Dyhydropyridines (DHPs) is a major class of L-type calcium channel (LCC) ligands, which have boat-like six-membered ring with NH-group at the stern, aromatic moiety at the bow, and various substituents at the port and starboard sides. DHPs demonstrate antagonistic or agonistic action, which was previously explained as stabilization or destabilization, respectively, of the closed-gate state by the hydrophilic or hydrophobic port-side substituent. Here we used Monte Carlo energy-minimizations to dock various DHPs in the open-LCC model (Tikhonov & Zhorov, 2008). The calculations suggest a novel structural model in which agonistic and antagonistic actions are determined by different parts of the DHP molecule and have different molecular mechanisms. In our model, DHP polar moieties at the stern, bow, and starboard form H-bonds with side chains of Tyr_IVS6, Tyr_IIIS6, and Gln_IIIS5, respectively. The aromatic moiety at the bow binds to Phe_IIIP. We propose that these contacts with the well-known DHP-sensing residues stabilize the channel's open-gate conformation. Since these contacts are common for various DHPs, our model explains why both agonist and antagonists increase probability of the long-lasting channel openings and why even partial disruption of the contacts eliminates the agonistic action. In our model, the port-side is exposed to the permeation pathway and approaches the selectivity filter. Hydrophobic ports-side group of antagonists may induce long-lasting channel closings by destabilizing calcium coordination with the selectivity-filter glutamates in domains III and IV. In contrast, agonists, which have either hydrophilic or no substituent at the port-side, lack this destabilizing effect. Our model explains action of DHPs with diverse substituents. Thus, long substituents at the port-side are readily accommodated in the pore. Long substituents at the starboard-side protrude in the III/IV domain interface, explaining activity of DHPs linked to a permanently charged group. Supported by CIHR.

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Reinterpretation of SCAM Data in View of Kv1.2-based Models of MTSET-Substituted CaV2.1 Channels

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Molecular modeling of calcium channels, which are important drug targets, relies on sequence alignments with potassium channels. Huber et al. (2000) and Zhorov et al. (2001) proposed alignments for outer and inner helices, respectively. Zhen et al. (2005) attempted to identify pore-lining residues in CaV2.1 channel using the substituted-cysteine accessibility method (SCAM) and interpreted their data as inconsistent with known sequence alignments. Indeed, the inner-helix residues in positions *i15*, *i18*, *i19*, and *i22* face the Kv1.2 pore. In agreement with this, 2-(trimethylammonium)ethyl methanethiosulfonate (MTSET) applications to CaV2.1 with engineered cysteines in positions *2i18* (domain 2, inner-helix position 18)*, 4i18,* and positions *i15* and *i19* of all four domains decreased current. However, CaV2.1 with cysteine substitutions in positions *1i18*, *3i18* and position *i22* of all four domains were not blocked by MTSET. Furthermore, despite the outer-helix positions *2o10* and *4o10* are far from the pore, corresponding cysteine substitutions were blocked by MTSET. Here we created the Kv1.2-based model of CaV2.1 using the above alignments. In this model, engineered cysteines in positions *i22* are surrounded by large hydrophobic residues, which would preclude cysteine ionization and hence reaction with MTSET. We further created CaV2.1 models with MTSET-substitutions in other positions and used Monte Carlo-energy minimizations to find energetically optimal conformations. The ammonium group of MTSET in positions *2i18* and *4i18* occludes the inner pore, while in positions *1i18* and *3i18* it protrudes in the domain interface. The ammonium group of MTSET in positions *2o10* and *4o10* approaches the pore, being closer to the pore axis than that in positions *1i18* and *3i18*. Thus, our model reinterprets experiments of Zhen et al. (2005), validates the above alignments, and suggests a similar folding of voltage-gated potassium and calcium channels. Supported by CIHR.

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Modeling L-type Calcium Channel with Phenylalkylamines

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Phenylalkylamines (PAAs), a major class of L-type calcium channel (LCC) blockers, have aromatic rings A and B connected by a flexible chain with cyano and ammonium groups proximal to rings A and B, respectively. Structural aspects of ligand-channel interactions remain unclear. We have built LCC models as in (Tikhonov and Zhorov, 2008) with KvAP, MthK, Kv1.2 and KcsA as templates and used Monte Carlo energy-minimizations to dock devapamil, verapamil, and gallopamil, which have three, four, and five methoxy groups, respectively. The PAA-LCC models have the following common features: meta-methoxy group in ring A accepts an H-bond from Y1179(3i10) in domain 3 inner-helix position 10, the ammonium group is stabilized at the focus of P-helices, and the cyano group coordinates a Ca2+ ion bound to the selectivity-filter glutamates in domains 3 and 4. The latter feature can explain the well-known effect of Ca2+ potentiation of PAA action. Our models are also consistent with structure-activity and mutational studies. For instance, mutation of Y1490(4i11) affects action of devapamil, but not verapamil and gallopamil (Johnson et al., 1996). In our models, the single meta-methoxy group in ring B of devapamil accepts an H-bond from Y1490(4i11), while meta- and para-methoxy groups in ring B of verapamil and gallopamil chelate the Ca2+ ion. Mutation T1066(3o14)Y in domain 3 outer-helix position 14 enhances action of devapamil and verapamil, but not gallopamil (Huber et al., 2004). Our models predict that para-methoxy group in ring A of devapamil and verapamil accepts an H-bond from Y3o14, while tri-methoxylated ring A of gallopamil is too bulky to approach Y3o14. Docking of devapamil in different models shows that the Kv1.2 template is most consistent with the experimental data. The closed (KcsA-based) model has the same ligand-channel contacts, but with weaker interaction energy. Supported by CIHR.

