ms and τ_{slow} ~900 ms). Slowing of deactivation by cAMP was slight for Mode I (~2-fold increase in τ_{fast}) in contrast with the ultra-sustained activation properties of Mode II (~9-fold increase in τ_{slow} to ~8 s). The voltage-dependence of Mode I versus Mode II deactivation was also markedly different (accelerating <1.5-fold versus ~4-fold respectively for 20-mV depolarization) near −40mV where ultra-sustained activation is most prominent. Thus, it is not Mode I states but specifically Mode II states whose kinetic stability shows the strong dual dependence on voltage and cAMP responsible for ultra-sustained activation.

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Electrophysiologic Characterization of a Complex hERG Channel Activator

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Novel and specific activators of the human ether-a-go-go-related gene (hERG) K^+ channel have been reported recently to enhance hERG current amplitude (4 synthetic small molecules and one naturally occurring substance). Here, we characterize the effects of a novel compound (Abbott-x) on atrial and ventricular action potentials (using microelectrode techniques) and cloned hERG channels stably expressed in HEK-293 cells (using whole cell patch clamp techniques). Abbott-x shortened cardiac action potentials and enhanced the amplitude of the hERG current in a concentration- and voltage-dependent manner. The fully activated current-voltage relationship revealed that this compound (60 uM) increased both outward and inward K^+ current. The slope conductance of the linear portion of the fully activated I-V relation was increased in the presence of the compound. Abbott-x significantly reduced the time constants (τ) of hERG channel activation at two example voltages tested (-10 mV: $\tau = 100 \pm 17$ vs 164 ± 24 ms, $n = 6$, $P < 0.01$; +30 mV: $\tau = 16.7 \pm 1.8$ vs 18.9 ± 1.8, $n = 5$, $P < 0.05$) and shifted the voltage-dependence for hERG activation in the hyperpolarizing direction by 9 mV ($n = 7$, $P < 0.01$). The time course of hERG channel deactivation was significantly slowed at multiple potentials tested (−120 to −70 mV). Abbott-x also significantly reduced the rate of inactivation and shifted the voltage dependence of inactivation in the depolarizing direction by 15 mV ($n = 5$, $P < 0.05$). Recovery of hERG channel from inactivation was not significantly affected by Abbott-x. In conclusion, Abbott-x enhances hERG current in a complex manner by facilitation of activation, reduction of inactivation, and slowing of deactivation, and abbreviates atrial and ventricular repolarization.

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Membrane Localization of S4 Transmembrane Segment of Voltage-Gated Ion Channels

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Voltage sensor domain (VSD) plays a key role in the channel conductance of the voltage-gated ion channels. Out of four transmembrane segments in VSD, cationic fourth transmembrane segment (S4) is the principal voltage sensor. Various models based on X-ray crystallography and site directed mutagenesis studies have shown that S4 segment, in response to the membrane potential, undergoes a conformational rotatory motion in the hydrophobic core of the lipid bilayer. Functional studies using whole-cell/ patch clamp techniques have detailed the conductance properties of the channel. However, the exact mechanism of membrane interaction and orientation of the cationic S4 segment in the non-polar lipid bilayer is yet to be understood. Direct experimental evidence using native S4 segment and liposomes, without any other energetic cofactors, would allow a direct test of the underlying mechanism of localization of S4 in the lipid bilayer. We used various fluorescence techniques to study the interaction and penetrative depth of the native S4 peptide in the lipid bilayer. Detail information concerning autonomous partition of S4 peptides in lipids will be discussed.

