characterized by chronic progressive weakness whose affected members harbor a novel $Ca_v1.1$ R1242G mutation affecting the third arginine in S4 of domain IV. Whole-cell patch clamp recordings of the R1242G mutant showed a strong hyperpolarized shift of steady-state inactivation in GLT mouse myotube expression system. The facilitated inactivation resulting in the reduction of channel availability may account for the chronic progressive weakness of the patients. In addition, we found an outward gating pore current through the mutant voltage sensor of R1242G which might induce a reduction in the amplitude and duration of the action potential and cause the failure of muscle fiber excitability.

1834-Plat

Bio-Inspired Voltage-Dependent Calcium Channel Blockers

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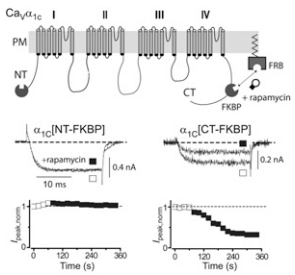
Blocking voltage-dependent Ca_V1/Ca_V2 channels is a prevailing or potential therapy for myriad diseases ranging from hypertension to Parkinson's disease, but a major limitation is lack of selective small-molecule inhibitors for distinct Ca_V1/Ca_V2 channels. Here, we report a general bio-inspired approach towards developing novel Ca_V1/Ca_V2 channel blockers (CCBs). We discovered that different proteins ($Ca_V\beta$, 14-3-3, camodulin, and calmodulin-dependent protein kinase II) that bind to spatially distinct regions of pore-forming α_1-subunit intracellular loops can be converted into CCBs, with tuneable selectivity and potency, simply by their anchoring to the plasma membrane. The principle is extendable to small molecules— engineering FKBP into specific sites within $Ca_V1.2$ α_{1C} intracellular loops permitted heterodimerization-initiated channel inhibition with rapamycin (Fig). The results reveal a universal method for developing novel genetically-encoded and small-molecule CCBs.



1835-Plat

Novel Modulatory Action of Calmodulin Complexation with L-Type Channels

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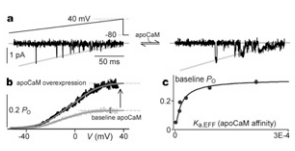
L-type Ca^{2+} channels convey vital Ca^{2+} inflow, which is critically down-regulated by intracellular Ca^{2+}. This Ca^{2+}-dependent inactivation (CDI) is only present in channels initially preassociated with Ca^{2+}-free calmodulin (apoCaM), where subsequent Ca^{2+} binding to this 'resident' calmodulin (CaM) inactivates channels (*Nature***463**:968). Channels lacking preassociated apoCaM fail to undergo CDI. Canonical L-type channels bind apoCaM so avidly that nearly all channels are 'charged' with apoCaM. However, natural variants feature potentially lower apoCaM affinity, so fluctuations in ambient apoCaM may tune overall CDI. Here, we demonstrate that channel preassociation with apoCaM does more than simply enable CDI, and strikingly enhances open probability (P_O). For example, in single-channel Ba^{2+} currents (to avoid CDI), taken from a variant with low apoCaM affinity, openings are sparse under baseline apoCaM (**a**, left), but prolific upon apoCaM overexpression (**a**, right). Corresponding P_O-V relations confirm this effect of apoCaM (**b**). Importantly, the baseline P_O of differing channel variants increases with channel/apoCaM affinity via a Langmuir function (**c**). Thus, P_O variability reflects differences in apoCaM preassociation, not contrasts in channel composition *per se*. These results suggest that ambient apoCaM levels can tune both CDI and P_O of channels.



1836-Plat

Discrete Conductance Levels in Calcium Channel Models: Multiband Calcium Selective Conduction

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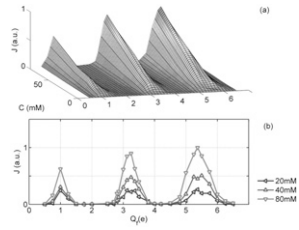
Brownian dynamics were simulated for the simple model of calcium channels introduced by Nonner and Eisenberg, computing electric forces from all charges. Permanent charge (of acidic side chains) was varied. Substantial conduction was found only at certain discrete values of permanent charge. Different conduction states had different selectivity, one resembling L-type Ca_V1 and the other RyR channels. We speculate that thermally activated switching between conductance values could produce some types of spontaneous gating. Below is calcium current J as a function of permanent charge Q_f and calcium concentration. Details at arXiv.org 1209.2381



1837-Plat

Pharmacological Correction of Gating Defects in the Voltage-Gated Cav2.1 Ca^{2+} Channel due to a Familial Hemiplegic Migraine Mutation

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Voltage-gated ion channels exhibit complex forms of gating, which can be targeted in pharmacological therapies for disease. Here, we report that the pro-oxidant, tert-butyl dihydroquinone (BHQ), modulates Cav2.1 Ca^{2+} channels in ways that oppose defects in channel gating and synaptic transmission resulting from a familial hemiplegic migraine mutation (S218L). BHQ inhibits voltage-dependent activation and unmasks slow deactivation that is enhanced by Ca^{2+} and more prevalent in S218L mutant than in wild-type channels. These actions of BHQ help reverse the gain-of-function and reduced Ca^{2+}-dependent facilitation of Cav2.1 channels with the S218L mutation. Transgenic expression of the mutant channels at the Drosophila neuromuscular junction causes abnormally elevated evoked postsynaptic potentials and impaired synaptic plasticity, which are largely restored to the wild-type phenotypes by BHQ. Our results reveal a new mechanism by which Cav2.1 gating modifiers can correct defects associated with disease-causing mutations in Cav2.1.

1838-Plat

Competition between Calcium Sensors on the Voltage-Gated Calcium Channel Cav1.2

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Voltage-gated calcium channel (CaV) activity is regulated by calcium sensors including calmodulin (CaM) and calcium-binding protein 1 (CaBP1). CaBP1 inhibits CaM-mediated calcium-dependent inactivation (CDI). We investigated the origins of functionally important differences between CaM and CaBP1 by creating a number of CaM/CaBP1 chimeras which suggest that both calcium sensors use their C-lobes for high affinity binding to the pore-forming CaV alpha-subunit, while their N-terminal lobe are responsible for the stark functional differences of the calcium sensors. CaBP1 and CaM are thought to modulate CaV function by competing for binding to the CaV C-terminal IQ-domain, but this assumption has never been tested directly. By determining CaV1.2 CDI in Xenopus oocytes under conditions with different ratios of CaM and CaBP1, we demonstrate direct competition between both calcium sensors for their CaV1.2 binding site. In order to extend our analysis of CaBP1/CaM competition we used isothermal titration calorimetry to determine the affinity of both CaM and CaBP1 in both calcium-bound and apo-states for the IQ domain, suggesting that competition occurs mainly in the apo-state. Overall, our data provide a framework for understanding how CaBP1 and CaM differentially regulate CDI on CaV1.2.

1839-Plat

Optically-Resolved Conformational Rearrangements of the Voltage-Sensing Domains of the Human $Ca_V1.2$ Channel

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Voltage-gated Ca^{2+} channels (Ca_V) consist of four tandem transmembrane repeats (I-IV), each including two pore-forming helices and four segments that make up a voltage-sensing domain (VSD). To understand voltage-dependent