Expression of modified CaV1.1 constructs in dysgenic (CaV1.1 null) myotubes has been useful in the determination of regions of the CaV1.1 that are important for skeletal muscle excitation-contraction (EC) coupling, however the use of this system to probe CaV1.1 channel gating has been problematic because dysgenic myotubes express a variety of other ion channels which conduct Ca^{2+} and contribute to intramembrane gating charge movement. This obstacle can be overcome by recording from tsA201 cells coexpressing CaV1.1 α1S, β1a, α2δ1 and Stac3 subunits. Our group has previously characterized, in dysgenic myotubes, a CaV1.1 mutation (R174W) which affects the innermost basic residue of the voltage-sensing S4 α-helix of Repeat I; R174W abolished activation of the L-type Ca^{2+} current in response to depolarization without affecting the magnitude or voltage-dependence of charge movement. Using tsA201 cells, we show that this dysfunction arises from an impaired gating transition of the RI voltage-sensor that is not detectable with short (20 ms) depolarizations. Specifically, we were unable to recruit the documented slow-moving gating charge component attributable to RI with longer (200 ms) test potentials. We next assessed the effects of corresponding mutations in Repeats II (K537W), III (R906W) and IV (K1245W) in order to determine the way in which translocation of these voltage-sensors affects channel opening. Currents and intramembrane charge movements produced by R906W were both smaller and shifted (~10 mV) to more depolarizing test potentials. In contrast to the severe loss of function observed with R174W and the somewhat impaired gating of R906W, the K537W and K1245W mutants produced L-type currents with enormous peak amplitudes almost three times greater than wild-type CaV1.1 and with greatly impaired deactivation. Supported by Conacyt 169006 (UM), the Boettcher Foundation (RAB), and NIH AR055104, NIH AR052354 and MDA 277475 (KGB).

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Rotenone, Stimulant of Superoxide Release from Mitochondrial Complex I, Transiently Augments L-Type Calcium Current in A7r5 Arterial Smooth Muscle Cells

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Rotenone (Rot) is a plant toxin that stimulates superoxide (O_2^-) release from complex I of mitochondrial respiratory chain. It is used as a pharmacological tool to study roles of O_2^- in redox-related pathophysiology represented by Parkinson's disease. However, its effects on ion channel currents have little been elucidated. To study O_2^--dependent regulation of L-type Ca^{2+} channel current (ICa,L) we examined acute effect of Rot on ICa,L in cultured A7r5 cells derived from embryonic rat aorta. ICa,L was recorded by ruptured whole-cell clamp technique with 10 mM Ba^{2+} as a charge carrier with high resistance pipettes that minimized run-down of the current. Rot dose-dependently (10nM-0.01mM) increased ICa,L. The increase was transient and was followed by the decrease of the amplitude and increase of voltage-dependent inactivation. It occurred with a characteristic shift of I-V relationship to the left and succeeding inhibition was accompanied with a shift of steady-state inactivation curve to the left. Dithiothreitol (DTT) induced similar increase of ICa,L but without any negative shift. Rot produced additional increase of ICa,L with the negative shift in the presence of DTT. Sustained pretreatment by1mM-tempol, scavenger of O_2^-, did not decrease basal ICa,L density but suppressed the Rot-induced increase of ICa,L. Since O_2^- is precursor of H_2O_2, we examined involvement H_2O_2 on the Rot-induced modulation of ICa,L. H_2O_2 (0.3 mM) significantly inhibited ICa,L. Also in the presence of H_2O_2, Rot increased ICa,L with a negative shift of I-V relationship and the increase diminished with time. We conclude that rotenone-induced augmented release of O_2^- from mitochondrial complex I increased ICa,L and the modulation is independent of H_2O_2 in A7r5 aortic smooth muscle cells. O_2^- may be involved in the regulation of basal ICa,L.

