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Molecular Weight and Volume at Position 46 in Transmembrane Domain 1 (TM1) are Important Determinants for Ethanol Sensitivity in P2X4 Receptors

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Purinergic P2X receptors (P2XRs) are a family of fast acting, cation-permeable ligand-gated ion channels, which are gated by synaptically released adenosine 5′-triphosphate (ATP). Building evidence supports the notion that P2XRs play a role in mediating and/or modulating behavioral effects of ethanol. However, this work is in the early stages and the sites and mechanisms of ethanol action in P2XRs are poorly understood. Recently, we identified an amino acid located in TM1 that was conserved across P2X3 and P2X4Rs that appeared to represent an important site for the action of ethanol (i.e., Trp41 and Trp46, respectively). The current study tests the hypothesis that physical-chemical properties of the residue at position 46 play a significant role in determining ethanol sensitivity in P2X4Rs. We expressed wildtype and mutated receptors in Xenopus oocytes and determined changes in ethanol sensitivity (200mM) using an 8-channel two-electrode voltage clamp system (OpusXpress 6000; -70mV). Exchanging Trp46 residue with other aromatic residues did not significantly alter ethanol sensitivity whereas replacing Trp46 with aliphatic residues significantly reduced the action of ethanol. Correlation analysis determined that molecular weight and volume of the residue at position 46 were significantly correlated with ethanol sensitivity whereas polarity or hydrophobicity was not. The findings suggest that the size and aromaticity of the residue at position 46 in TM1 play an important role in determining ethanol sensitivity of P2X4Rs. Identifying physical-chemical properties of residues that are important for ethanol sensitivity will increase our knowledge regarding structural requirements that are necessary for ethanol to cause changes in modulation and/or transduction of agonist action in P2X4Rs. Support: NIAAA/NIH F31 AA017029, KO1 AA017243-01A1, AA013922, AA03972 and USC School of Pharmacy.

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Lack of Ethidium Bromide Uptake during Hypotonic Stress in HEK 293 Cells that Express P2X7 Receptors

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Recent reports indicate that the long known ethidium bromide uptake resulting from prolong stimulation of cell surface $P2X_7$ receptors is due to Pannexin-1 (Panx-1) hemichannels, which are probably activated by the carboxy terminus of the receptor itself. It has also been proposed that Panx-1 is activated by exposing the cells to hypotonic solutions without the need for $P2X_7$ receptor activation and that this maneuver results in ATP release from cells probably via Panx-1. However, other groups have previously proposed that the hypotonic-induced ATP release is via cell swelling-activated chloride channels. In this work we explored activation of endogenous Panx-1 in HEK 293 cells (untransfected or transfected with $P2X_7$ receptors) exposed to hypotonic solutions, while simultaneously measuring whole cell chloride current and fluorescence signals resulting from ethidium bromide uptake (an index of Panx-1 activation). When HEK cells were exposed to hypotonic solutions that induced activation of cell swelling-activated chloride current ($I_{Cl,swell}$) no ethidium bromide uptake was detected. Treatment of the cells with the Panx-1 inhibitor mimetic peptide ^{10}panx1 resulted in complete inhibition of ethidum bromide uptake induced by ATP activation of $P2X_7$ receptors without affecting $I_{Cl,swell}$. Pretreatment with carbenoxolone (CBX), another blocker of Panx-1, inhibited ethidium bromide uptake induced by stimulation of HEK cells expressing $P2X_7$ receptors and at the same time inhibited $I_{Cl,swell}$ in a dose-dependent manner. We conclude that hypotonic conditions most likely induce ATP release by via activation of CBX-sensitive volume-sensitive chloride channels without participation of Panx-1 hemichannels.

