by a conformational change in the SNARE complex. Although the structure of the fusion pore remains elusive, current fusion pore models place the C-termini of all three SNAREs in or near the fusion pore. Mutations in the C-terminus of SNAP-25 affects the rate at which fusion pores are formed (1). To study the role of the SNAP-25 C-terminus in determining the structure and dynamics of the fusion pore, SNAP-25 coupled to GFP at its N-terminus was overexpressed in bovine chromaffin cells. Single exocytotic events were characterized by carbon fiber amperometry and cell-attached patch capacitance measurements. Cells overexpressing SNAP-25 delta9 (lacking last nine C-terminal residues) displayed smaller amperometric "foot-currents" indicating reduced flux of transmitter through the fusion pore. Measurements of fusion pore conductance revealed that this is due to reduced fusion pore conductance and lower fusion pore expansion rate. Fusion pores involving SNAP-25 delta9 also showed a markedly prolonged lifetime from formation to rapid expansion. These results indicate that the C-terminus of SNAP-25 not only affects the rate of fusion pore formation, but also determines the structure of the initial fusion pore, the dynamics of fusion pore expansion, and the rate of transmitter release.

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1297-Pos Does the Ca^{2+} Activated Potassium Channel *h*SK3 Play a Functional Role in Endocytosis?

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Board B273

Endocytosis is a mechanism for selective internalization of plasma membrane and membrane proteins. In the nerve terminal, this pathway takes part in the recycling of synaptic vesicles. For clathrin-mediated endocytosis an array of proteins, including dynamin, amphiphysin, synaptojanin, and endophilin, have been implicated as accessory factors in the early steps of clathrin-coated pit formation. Using a *LexA*-based yeast two-hybrid system we identified *h*SK3, a Ca^{2+} activated K^+ channel, as interaction partner of endophilin 3. This interaction was verified by pull-down experiments. The human SK3 channel (*h*SK3) belongs to a family of Ca^{2+} activated K^+ channels that play a role in neuronal function by shaping single action potentials and modifying firing patterns. Sugiura *et al*., 2004 (*J Biol Chem* ***279***:23343) repoted a reduction of endocytosis in COS7 cells expressing endophilin 3. Therefore, in order to determine a physiological consequence of the *h*SK3/endophilin 3 interaction we initially established an endocytosis assay using scanning confocal microscopy to monitor endocytosis in COS7 cells, lacking *h*SK3 channels. The lipophilic styryl dye FM4-64, a general endocytosis marker, was used in order to visualize selectively the plasma membrane and internalized membrane. Cells were incubated with FM4-64 for 30 min either at 4°C (control) or at 37°C and the fluorescence signal of the internal cell lumen was then quantified. Using this assay we did not observe a difference in FM4-64 uptake in COS7 cells expressing EGFP-endophilin 3 and EGFP. This is in contrast to Sugiura et al., (*ibid*) who used labelled transferrin as an endocytotic marker. Future experiments with transferrin will clarify this discrepancy.

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Ligand-gated Channels

1298-Pos Cation and Anion Binding Sites in the Ligand-Binding Domain of Glutamate Receptors of the Kainate Subtype

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Board B274

Ionotropic glutamate receptors (iGluR), activated by the amino acid L-glutamate, form a large family of ligand gated ion channels that mediate the majority of excitatory neurotransmission in the brain. Pharmacologically, the iGluR family is categorized by the selectivity shown by individual proteins towards alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) and kainate. Ions modulate the behaviour of many receptors. Recently Plested & Meyer (Neuron 2007, *53*:829) showed that kainate receptors, but not AMPA or NMDA receptors, require both Na+ and Cl– in the extracellular region to function. Whilst only the anion binding site was identified, it was suggested that both anions and cations bind in the ligand binding domain. Using computational approaches, we have identified two cation binding sites located symmetrically opposite the anion binding site and within the same dimer interface cavity. Multiple molecular dynamics simulations and relative binding free energy calculations using thermodynamic integration were performed to study the anion and cation binding sites in detail. Simulations confirm that the identified locations are indeed cation binding sites. The rank order of binding for halide and alkali-metal ions were determined which is indicative of their binding affinities. The computational results agree well with mutagenesis and crystallographic studies.

