Cys-loop receptors are a superfamily of ligand gated ion channels comprising such receptors as acetylcholine, serotonin, Glycine and GABA. Despite the low level of sequence conservation all these receptors are thought to share common structural characteristics. Numerous experiments describe in detail the pore channel and the extracellular domain. However due to the low sequence conservation between members of Cys-loop family, and the lack of any high resolution structures of these receptors in the trans-membrane domain (TMD), little information is available concerning the potential allosteric binding site. This potentiating binding site is of extreme importance for a pharmacological perspective, as it is the target of anesthetic an alcohol molecules.
Recently the crystal structure of the open form of Gloeobacter violaceus pentameric ligand-gated ion channel (GLIC) has been solved [1]. As Glycine receptors (GlyR) are the closest receptors in terms of sequence, to GLIC, we decided to create a homology model of the human homomeric alpha-1 GlyR. Furthermore we chose the well-known ethanol molecule as a ligand target for studying the potentiating effect of such molecules on Cys-loop receptors [2]. In total we present 2 microseconds of molecular dynamics simulations, the longest simulation ever presented for Cys-loop receptors. Our simulations show a spontaneous binding of ethanol in cavities of the TMD. These cavities are located between subunits of GlyR TMD, and involve several residues previously identified by mutations as crucial for the potentiating effect of GlyR by ethanol. We also show that ethanol is stabilizing the open form of the GlyR, which could explain the effect of allosteric ligand on Cys-loop receptors.
[1] Bocquet, N, et al. Nature (2009): (457)111-114.
[2] Harris, RA, Trudell, JR and Mihic, SJ. Sci Signal. (2008): 1(28):re7.

3669-Pos
The Activation Mechanism of the Rat Homomeric alpha2 Glycine Receptor
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The alpha2 glycine receptor subunit, abundant in embryonic neurons, is replaced by alpha1 in the adult nervous system. Glycine-activated single-channel currents were recorded in the cell-attached configuration at +100 mV pipette holding potential from alpha2 homomeric receptors expressed in HEK293. At all glycine concentrations (0.02 - 10 mM), openings were grouped in long (> 300 ms) bursts/clusters with high open probability (0.99). The mean duration of individual apparent openings was c. 30 ms. Shut-time intervals within groups of openings were dominated by short shuttings of 5-10 μs. The properties of these groups of openings appeared to have an unusually steep concentration dependence and it is unclear whether they represent receptor activations (i.e. bursts) or clusters. Several mechanisms were fitted by maximising the likelihood of the entire sequence of open and shut times, with exact allowance for missed events (program HJCFIT, ref.1). Several records obtained at different glycine concentrations were fitted simultaneously. Good fits were obtained with several qualitatively different mechanisms incorporating 2 or 3 binding sites. In fits with the flip mechanism (2), we found that the closing rate constant of alpha2 glycine receptors is slow (about 500 s-1) and thus the efficacy for the final opening step in the activation is >10 fold higher than that of alpha1 beta channels, the receptor in adult synapses. On the other hand, flipping efficacy and binding affinity were lower for alpha2 than for alpha1 beta channels. These differences confirm that the alpha2 glycine receptor properties make it less suitable than alpha1 beta channels to mediate fast synaptic transmission (3).
1. Colquhoun et al. (2003) J Physiol 547, 699-728.
2. Burzomato et al. (2004) J Neurosci 24, 10924-10940.
3. Mangin et al. (2003) J Physiol 553, 369-386.

3670-Pos
Transient Currents from Glycine Receptors Depend on Intracellular Chloride
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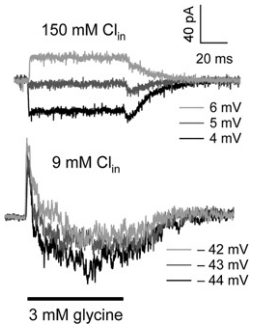
We transfected HEK-293 cells with cDNA for homomeric human α1 glycine receptors. 2-3 days after transfection, we recorded currents and used a submillisecond perfusion system to apply glycine to outside-out patches. The extracellular solution contained 150 mM chloride but the intracellular solution contained either 150 mM chloride (symmetric) or 9 mM chloride and 127 mM acetate ("physiological"). We clamped at a range of potentials near the current reversal potential for each condition. With symmetric chloride, currents were nearly constant during the 70 ms application of 3 mM glycine. With "physiological" chloride, currents reversed direction from inward to outward on the 5 ms time scale during agonist application. Similar biphasic currents were seen with gluconate as the substitute intracellular anion. Biphasic currents were observed at external pH values from 6.4-8.0, with HEPES, TRIS or PIPES as external buffer and with external choline replacing sodium. Activation of GlyR channels is dependent on Cl_{in} (J Neurosci 28:11454, 2008) but this cannot explain the observed reversal of currents. We are considering that local changes in Cl_{in} can cause this effect or that time-dependent changes in pore selectivity are possible.



