both, CaVβ1a- and CaVβ2-subunits, fraction of low-P(O) mode periods increased when more CaVβ1a-subunits in relation to CaVβ2-subunits were expressed. As recordings were split into low-P(O) (CaVβ1a-like) and high-P(O) (CaVβ2-like) periods, the extent of inactivation was higher for the low-P(O) (CaVβ1a-like) mode.

Our results illustrate a functionally effective competition between CaVβ1a-subunits, suggesting that in cells that express different CaVβ isoforms, activity of a given channel can be dynamically regulated.

3089-Pos Board B194
Biophysical Properties of a Human Disease-Causing Mutation in CaV1.3 L-Type Calcium Channels

Mice lacking functional CaV1.3 L-type Ca\(^{2+}\)-channels show sinoatrial node dysfunction and are congenitally deaf. Here we investigated the functional consequences of a homozygote mutation (insertion of an amino acid residue in a pore-forming S6-helix) in the alpha1-subunit encoding the CACNA1D gene which causes bradycardia and deafness in humans.

We expressed human wildtype (WT) and mutant channel complexes (MUT) in tsA-201 cells (with alpha2delta and beta2-subunits) and recorded ON-gating (Q\(_{\text{on}}\)) and intrinsic Ca\(^{2+}\)-currents (I\(_{\text{Ca}}\)) using the whole-cell patch-clamp technique. Full length WT and MUT channel alpha1 subunit proteins were expressed at equal levels in tsA-201 membranes. In contrast to WT, MUT channels did not conduct significant I\(_{\text{Ca}}\), but gave clear rise to Q\(_{\text{on}}\). WT and MUT Q\(_{\text{on}}\) exhibited a typical nonlinear voltage-dependence of activation. Under identical experimental conditions (block of I\(_{\text{Ca}}\) by replacing Ca\(^{2+}\) with Mg\(^{2+}\) and addition of La\(^{3+}\) and Cd\(^{2+}\) in the recording solution) the half maximal activation voltage (V\(_{1/2}\)) of MUT Q\(_{\text{on}}\) was significantly shifted by 16.2 ± 2.8 mV (n=6-8, p<0.0001) to more negative voltages compared to WT. Toward I\(_{\text{Ca}}\), WT activated 11.5 ± 2.9 mV more positive than WT Q\(_{\text{on}}\) (n=7-8, p=0.0016). In addition, MUT Q\(_{\text{on}}\) kinetics were significantly faster than for WT e.g. as shortage time constants for gating current decay over a large voltage range (~10 ~ -50 mV).

The presence of charge movement in the absence of ionic currents implies that voltage-sensors in MUT channels move, but either fail to trigger pore opening or prevent conduction through open channels. The mutation is not located within voltage-sensing S4-helices, but within a region of S6 predicted to interact with the voltage-sensor. Compromising this functional module may uncouple voltage-sensor function from pore opening and allow voltage-sensor movements to occur faster and at more negative voltages.

3090-Pos Board B195
Role of the Putative Glycines Hinge of CaV3.3 Channels

Voltage-gated ion channels (VGIC) are proteins that form transmembrane pores in cell membranes. Based on the crystal structure of an open potassium channel (K\(_{\text{v}}\)) that reveals the binding of a glycine residue in the inner helix that lines the pore (M2 or S6), and the conserved pore sequences in many members of the VGIC superfamily, it is thought that the opening mechanism might be quite similar within the family. In fact, this has been demonstrated for a bacterial sodium channel (Nav). However, in LVA calcium channels, the mid-S6 hinge glycine residue is present only in IS6 and IS6, suggesting a likely different opening mechanism. Here, we explored this possibility in the CaV3.3 member of LWCA channels by performing a mutational analysis of the two conserved glycines (Gly385 and Gly814), and a valine (Val1383) in IS6 which, according to sequence similarity, corresponds to a hinge glycine in K\(_{\text{v}}\) and Nav channels.