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A symmetric structural model for Acid sensing ion channel-1: Transmembrane domain dynamics and Implications to Gating

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Acid sensing ion channels (ASICs) are cation-selective neuronal membrane channels, activated by H+ binding upon decrease in extracellular pH. The mechanistic and structural details of channel activation and ion permeation in ASICs are only partially understood. The only known crystal structure for ASICs is in the desensitized, non-conducting state, and the open and closed structures remain unsolved. The crystal structure reveals ASIC1 to be a trimer with 3-fold symmetry, but reports significant asymmetry in the transmembrane (TM) region, suggested to be induced by crystal lattice contacts, hence restricting further hypotheses about TM behavior. In order to enable a study of TM domain dynamics, we sought to derive models from the crystal structure, where this asymmetry was corrected using a simulation based approach. We designed a model target based on normal mode analysis, and employed targeted molecular dynamics to generate the initial model from the crystal structure followed by a series of simulations to equilibrate the structure. The resulting model, though starting with nearly straight TM helices, exhibits kinks as the simulations proceed, suggesting dynamic hinges in the TM pore, which could be participants in gating. Observations about channel behavior from these models show consistence with experimental data. An interesting revelation from the model is the identification of a possible gating region near the cytoplasmic end of the TM pore constituted of highly hydrophobic residues. Overall the study reveals several details about the putative cation permeation pathway in ASICs and also provides a symmetric structural model to test further hypotheses on ASIC channel function.

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Analysis of IP_3 Receptor Activation Using Novel Partial Agonists

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Inositol 1,4,5-trisphosphate receptors (IP_3R) are ubiquitous intracellular Ca^{2+} channels. Each subunit of the tetrameric IP_3R comprises a N-terminal IP_3-binding site, a cytosolic domain and six transmembrane domains (TMD) near the C-terminal. TMD5 and 6 form the pore. IP_3 binding to the IP_3-binding core (IBC, residues 224-604) initiates conformational changes that lead to opening of the pore. The mechanisms are unresolved but require the suppressor domain (SD, residues 1-223) and interactions between N and C termini. We have synthesized a family of 2-*O*-modified IP_3 analogues that are partial agonists of the IP_3R. In defining their properties, we identify a novel form of partial agonism that allows us to define key steps in IP_3R activation. By combining analysis of ΔG for ligand binding, single-channel recordings and molecular modelling we show that these partial agonists are IP_3-like in their interactions with the IBC, but they less effectively rearrange its relationship with the SD. The partial agonists open the channel at slower rates than full agonists. IP_3R with a point mutation in the SD that occupies a position similar to the 2-*O*-substituent of the partial agonists has reduced open probability that is similar for full and partial agonists. This suggests that bulky or charged substituents at the IBC-SD interface, whether provided by the ligand or SD, frustrate an obligatory coupling between IBC and SD. Our analysis of ΔG for ligand binding to truncated IP_3R shows that conformational changes initiated by IP_3 binding propagate entirely via the SD to the pore. We suggest that IP_3 first closes the clamp-like structure of the IBC, then the IBC interacts with the SD to give a compact structure that allows the SD alone to signal onwards to the pore.

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Single-Channel Kinetic Analysis for Activation and Desensitization of Homomeric 5-HT_{3A} Receptors

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The 5-HT_{3A} receptor is a member of the Cys-loop family of ligand-gated ion channels. Due to its low conductance, kinetic analysis of this receptor has been restricted to the macroscopic level. We introduced mutations in the 5-HT_{3A} subunit to obtain a high-conductance form so that single-channel currents can be detected. At all 5-HT concentrations (>0.1 μM) channel activity appears as opening events in quick succession forming bursts, which, in turn, coalesce into clusters. By combining single-channel and macroscopic data we generated a detailed kinetic model that perfectly describes activation, deactivation and desensitization. The model shows that full activation arises from receptors with three molecules of agonist bound. It also reveals an earlier conformational change of the fully-liganded receptor (flipping) that occurs while the channel is still closed. From this pre-open state the receptor enters into an open-closed cycle involving three open states, which conforms the cluster whose duration parallels the time constant of desensitization. This suggests that at a synapse the lifetime of the elementary response of 5-HT_{3A} receptors is determined mainly by desensitization. Since the desensitized state is a stable state, the inter-response latency is expected to be prolonged. The present kinetic model provides a foundation for studying molecular mechanisms of drug action. We show that mutations at valine 10′ of M4 affect opening and closing rates