was subjected to in vitro cRNA synthesis. The resulting cRNA was subsequently injected into Xenopus oocytes, and channel currents were examined using Two-Electrode Voltage Clamp. Four days later. We thus compared the biophysical properties of WT and mutant channels. We found that the R477H mutation shifts the voltage dependence of inactivation to more depolarized voltages, rendering the mutant channels more difficult to inactivate compared to the WT channel. In addition, the time constant of inactivation was slower for the mutant. At the same time, the voltage dependence of activation was unchanged. These results suggest a potential mechanism for epileptogenesis: reduced channel inactivation would contribute to increased excitability and excitotoxicity - two hallmarks of epilepsy. The results also highlight the role of the II-1 loop of VGCC in channel inactivation. As for the Q1957X mutation, our current hypothesis is that this mutant generates no currents. In addition, we hypothesize that the Q1957X mutant will act as a dominant negative regulator of coexpressed WT channels via the unfolded protein response.

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β2A Subunit Efficiently Modulates Voltage-Dependent Properties of Individual Voltage Sensors in the Human CaV 1.2 Channel

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The human L-type calcium channel (CaV 1.2) is a multi-protein complex consisting of the pore-forming α1c subunit and at least two auxiliary subunits, β and σ2, which assemble in a 1:1:1 stoichiometry. β subunits regulate multiple aspects of CaV 1.2 channel physiology, including surface expression, degradation and gating. Investigating the effect of β2a and β3 subunits on human CaV 1.2 channels (α1c-β2a) expressed in Xenopus oocytes, we found that the voltage dependences of total charge displacement (QV) and conductance (G) were 10mV more negative in the presence of β2a. We tested the hypothesis that β2a and β3 subunits differentially modulate the activation of the four voltage-sensor domains (VSDs) in the α1c subunit. Using the Voltage-Clamp Fluorometry technique, we fluoroescence tracked the movement of individual VSD in conducting human CaV 1.2 channels. We found that, in the presence of β2a, the voltage-dependent activation of VSD II and III was left-shifted as compared to β2a-associated CaV 1.2 (VSD II with β2a: Vhalf=−32.1±.1 mV, z=4.5±.8 e1, VSD II with β3: Vhalf=−26.5±.8 mV, z=2.4±.0 e1, VSD III with β2a: Vhalf=−36.6±.3 mV, z=1.8±.0 e1, VSD III with β3: Vhalf=−16.9±.4 mV, z=1.2±.0 e1). Moreover, activation and deactivation kinetics of VSD II in channels associated with β2a were dramatically slower than in the presence of β3, while voltage- and time-dependent properties of VSD IV remained the same in the presence of either Cav β2a or β3. These results suggest that α1c subunit is VSD IV, which does not show evidence of functional interaction with α1c-β2a subunits.

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The z28 Subunit Efficiently Couples VSDs Activation to Pore Opening in Human CaV 1.2 Channels

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The z2-1 auxiliary subunit binds to the pore-forming α1c subunit of voltage-gated L-type calcium channels (CaV 1.2) and facilitates both membrane trafficking and voltage-dependent activation. Using voltage-clamp fluorometry to study human CaV 1.2 channels expressed in Xenopus oocytes, we have recently found that z2-1 association, in the presence of β2a subunits, causes voltage sensor domain (VSDs) I, II and III, but not IV, to activate at more hyperpolarized potentials (ΔVhalf=−39mV, −32mV, −18mV for VSDs I-III respectively) and with steeper voltage-dependence (z fold increase with z2-1: 1-1.9, 2.2 and 1.6 for VSDs I-III respectively). Thus, the z2-1-induced facilitation of CaV 1.2 voltage-dependent activation seems to be mediated through the remodeling of three VSDs. We analyzed our voltage-clamp fluorometry data with a 32-state allosteric model for CaV 1.2 activation, consisting of five gating particles (one pore, four VSDs). By simultaneously fitting kinetic and steady-state data from the pore (ionic currents) and from the individual VSDs (fluorescence), we estimated the energetic contribution of each VSD to pore opening in the presence and in the absence of z2-1. The model predicts that, in channels not associated with z2-1, all VSDs were poorly energetically-coupled to pore opening. The association of z2-1 with z1-1 specifically increased the coupling energy of VSDs II and III to the pore. In agreement with the model prediction, we found that without z2-1 subunits the ion current deactivation is largely voltage-independent. A low-resolution structure of CaV 1.2 channel complexes shows that the z2-1 subunit is cap that embraces 1/3 of the extracellular surface of the z1-1 subunit (Walsh et al., JBC 2009). Based on our results, we propose that the exposed ⅔ of the z1-1 subunit is VSD IV, which does not show evidence of functional interaction with z2-1 subunits.

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Genetic Ablation of KLHL1 Alters CaV 3.2 Expression in DRG Neurons and Mechanical Pain Transmission

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Duchenne Muscular Dystrophy (DMD) is a fatal muscle wasting disorder. It is commonly associated with cardiomyopathy that is characterised by disrupted cytoskeletal architecture and mitochondrial dysfunction. Activation of the L-type Ca2+ channel (LTCC) provides the Ca2+ influx required for contraction, but also causes an increase in mitochondrial membrane potential (ΔΨm) in a calcium-independent manner. This involves the movement of cytoskeletal proteins and regulation of mitochondrial VDAC. The increase in ΔΨm (JC-1 fluorescence) after activation of LTCC is absent in cardiomyocytes isolated from hearts of mdx mice, a murine model of DMD (1.5±1.0%, n=4 vs. 12.1±1.4%, n=10 in C57BL/10ScSnArc controls) but can be restored using antisense morpholino oligomers to induce exon skipping of dystrophin exon 23 (M23D). Here we investigate the effects of M23D peptides on changes in ΔΨm and mitochondrial oxygen consumption (flavoprotein autofluorescence) induced by LTCC activation. Male (M) and female (F) neonatal mdx mice were injected (i.p.) for 3 weeks and divided into two treatment groups: (i) 120mg/kg once per week; (ii) 30mg/kg four times per week. Activation of LTCC with the dihydrodipyrudine agonist BayK(+) induced an increase in ΔΨm in cardiomyocytes from animals treated with a single weekly dose (M: 17±2%, n=5; F: 16±3%, n=12) and with multiple weekly doses...