The molecular details of ion channel regulation by G proteins remain unknown. A first step in this direction is to define the characteristics of interacting proteins of known structure in isolation and in complex form. The three-dimensional structure of a GIRK1-chimera determined by single particle electron microscopy at 25Å is consistent with the crystal structure (Nishida et al., 2007, EMBO J 26:4005-15). We have functionally reconstituted this GIRK1-chimera into a 1:1 ratio of phosphatidylethanolamine to phosphatidylserine planar lipid bilayers. The GIRK1-chimera produces a conductance of approximately 23 pS that shows $Mg^{2+}$-dependent inwardly rectifying $K^+$ currents and an absolute requirement on the presence of phosphatidylinositol-4,5-bisphosphate for activation. These currents are blocked by $PIP_2$ antibody and poly-lysine applied from the cis but not the trans side. Moreover, the channel shows a high affinity for diC8-$PIP_2$ ($EC_{50}$ ~ 7.5 μM). GIRK1-chimera channel currents are blocked by $Ba^{2+}$ and the GIRK peptide blocker tertiapin when applied from the trans but not the cis side. Interestingly, Gβγ applied from the cis side inhibits GIRK1-chimera currents and shifts phosphoinositide sensitivity by decreasing the apparent affinity to $PIP_2$. This is in contrast to the Gβγ effects on full-length GIRK1* channels assayed in *Xenopus* oocytes or planar lipid bilayers.

**3649-Pos**

**Expression and Purification of Recombinant Human Inward Rectifier $K^+$ (KCNJ) Channel**

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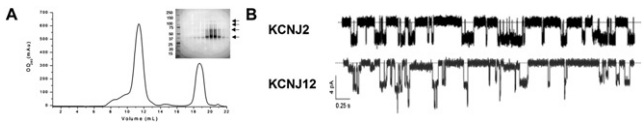
Inward rectifier potassium (KCNJ) channels regulate vital cellular processes including cell volume, electrical excitability, and insulin secretion. Dysfunction of different isoforms has been linked to numerous diseases including Bartter's, Andersen-Tawil, Smith-Magenis Syndromes, diabetes, and epilepsy. We succeeded in expressing 10 of 11 human KCNJ channels tested in *Saccharomyces cerevisiae* under Gal1-inducible promotion. GFP-fusion proteins are located in the plasma-membrane, suggesting the protein is correctly folded and trafficked. Following large scale expression of Kir2.x family members, a 2-step purification process can be used to isolate protein to >95% in a mono-dispersed form (Fig.1A). $^{86}Rb^+$ flux assays and patch clamp analysis on reconstituted proteins confirm the functionality of the purified proteins as inward rectifier potassium channels. For KCNJ2 (Kir2.1) and KCNJ12 (Kir2.2) channels, the unitary conductance in 150mM symmetrical [$K^+$] (~33pS and ~40.5pS, respectively, Fig.1B at −100mV), sensitivity to spermine block, and activation by $PIP(4,5)_2$ resemble those observed in eukaryotic membranes. The high-level purification and reconstitution of these proteins makes feasible not only ongoing biochemical and structural analysis of eukaryotic KCNJ channels, but also the analysis of channel function in the absence of modulator proteins, and in membranes of defined composition.




**3650-Pos**

**Purified Hetero-Tetramers of the Potassium Channel Kcv Revealing Independent Subunit Contribution to the Tea Block**

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Tetraethylammonium (TEA) is a common molecular probe in detecting potassium channel blocking. The external TEA binding affinity has been proposed to be highly related to the aromatic residue located at the outer mouth of the potassium channels such as Y82 in KcsA and Y449 in Shaker, probably due to the π-cation interaction between TEA and the aromatic side chain. In this report, we identified the highly sensitive TEA block for the chlorella virus-encoded Kcv, a miniature model K+ channel that only consists of 94 amino acids with two transmembrane domains and a conservative selectivity filter. By mutagenesis screening at Leu70 of Kcv, which is equivalent to the TEA site Y82 in KcsA, we found substitution of Leu70 to all other amino acids including Tyr, Phe and His will reduce the TEA affinity, suggesting a more complicated mechanism beyond cation-π interaction involved in TEA blocking. We further developed a novel functional stoichiometric approach to exploring how each individual subunit contributes to the TEA binding. We co-expressed the mutant Kcv and a mass-tagged wild-type Kcv, to form hetero-tetramers that can be electrophoretically separated. Because Kcv is able to retain the channel-forming function in detergent SDS [*FEBS Lett.* 581, 1027-1034 (2007)], we can purify all types of hetero-channels directly from the SDS gel, and subject to single channel recording. Through this approach, we established a linear correlation between the free energy for TEA blocking and the number of mutant subunits in a tetramer, which infers that each subunit independently interacts with one ethyl group of TEA and contributes equal energy to the overall TEA affinity. The functional stoichiometric approach we developed with purified hetero-channels can be applied to the mechanism study of many K+ channel drugs and inhibitors.