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Effect Of $Ca_V\beta$ Subunits On Structural Organization Of $Ca_V1.2$ Calcium Channels As Revealed By Three-color Fret Microscopy

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Voltage-gated $Ca_v1.2$ calcium channels play a crucial role in Ca^{2+} signaling. The poreforming α_{1C} subunit is regulated by accessory $Ca_v\beta$ and $\alpha_2\delta$ subunits. $Ca_v\beta$'s are cytoplasmic proteins of various size encoded by four different genes ($Ca_v\beta_1$ - β_4). Here we investigated the effect of three major $Ca_v\beta$ types, β_{1b}, β_{2d} and β_3, on the structure of $Ca_v1.2$ by measuring inter and intramolecular distances between α_{1C} and β in the plasma membrane of COS1 cells using three-color FRET microscopy. The results show that $Ca_v1.2$ channels are in close proximity in the plasma membrane. The presence of different $Ca_v\beta$'s does not result in significant differences in intramolecular distance between the termini of α_{1C}, but significantly affects intermolecular distance between the termini of neighbor α_{1C} subunits, which varies from 67 Å (β_{1b}) to 79 Å (β_3). Thus, our results show conclusively that plasma-membrane density of $Ca_v1.2$ channels depends on the type of $Ca_v\beta$'s present, suggesting a possible mechanism contributing to differences in Ca^{2+} signaling between various cell types.

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Quantification Of L-type Ca Current Inactivation Mechanisms In Trout Ventricular Myocytes.

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Inactivation of L-type calcium current (I_{Ca}) is due to two mechanisms: voltage-dependent inactivation (VDI) and calcium-dependent inactivation (CDI). In fish cardiac myocytes, it is unknown whether Ca release from the sarcoplasmic reticulum (SR) participates in CDI of I_{Ca}. This study assesses the relative contribution of different inactivation mechanisms of I_{Ca} in trout ventricular myocytes. Trout ventricular myocytes were enzymatically isolated. I_{Ca} was recorded using whole cell patch clamp with Na- and K-free solutions to avoid contaminating currents. With a low concentration of a slow Ca buffer (EGTA 2mM) in the pipette solution, I_{Ca} inactivated slowly (compared to mammalian cardiac myocytes): the time to reach 37% of peak current (T_{37}) was 26.2 ± 2.4 ms (mean $\pm$ SEM, n=14). When a fast Ca buffer (BAPTA 10 mM) was present in the pipette solution I_{Ca} decay was similar to the decay in the presence of EGTA (T_{37}: 25.4 ± 1.5 ms, NS, t-test, n=9). When barium was used as a charge carrier, I_{Ba} inactivates mainly via VDI and T_{37} was significantly increased (43.7 ± 3.1 ms, n=9, $p<0.05$, t-test), albeit T_{37} is twice faster than in mammalian cardiac myocytes. We quantified the relative contribution of VDI and CDI according to the method previously described [Brette *et al.* (2004). *Circ Res;* **95;** e1-7]. We measured the fraction of current remaining 20 ms after its peak (I_{R20}). I_{R20} was 0.39 ± 0.03 in EGTA, 0.42 ± 0.02 in BAPTA and 0.64 ± 0.03 in barium. We estimated that CDI represents ~39% of total fast I_{Ca} inactivation, and that SR Ca release causes only ~12% of CDI. We conclude that the main inactivation mechanisms in the trout myocyte are due to VDI and CDI from Ca entering the cell via I_{Ca} and not SR Ca release. *Supported by the Wellcome Trust and the BBSRC.*

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Egg Coat Proteins Activate Ca^{2+} Entry into Mouse Sperm via CATSPER Channels

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During mammalian fertilization, the contact between sperm and egg triggers increases in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in sperm. Voltage-gated Ca^{2+} channels (Ca_Vs) are believed to mediate the initial phase of $[Ca^{2+}]_i$ increases in sperm induced by egg coat (zona pellucida, ZP) glycoproteins, while