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CaMKII Regulates L-type Ca^{2+} Current during Action Potential Plateau and Repolarization _ a Direct Link to Arrhythmias

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During the cardiac action potential (AP), the L-type Ca^{2+} current ($I_{Ca,L}$) is the main source of Ca^{2+} entry into the cell that triggers excitation-contraction coupling, and it is also the main inward current that contributes to the depolarization during AP plateau and later phases. Derangement of $I_{Ca,L}$ has been proposed to cause early or delayed afterdepolairzation (EAD, DAD) that lead to arrhythmias. Previous studies have characterized the steady state $I_{Ca,L}$ activation, inactivation and recovery kinetics. However, the non-steady state dynamics of $I_{Ca,L}$ during AP still remains incompletely understood. Here we study the dynamics of $I_{Ca,L}$ during the cell's own AP under physiological condition. **Method:** Unlike previous studies using an averaged AP waveform, we used each cell's own AP to perform individualized AP-clamp (iAP-clamp) study of $I_{Ca,L}$. This allows us to examine the dynamics of $I_{Ca,L}$ during AP with physiological ionic compositions. **Results:** (1) $I_{Ca,L}$ took the form of a spike at AP phase-1, followed by a dome at phase-2 . Now we report a newly observed inward tail current at phases-3&4 which is composed of $I_{Ca,L}$ and Na^+/Ca^{2+} exchange current. This inward tail current is important because it causes depolarization and directly links $I_{Ca,L}$ to the generation of EAD or DAD. (2) Furthermore, Ca^{2+}-calmodulin dependent kinase II (CaMKII) modulates this tail current; inhibition of CaMKII abolished the tail current. (3) The tail current was also abolished by using exogenous Ca^{2+} buffer to blunt SR Ca^{2+} release and prevent activation of CaMKII. **Conclusion**: The iAP-clamp reveals, for the first time, an inward $I_{Ca,L}$ associated tail current at the late phases of AP. CaMKII modulation of this tail current should play a critically important role in generating the afterdepolarizations that lead to cardiac arrhythmias.

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Simultaneous Recordings of Cell Shortening and cAMP or Calcium Transients Reveal Differential Regulation of Cardiac Contractility by Specific Phosphodiesterases

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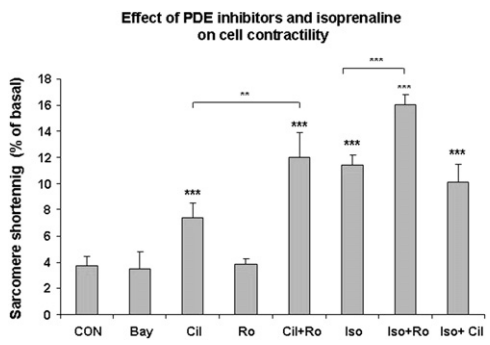
Multiple cyclic nucleotide phosphodiesterases (PDEs) belonging to four families (PDE1 to PDE4) hydrolyze cAMP in cardiac cells, but the functional significance of this diversity is not well understood. The goal of this study was to characterize the involvement of different PDEs in excitation-contraction coupling in cardiomyocytes. For this, sarcomere shortening and Ca^{2+} transients were recorded simultaneously in rat ventricular myocytes field stimulated at 0.5 Hz with an IonOptix system. As shown in the figure, selective inhibition of PDE2 with Bay 60-7550 (Bay, 100 nM) or PDE4 with Ro-201724 (Ro, 10 μM) had no effect on basal cell contraction, whereas selective inhibition of PDE3 with cilostamide (Cil, 1 μM) or β-adrenergic stimulation with isoprenaline (Iso, 1 nM) increased myocyte shortening. Inhibition of PDE4 potentiated the response to Cil and Iso, showing that PDE4 becomes important when cAMP is prestimulated. Similar results were obtained on Ca^{2+} transients. cAMP measurements by FRET in beating cardiomyocytes indicate that Iso strongly increases cAMP levels. Effects of selective PDE inhibitors are under investigation. These results show that PDE2, PDE3 and PDE4 differentially regulate excitation-contraction coupling in cardiomyocytes.



