the same receptor mediates such opposing effects. To address this question, in this study we combined electrophysiological recordings of rat P2X7R current using an ultrafast application system for agonist delivery and removal and confocal imaging studies using the YFP-tagged P2X7R and CFP-tagged endoplasmic reticulum (ER) and Golgi markers. The rates of receptor activation and deactivation were consistent with a previously proposed hypothesis of high and low affinity ligand binding sites at P2X7Rs. Activation of high affinity sites resulted in low amplitude slowly desensitizing currents and internalization of receptors. On the other hand, activation of low affinity sites led to a secondary current growth and a sustained rise in calcium, the plasma membrane blebbing, and increase in the cell volume, resulting in cytolysis during the sustained receptor occupancy. These plasma membrane events were associated with broadening and separation of ER tubes, their fragmentation, vesiculation, and fusion of vesiculated ERs, whereas the structure of Golgi apparatus was not affected. Removal of agonist facilitated retraction of blebs and reversed the cytolytic cascade but did not stop the ongoing disruption of the ER morphology. These results suggest that the level of saturation of the ligand binding sites and duration of stimuli determines the nature of the P2X7R gating and actions. The results further indicate that the plasma membrane blebbing and cytolytic effects are independent of disruption of ER morphology and that the ER stress response is probably coupled with apoptosis.

3654-Pos

Redox Modulation of ATP-Gated P2X7 Currents

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Activation of P2X7 receptors is known to initiate downstream signaling processes including the release of proinflammatory cytokines and reactive oxygen species (ROS). Recombinant and native P2X7 receptors have been observed to exhibit time-dependent changes in current amplitude, an effect observed both during prolonged continuous ATP exposure and during short repeated applications of adenosine 5′-triphosphate (ATP). We used patch clamp electrophysiology in the whole cell perforated patch configuration to test the hypothesis that this time-dependent change in current amplitude reflected changes in the redox environment of membrane P2X7 receptors. In HEK293 cells expressing recombinant P2X7 receptors, we found that short repeated applications of ATP (1 s exposure every 60 s) evoked currents that increased and/or decreased in peak amplitude for several minutes before reaching a reproducible steady state amplitude. In cells that ultimately exhibited a net reduction in peak current amplitude over time, we observed that exposure to the membrane permeable reducing agent, DTT (1 mM, 1 min), significantly increased the peak current amplitude of subsequent ATP-evoked responses. We repeated the experiment with the endogenous reducing agent, glutathione, and this chemical also potentiated the amplitude of ATP-gated currents in these cells. In all cells, exposure to the oxidizing agent, hydrogen peroxide, was observed to reduce the amplitude of ATP-gated currents. In summary, ATP-gated currents through P2X7 receptors appear to be sensitive to modulation by redox chemicals.

3655-Pos

Identifying the Ion Access Pathway to the Transmembrane Pore in P2X Receptors

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P2X receptors are trimeric ion channels activated by extracellular ATP. Upon activation, P2X receptors promote inward current of cations to evoke action potentials or trigger calcium mediated signaling that are important for pain sensing, inflammation, and the synaptic transmission. However, the molecular mechanism of how extracellular ions access the transmembrane pore of P2X receptors is unknown. According to the zebrafish P2X4 crystal structure in the closed state, extracellular ions appear to be readily accessible to the pore through three identical fenestrations located right above the membrane leaflet (lateral pathway). In addition, ions may access the pore through a second possible pathway that runs through the central voids along the molecular three-fold axis of symmetry (central pathway). While this pathway is hypothetical, as the constrictions flanking the central voids are too narrow for hydrated ions to pass (~2.3 Å), agonist binding may expand these constrictions to enable ions to access the transmembrane pore. We have begun to explore the pathway ions use to move through the extracellular domain to enter the pore by inserting cysteine residues into rat P2X2 receptor channels and measuring reaction rates with a range of thiol-reactive reagents and ions. We found that MTSEA-Texas Red (MW=~750) can access T336 in the transmembrane pore with an apparently fast modification rate when the channel is open. These results suggest a large access pathway exists between the extracellular solution and the transmembrane pore, consistent with the fenestrations observed in the crystal structure. We are currently testing whether reagents of various size may also move through the central pathway.