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CaV1.2 Interaction with AT1R Reduces Receptor Internalization

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The cardiac L type calcium channel is a multi-subunit complex that includes CaV1.2 as the pore-forming subunit that co-assembles with the auxiliary CaVa2d1 subunit and with the CaVb subunit. The main role of these auxiliary subunits is to modify the amount of channels inserted in the plasma membrane. Similarly, trafficking of these channels is controlled by direct interaction with other proteins such as some G-protein coupled receptors (GPCR). Angiotensin II (AngII) type I receptor (AT1R) is a GPCR well known to be involved in the intracellular calcium regulation at the heart, modulating cardiomyocytes contractibility. In this work we show, by co-immunoprecipitation, that AT1R forms a macro-complex with CaV1.2 in primary culture of newborn rat cardiomyocytes and in an heterologous system. Standard biochemical approaches were used to demonstrate that these proteins colocalize at the plasma membrane and to determine the region of CaV1.2 responsible for this interaction. The functional impact of this complex was studied in terms of L-type currents inhibition and in terms of internalization of AT1R. Although inhibition of endogenous L-type current in cardiomyocytes was found to be dependent on the CaVb2 transcriptional start site (TSS) expressed, the presence of the macro-complex was found to be independent on the variant expressed. The effect of this interaction over AT1R internalization was studied in cells stimulated with AngII at different times. As expected, in cell expressing only the AT1R and stimulated with AngII, the receptor is readily internalized after 15 min, however, in cells expressing also CaV1.2, AT1R internalization (and CaV1.2) is observed only after 30 minutes of AngII stimulation. Overall, our results demonstrate that AT1R interaction with CaV1.2 increases the residence time at the plasma membrane of the receptor and induce L-type channel internalization after prolonged AngII exposure.

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Posttranslational Proteolytic Cleavage of α2δ Subunits: Functional Implications for High Voltage-Gated Calcium Channels

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CaV1 and CaV2 voltage-gated calcium channels contain an α1 subunit, which forms the selective pore and determines the functional properties of the channel. The α1 subunit is associated with auxiliary β and α2δ subunits, which modulate the trafficking and functional properties of the channels.

α2δ subunits are membrane-associated proteins, which are highly glycosylated and possess multiple disulfide bonds. The α2 and δ peptides are encoded by a single gene as an uninterrupted α2δ pre-protein, which is cleaved post-translationally by a yet unidentified protease. The highly glycosylated α2 polypeptide, which is entirely extracellular, remains disulfide-bonded to the smaller δ moiety, which links the protein to the plasma membrane. All four known α2δ subtypes are cleaved into α2 and δ in vivo, but the significance of the proteolytic processing with regard of the function of α2δs as voltage-gated calcium channel subunits has remained elusive.

We now provide evidence that preventing the cleavage, by mutating the proteolytic site in α2δ-1 and α2δ-3 subtypes, abolishes any effect of α2δ on CaV currents in tsA −201 cells expressing CaV2.2/β. In contrast, our experiments indicate that the trafficking of Cav2.2 channels by α2δ-1 is not affected by the lack of cleavage. In neurons, we find that all α2δ-1 in DRG neuronal axons is proteolytically cleaved; however, in cell bodies not all α2δ-1 is cleaved, indicating that there is a potential modulatory role for unprocessed α2δ.

We are currently examining the nature of the protease(s) involved in the processing of α2δ-1 by application of selected chemical protease inhibitors.

Targeting the protease responsible for the cleavage of α2δ-1 could provide a novel avenue for therapy of neuropathic pain, which would act on the same target as gabapentinoids, but by a completely different mechanism.

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Bin1 Regulates $Ca_V1.2$ Channel Clustering in Ventricular Myocytes

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L-type $Ca_v1.2$ channels are expressed in the plasma membranes of excitable cells including cardiac myocytes where they play a key role in excitation-contraction coupling. Recently, we reported that $Ca_v1.2$ channels form clusters that undergo dynamic, reciprocal, allosteric interactions. This 'functional coupling' facilitates Ca^{2+} influx by increasing activity (NP_o) of adjoined channels and occurs through C-terminal-to-C-terminal interactions. Physical proximity of $Ca_v1.2$ channels on the plasma membrane is an essential requirement for functional interactions of the channels. In the present study, we investigated the role of two scaffolding proteins, A-Kinase Anchoring Protein 150 (AKAP150) and Bridging