Platform F: Voltage-gated Na Channels

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How Myasthenia Gravis Alters the Safety Factor for Neuromuscular Transmission

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Myasthenia gravis (MG), the best understood autoimmune disease, is characterized by antibodies directed against the skeletal muscle acetylcholine receptors (AChRs). An elevated concentration of Na+ channels at the endplate ensures the efficiency of neuromuscular transmission by reducing the threshold depolarization needed to trigger an action potential (E_{AP}). Postsynaptic AChRs and voltage-gated Na+ channels are lost from the neuromuscular junction in MG. We studied the impact of postsynaptic voltage-gated Na+ channel loss on the safety factor for neuromuscular transmission (SF). Comparing intercostal nerve-muscle preparations from controls and MG patients, endplate AChR loss decreased the size of the endplate potential (EPP) from 40.2 ±1.3 mV to 23.5 ±1.7 mV ($p < 0.001$). Endplate Na+ channel loss in patients with MG increased E_{AP} from −71.9 ±2.2 mV to 62.3 ±2.7 mV ($p < 0.001$). The SF was reduced from 2.98 to 1.09. If the EPP was the same as control, the SF for MG fibers would have been 1.86 rather than the observed value of 1.09. If E_{AP} for MG fibers were the same as control fibers, the SF for MG fibers would have been 1.74. The reduction in EPP accounted for 59% of the reduction in SF and the increase in E_{AP} produced 40% of the SF reduction. To evaluate whether AChR-specific antibody impaired the function of Na^+ channels, we tested omohyoid nerve-muscle preparations from rats injected with monoclonal myasthenogenic IgG (passive transfer model of MG [PTMG]). AChR antibody that produced PTMG did not alter the function of Na^+ channels. We conclude that loss of endplate Na^+ channels in MG is due to complement-mediated loss of endplate membrane rather than a direct effect of myasthenogenic antibodies on Na^+ channels. Loss of endplate Na+ channels caused 40% of the SF reduction in MG.

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$Na_V1.7$ Gain-of-function Mutations As A Continuum: A1632E Displays Physiological Changes Associated With Erythromelalgia And Paroxysmal Extreme Pain Disorder Mutations And Produces Symptoms Of Both Disorders

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Gain-of-function mutations of $Na_V1.7$ have been shown to produce two distinct disorders: $Na_V1.7$ mutations which enhance activation produce inherited erythromelalgia (IEM), characterized by burning pain in the extremities; $Na_V1.7$ mutations which impair inactivation produce a different, non-overlapping syndrome, paroxysmal extreme pain disorder (PEPD), characterized by rectal, periocular and perimandibular pain. Here we report a novel $Na_V1.7$ mutation associated with a mixed clinical phenotype with characteristics of IEM and PEPD, with an alanine 1632 substitution by glutamate (A1632E) in domain IV S4-S5 linker. Patch-clamp analysis shows that A1632E produces changes in channel function seen in both IEM and PEPD mutations: A1632E hyperpolarizes (-7 mV) the voltage-dependence of activation, slows deactivation, and enhances ramp responses, as observed in $Na_V1.7$ mutations that produce IEM. A1632E depolarizes (+17mV) the voltage-dependence of fast-inactivation, slows fast-inactivation and prevents full inactivation, resulting in persistent inward currents similar to PEPD mutations. Using current-clamp, we show that A1632E renders dorsal root ganglion (DRG) and trigeminal ganglion neurons hyperexcitable. These results demonstrate a $Na_V1.7$ mutant with biophysical characteristics common to PEPD (impaired fast-inactivation) and IEM (hyperpolarized activation, slow deactivation and enhanced ramp currents) associated with a clinical phenotype with characteristics of both IEM and PEPD, and show that this mutation renders DRG and trigeminal ganglion neurons hyperexcitable. These observations indicate that IEM and PEPD mutants are part of a physiological continuum that can produce a continuum of clinical phenotypes.