## Ligand-gated Channels

**3651-Pos**

**Desensitization Contributes to the Postsynaptic Response of Ionotropic Receptors; A Comparative Study of Cys-Loop, Purinergic, and Glutamate Receptor-Channels**

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All naturally-occurring ionotropic receptors desensitize significantly in the continuous presence of a sufficiently high concentration of agonist. However, the neurotransmitter lifetime in the synaptic cleft is limited by diffusion, neurotransmitter reuptake, and/or enzymatic cleavage, to an extent such that desensitization does not appreciably occur during the agonist pulse. Nevertheless, channels remain prone to desensitization during the much longer interpulse intervals while they deactivate. As a result, it is of interest to determine the extent to which ligand-gated ion channels (LGICs) known to participate in fast synaptic transmission undergo desensitization upon ligand removal, since entry into these refractory states would progressively decrease the postsynaptic-current response. To address this problem, we have applied high-frequency trains of brief (approximately 1 ms) agonist pulses to outside-out membrane patches expressing these LGICs; receptors under study include the rat purinergic P2X receptor, the rat AMPA-type glutamate receptor, the rat gamma-aminobutyric acid receptor (GABAR), the human glycine receptor (GlyR), and the human ganglionic (alpha3-beta4) and mouse-muscle nicotinic acetylcholine receptors (nAChRs). Our results indicate that all tested receptors exhibit increasingly reduced peak responses in a train-frequency- and receptor-dependent manner, consistent with the notion that the extent of desensitization upon deactivation is substantial. These findings suggest that a) receptor desensitization may contribute to limit the in-vivo postsynaptic response mediated not only by glutamate receptors (which has been proposed earlier), but also by all of the other ionotropic receptors studied here, and b) that the occurrence of desensitization cannot be neglected (as it often is) in attempts to characterize the kinetic behavior of these channels.

**3652-Pos**

**Effects of Protons on Macroscopic and Single-Channel Currents Mediated by the Human P2X7 Receptor**

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Human P2X7 receptors (hP2X7Rs) belong to the P2X family, which opens an intrinsic cation channel when challenged by extracellular ATP. hP2X7Rs are expressed in cells of the inflammatory and immune system. During inflammation, ATP and protons are secreted into the interstitial fluid. Therefore, we investigated the effect of protons on the activation of hP2X7Rs. hP2X7Rs were expressed in *Xenopus laevis* oocytes and activated by the agonists ATP or benzoyl-benzoyl-ATP (BzATP) at different *pH* values. The protons reduced the hP2X7R-dependent cation current amplitude and slowed the current deactivation depending on the type and concentration of the agonist used. These effects can be explained by (i) the protonation of ATP, which reduces the effective concentration of the genuine agonist, free $ATP^{4-}$, at the high- and low-affinity ATP activation site of the hP2XR, and (ii) direct allosteric inhibition of the hP2X7R channel opening that follows ATP binding to the low-affinity activation site. Due to the hampered activation via the low-affinity activation site, a low *pH* (as observed in inflamed tissues) leads to a relative increase in the contribution of the high-affinity activation site for hP2X7R channel opening.

**3653-Pos**

**P2X7 Receptor-Mediated Disruption of the Plasma Membrane and Endoplasmic Reticulum Morphology and Cell Survival**

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The cation-conducting P2X7 receptor channel (P2X7R) operates as a cytolytic and apoptotic nucleotide receptor but also controls sustained cellular responses, including cell growth and proliferation. However, it has not been clarified how

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 $3.5 \pm 0.2$ s-1 to $1.6 \pm 0.2$-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