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3671-Pos
A Novel Mechanism for the Inhibitory Action of Hydrocortisone at 5-HT$_3$A Receptor
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The 5-HT$_3$A receptor is a member of the Cys-loop family of ligand-gated ion channels. Due to its low conductance, analysis of this receptor has been restricted to the macroscopic level. We introduced mutations in the 5-HT3A subunit to obtain a high-conductance form so that single channels can be detected. We studied the actions of the neuroactive steroid, hydrocortisone (HC), in the high-conductance form of the 5-HT$_3$A receptor. Channel activity elicited by 1 μM 5-HT appears as opening events of 4.6 ± 0.4 pA (−70 mV) forming bursts, which coalesce into long clusters. A minor population of lower amplitude events (~2.8 pA) is observed, which corresponds to 0-10 % of the total events in all recordings. HC produces a concentration-dependent reduction in the duration of bursts and of the slowest open component (from ~100 ms in the control to ~3.6 ms at 400 μM HC), which can be explained on the basis of a slow block mechanism. Interestingly, amplitude histograms reveal a concentration-dependent increase in the relative area of the low-amplitude component without changes in its mean value. At 400 μM HC, the low-amplitude events correspond to 40 % of the total events. This channel population shows an amplitude of 2.8 ± 0.5 pA (−70 mV). Macroscopic currents elicited by 5-HT in the presence of HC show reduced peak currents (~50% at 400 μM HC) and increased decay rates compared to those recorded in the absence of the steroid. Taken together, our results reveal that hydrocortisone negatively modulates 5-HT$_3$A receptors and show a novel mechanism which involves the stabilization of a subconductance state.

3672-Pos
Analysis of CorA-Catalyzed Mg^{2+} Selective Currents in Xenopus Oocytes
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In prokaryotes, Magnesium homeostasis is tightly controlled through the workings of three distinct transport systems, with CorA being the primary source of Mg^{2+} uptake. The recent structures of CorA from the thermophilic eubacteria *Thermotoga maritima* (TmCorA) have provided an excellent model for a molecular understanding of Mg^{2+} transport. Yet, a detailed characterization of CorA function has been conspicuously missing so far, as only *in-vivo* bulk assays have been available to evaluate Mg^{2+} translocation leaving unanswered fundamental questions related to CorA ion selectivity and specificity, gating mechanism and regulation. Here we have developed a system for high-level expression of TmCorA in *Xenopus* oocytes that allows for an accurate functional readout of Mg^{2+} transport through several electrophysiological techniques. Mg^{2+} currents from TmCorA in the order of 1 to 10 μA were routinely obtained with the cut-open voltage clamp technique and two electrodes Voltage clamp. We demonstrate that CorA act as a strong inward rectifier, an observation in agreement with its physiological role as the primary source of Mg^{2+} uptake. As expected, Mg^{2+} currents are blocked by Co(III) hexamine, a structural analog of a hydrated Mg^{2+}. CorA selectivity towards Magnesium *vs.* other divalent and monovalents was characterized using a perfusion TEV setup. The divalent selectivity series for CorA was determined as $Co^{2+} >= Mg^{2+} > Ni^{2+} > Zn^{2+} >>> Ca^{2+} = Ba^{2+}$. Macroscopic CorA currents record from oocytes macropatches show that Mg^{2+}-binding to the cytoplasm domain act as a gating factor. Single channel transitions are still below our detection capabilities and efforts are ongoing to characterize the conductance of TmCorA through noise analysis. The ability to evaluate CorA function by means of electrophysiological methods will allow for a detailed analysis of structure/function relationships in CorA.