx through the LTCC in the post MI heart increases myocyte contractility but depresses cardiac pump function, possibly by increasing myocyte death.

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Chemical Ablation Of Purkinje Fibers Diminishes Spontaneous Activity In A Rat Model Of Regional Ischemia And Reperfusion

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Spontaneous activity and arrhythmias associated with acute local ischemia and reperfusion were studied in isolated Langendorff-perfused hearts from healthy Sprague-Dawley rats (n=16). Epicardial fluorescence imaging of transmembrane potential and NADH were used to relate sources of electrical activity to changes in mitochondrial redox state caused by local ischemia. The left anterior descending coronary artery was cannulated and the flow of perfusate to the cannula was controlled by a high-pressure/low-flow HPLC pump. Studies were conducted using a local ischemia/reperfusion protocol that consisted of 10 min of normal flow, 20 min of regional LV ischemia, followed by 20 minutes of reduced flow reperfusion, and then 20 min of normal flow reperfusion. Control hearts (n=9) were compared with hearts in which the endocardium (containing the Purkinje fibers) was chemically ablated by applying a Lugol's iodine solution to the ventricular cavities (n=7). The ablation significantly reduced spontaneous activity in each phase of the protocol. Specifically, during acute regional ischemia, spontaneous activity was reduced by 80% ($p<0.005$); by 70% during low-flow reperfusion ($p<0.005$); and by 85% during full-flow reperfusion ($p<0.001$). Omission of blebbistatin, an electro-mechanical uncoupling agent, did not