As expected, when recordings were split into low-P(O) (CaVβ1a-like) and high-P(O) (CaVβ2-like) periods, the extent of inactivation was higher for the low-P(O) (CaVβ1a-like) mode. Substitutions by proline for glycines (G814P) and alanine (G814A), as well as the mutant V1383G, drastically decreased whole-cell current density, with diminishing current inactivation kinetics was slowed down by 50%, and the recovery was also more than two-fold slower. The substitution by proline also increased the channel closing time constant by 60%; this effect was also expressed by mutant V1383G, which in addition induced the strongest effect on the inactivation kinetics slowing (≥ 2-fold). Our preliminary results suggest that residues Gly814 and Val1383 are mostly involved in the inactivation gating and, in a lesser degree, stabilizing the open state of Cav3.3. Additional mutations, including double and triple substitutions, are currently under investigation.

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Mechanical Induced Inhibition of L-Type Calcium Channels
Angelo O. Rosa, Naohiro Yamaguchi, Martin Morad.

L-type calcium channels are modulated in different fashions by Ca\(^{2+}\) (Ca\(^{2+}\) dependent inactivation, Ca\(^{2+}\) dependent facilitation), cytosolic proteins (CAM, CAMKII, PKA, PKC, etc) and voltage (voltage dependent inactivation).

Here we describe a novel modulation of Ca\(^{2+}\) channel exerted by pressure/flow (PF) forces where 35-60% inhibition of IBa occurs when cells were exposed to 30 cm of PF forces. Only brief periods (100ms) of high PF applications were required to activate the response, but the effect was reversible and had a latency of ~500-700 ms. Similar data was obtained in HEK cells expressing all the recombinant subunits of Ca\(^{2+}\) channel. To determine the mechanism underlying the PF effect, the current through the channel was measured in cells treated with 10μM of thapsigargin, or ifP3R blocker APB-2 (10μM), or mitochondrial protonophore, FCCP + oligomycin, or high concentrations of BAPTA. We found no significant difference in effectiveness of PF pulses to inhibit Ib, or Ica in cell exposed to, thapsigargin. APB-2, FCCP, BAPTA, or a mixed cocktail of them. We concluded that native Ca\(^{2+}\) channel of rat ventricle myocyes or recombinant human variants of L-type Ca\(^{2+}\) expressed in HEK cells can be modulated by PF forces. This mechanism may represent a different physiological regulation of calcium channels in the heart and blood vessels.

3092-Pos Board B197
A Model of the Inner Pore of Ca Channels in the Open State
Gregory M. Lipkind, Harry A. Fozzard.

Known open state structures of K channels do not provide a useful template for open Ca channels, because the latter do not contain the hinge residues Gly and Phe which form the conserved pore sequence, but are in a different family. However, with the closed KvA structure (Doyle et al, Science 280:69, 1998) and sequentially outwardly displaced amino acids near the bundle crossing (Thr107-Ala111) and optimized the structures (restricted minimization with distance constraints). We then populated open channel structures with amino acid residues of T and P/Q channels and re-optimized. The Ca channel structures with openings of ~11 Å were similar and allowed both symmetrical TEA and verapamil to enter and block from inside. However, simulations were in disagreement with the MTSET accessibility data for the P/Q channel (Zhen et al, JGP 126:193, 2005). Noting that amino acid residues near C-termini of S6 helices of Ca channels have side chains facing the inner pore that are quite different from those in K channels, we speculated that these segments might contain intra-molecular deformations that lead to the reorientation of their side chains. We modeled these deformations by π-bulges, which produced wide turns, containing an additional amino acid residue. The very conserved Asn residues of Ca channels initiated the formation of π-bulges in the direction of the C-ends in all 4 S6 α-helices. Introduction of π-bulges achieved agreement between amino acid residues predicted to face the pore and MTSET accessibility data. Formation of π-bulges would be expected to stabilize the open state of the Ca channel, and MTSET modification of single cysteines at the C-ends of S6’s could produce physical occlusion of the inner pore, i.e. full block of Ca current as observed experimentally. Supported by R01HL065680.

3093-Pos Board B198
Signaling Complex of Calcium/Calmodulin-Dependent Protein Kinase II Associated with the C-Terminal Domain of CaV2.1 Channels

Abstract:
Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) forms a major component of postsynaptic density, where its functions are well established but the presynaptic actions of CaMKII are poorly defined. We show that CaMKII constitutively binds and modulates presynaptic voltage-gated calcium channels (CaV2.1) channels that conduct P/Q type calcium currents. Using co-immunoprecipitation methods, we isolated a signaling complex of Cav2.1/CaMKII containing synapsin 1. We observed more than a six-fold increase in the synapsin 1