3656-Pos

Characterization of Shark ASIC1b, an Ancient Form of an Acid-Sensing Ion Channel

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Acid-sensing ion channels (ASICs) are cation-permeable membrane proteins activated by extracellular H^+. They belong to the class of DEG/ENaC channels and share a common topology with cytosolic termini, two transmembrane domains and a large extracellular loop. ASICs are present in the genome of chordates but are absent in other animals. So far, functional ASICs that are gated by protons were cloned from bony fish, chicken and mammals. In contrast, ASICs from urochordates, jawless vertebrates, cartilaginous shark were shown to be H^+-insensitive, suggesting that proton-gating evolved relatively late in bony fish and that primitive ASICs have a different gating mechanism. Recently, amino acids that are crucial for proton-gating of ratASIC1a have been identified; these amino acids are conserved in an ASIC from the shark *Squalus acanthias* (sharkASIC1b).

Here we show that, contrary to previous findings, sharkASIC1b is gated by protons. This result shows that the conservation of the amino acids crucial for proton-gating can predict proton-sensitivity of an ASIC. The sharkASIC1b current is half-maximally activated pH 6.0 and is blocked by amiloride. It desensitizes quickly but incompletely, efficiently encoding transient as well as sustained proton signals at pH values between 7.0 and 6.2.

Since ratASIC1a desensitizes approximately 100-fold slower than sharkASIC1b but completely, we started a chimeric approach swapping regions between sharkASIC1b and ratASIC1a to identify the amino acids determining speed of desensitization and the unique sustained current. Functional chimeric channels point towards two separate regions in the large extracellular domain accounting for these two characteristics.

3657-Pos

The β1-β2 Linker in the Extracellular Domain of ASIC1 Determines Desensitization of ASIC1

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ASICs are proton-activated channels expressed in the nervous system of all chordates. Despite high amino acid conservation of the ASICs we have observed significant functional differences among these channels. For instance, ASIC1 from fishes and amphibian are more sensitive to desensitization by preconditioning pH than the mammalian channels. Studies of fish, shark, and frog ASIC1 show that these channels are completely desensitized by pH $\geq$7.3. We identified three residues in the linker connecting b1 to b2 in the extracellular domain of ASIC1 (corresponding to positions P82, N83, and M84 in the frog sequence) that are responsible to this property. Mutations of those residues for the corresponding ones in rat ASIC1 shift the preconditioning pH from 7.5 to 7.3 and decrease the rate of decay of the peak currents from 3.5 ± 0.2 s-1 to 1.6 ± 0.2-1. Out of the three indicated residues, the one in position 84 has the largest effect in desensitization. Similar results were obtained in shark ASIC1 and in elephant shark ASIC1. We conclude that the b1-b2 linker is an important determinant of the rate of ASIC1 desensitization thereby it may undergo conformational changes during the desensitization process. From a physiological point of view, the b1-b2 linker sets the pH range wherein these channels are functional. The results are also consistent with the notion that the b1-b2 linker has evolved to optimize the response of ASIC1 to the range of physiological extracellular pH of each species, which is higher in amphibians and fishes than in mammals.

3658-Pos

Multisite Binding of Anesthetics to GLIC, a Pentameric Ligand-Gated Ion Channel

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Volatile and intravenous anesthetics inhibit channel function of nicotinic acetylcholine receptors (nAChRs).Here we report the putative general anesthetic binding sites in *Gloeobacter vioaceus* pentameric ligand-gated ion channel (GLIC), a bacterial homolog of nAChR, using fluorescence quenching, multi-ns molecular dynamics (MD), and docking analysis. Fluorescence