Ligand-gated Channels

2503-Pos Board B473

Accessibility of Ag^+ in the Pore of P2X Receptor Channels

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P2X receptor channels are extracellular ATP gated cation channels composed of three identical or related subunits. In mammals, seven subunits have been cloned ($P2X_1$-$P2X_7$). Each subunit has two transmembrane (TM) helices flanking a large extracellular domain that contains the ATP binding site, with the NH_2 and COOH termini on the intracellular side of the membrane. To investigate the mechanism underlying gating, we recently mutated one at a time each residue in the two TMs to cysteine, and measured the rate of modification by extracellular methanethiosulfonate (MTS) when channels are either closed or activated by ATP. Whereas only one residue in the extracellular end of TM1 is modified by MTSET, several residues are modified in TM2 in a state-dependent manner. These results suggest that TM2 makes substantial contribution to lining the pore and that the external region of TM2 forms a barrier for MTSET [1]. We now report on the accessibility of Ag. Several residues in both TMs are modified by Ag in the open state, although high modification rates were only seen at a number of positions in TM2. The modification pattern by Ag supports the results with MTSET that the pore is primarily formed by TM2 and that the external region of TM2 forms a gate for small ions. In addition, the results suggest that in the open state there is a crevice between TM1 and TM2 that is accessible to ions. The implications of these findings for the gating motion of the TMs will be discussed.

1. Li, M., Chang, T.-H., Silberberg, S.D. and Swartz, K.J. (2008) Gating the pore of P2X receptor channels. Nature Neuroscience 11, 883-887.

2504-Pos Board B474

Mutation Analyses Of The Critical Regions For The Voltage And [ATP] Dependent "Gating" Of $P2X_2$ Receptor Channel

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$P2X_2$ is an extracellular [ATP] gated cation channel with 2 transmembrane (TM) regions. In spite of lacking a voltage sensor domain, inward current through it in the steady state after ATP application shows gradual increase upon hyperpolarization, implying "voltage-dependent gating". We analyzed the tail current and activation time constant using Xenopus oocytes under two electrode voltage clamp and observed that increasing [ATP] shifted conductance (g)-V relationship towards depolarized potentials and accelerated activation kinetics. The results show that in steady state after ATP application, the gating was dependent on both voltage and [ATP]. We aimed at identifying the critical region for the gating and first introduced mutations to the ATP binding region with a hypothesis that a negatively charged ATP itself or its complex with the binding site could be an origin of voltage dependency and introduced K71A/R, K69A/R, R290A/K, K308A/R mutants. Analyzed mutants showed no clear [ATP] dependent acceleration of the activation upon hyperpolarization. Except for K69R, no remarkable [ATP] dependent shift of g-V relationship was observed. Secondly, we focused on the linker region between the ATP binding site and 2nd TM. In G311A and G320A, [ATP] dependent g-V shift to depolarized potentials was much more prominent than in WT. In contrast, G311P and G320P had no clear g-V shift by [ATP], indicating an essential role of the glycine residues in the linker region. Thirdly, introduced mutations in TM regions F44C, Y47C in TM1 and T339S in TM2 were devoid of activation phase and no more voltage dependent gating was observed at higher [ATP]. Taken together, these results suggest the critical involvement not only of the ATP binding region but also the linker and the TM regions in voltage and [ATP] dependent "gating".

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A Single Amino Acid Mutation Turns a P2X3 Antagonist into an Agonist

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Purinergic P2X3 receptors (P2X3Rs) are ATP-gated cation channels expressed mainly in sensory neurons. Suramin inhibits P2X3R currents of several species and provides a useful tool for *in vitro* studies of P2X antagonism. A rhesus homolog of P2X3R was cloned, stably expressed, and pharmacologically characterized. Suramin was a more potent inhibitor of rhesus P2X3R (IC50: 0.4 μM) than of human P2X3R (IC50: 8.6 μM). Surprisingly, suramin activated rhesus P2X3R at higher concentrations (EC50: 4.7 μM). In contrast, suramin did not activate human P2X3R at similar concentrations. Other than differences in suramin agonism, the kinetic and pharmacological profile of rhesus and human P2X3Rs were similar. To investigate the molecular basis of suramin agonism with P2X3R, we generated single-point mutations in the P2X3R. Only four amino acid differences exist between rhesus and human P2X3Rs (S67F, L127F, L144F, and T162M). Mutant receptors were transiently expressed in HEK293 cells and their currents measured using an automated patch clamp instrument (PatchXpress, MDS Analytical Technologies, USA). A single amino acid mutation of human P2X3R (S67F) allowed suramin to act as an agonist and increased the potency of suramin. A corresponding mutation (F67S) in the rhesus P2X3R resulted in a loss of suramin agonism and a decrease of its inhibitory activity. This suggests that position 67 is critical for suramin modulation of P2X3R activity and that inhibition and activation by suramin may be mechanistically related.