1299-Pos Investigating the Role of Electrostatic Interactions in Glutamate Receptor Functioning

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Board B275

Elevated levels of glutamate during cerebral ischemia play a major role in "excitotoxicity" of glutamate receptors (GluRs) leading to neuron death and devastating effects on the central nervous system. Clearly understanding the mechanism by which GluRs function is critical for rational drug design while exploring subtle differences in receptor subtypes provide a means of drug specificity, eliminating

potential side effects. Structural and theoretical (Speranskiy et al. Biochem. 2005 Aug 30;*44*(34):11508) studies of AMPA receptors indicate that electrostatic interactions play an important role in ligand attraction to the ligand binding S1S2 extracellular domain. Recent studies of the conformational change in S1S2 that occurs upon ligand binding indicate the ligand bound conformation may be stabilized by hydrogen bonding and the conformational change controlled electrostatically (Mamonova and Kurnikova 2007 submitted). These results contribute to the current working hypothesis that electrostatic interactions play an important role in the glutamate receptor function. Continuum electrostatics calculations of momomeric GluR2 reveal a binding cleft glutamate residue (E705) plays a major role in the stabilization of the ligand binding domain conformational state. Small modulation of the charge on E705 destabilizes the closed conformation allowing transition to the open conformation of the binding domain. These calculations suggest E705 may contribute to the size of the free energy barrier between the closed and open conformations of the ligand binding domain. Guided by experimental results, studies of various GluR ligands and subtypes are currently being conducted to investigate electrostatic interactions as the main driving force in the conformational change that accompanies ligand binding.

1300-Pos Kinetic Mechanism of Partial Agonism at the NR1 Subunit of NMDA Receptors

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Board B276

NMDA receptors are ligand-gated ion channels composed of two NR1 and two NR2 subunits. They become active only after the full-agonists glycine and glutamate bind to their cognate ligand-binding domains (LBDs) located on extracellular portions of homologous NR1 and NR2 subunits, respectively. Several crystal structures of glutamate receptor LBDs, expressed as engineered soluble proteins, have revealed that ligands bind in a cleft between two mobile lobes. Based on these findings it was proposed that an agonist's potency to promote channel opening correlates with the degree of LBD cleft closure they induce. In contrast, crystal structures of the NR1-LBD with partial agonists (ACPC and ACBC) resemble the glycine-bound structure and reveal a fully closed cleft. We hypothesize that partial agonists at the glycine site, although capable of inducing full cleft closure, do so less often or for shorter durations than the full agonist glycine. To investigate this hypothesis we used kinetic analyses and modeling of single-channel current recordings obtained in the presence of glycine, ACPC, and ACBC, to measure gating rate constants for NR1-1a/NR2A receptors expressed in HEK cells. Consistent with the lower efficacy of ACPC and ACBC reported for macroscopic currents, we find that closed dwell times are longer and open dwell times are shorter in the presence of ACPC and ACBC as compared to glycine. Specifically, when we fit our data with a linear state model composed of 3 closed and 1 open (3C1O) states, we find that receptors with bound partial agonists dwell longer in the closed state C2 due to increased exit energy barriers from this state. These results support the hypothesis that, when compared to glycine, partial agonists promote cleft closure less often.

1301-Pos Endogenous Synaptic Fluctuation of Proton Concentration Modulates Post-Synaptic NMDA Currents

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Board B277

Synaptic vesicles are known to maintain an internal pH of 5.5. It is possible therefore that the release of vesicular contents may include release of protons along with the neurotransmitter. Though there is significant evidence that exocytosed protons serve to inhibit presynaptic voltage-gated calcium channels, there is little independent evidence to suggest that acidification of the synapse may have direct post-synaptic effects. To investigate the potential post-synaptic modulatory role of exocytosed protons, we have created functional solitary autaptic cerebellar granule cell cultures from rat brain and recorded the spontaneous miniature and evoked NMDA post-synaptic currents (mPSCs and ePSCs, respectively). At DIV 8, we find that increasing proton buffering to 30mM HEPES from 3mM control results in a significant ($p<.01$) increase in average mPSC amplitude (29.2 pA from 21.4 pA respectively), while the decay kinetics of the mPSCs do not change significantly (tw=151ms and 161ms respectively). We also observe a significant increase in the amplitude of ePSCs ($p<.01$). We find no evidence of positive modulatory effects of HEPES buffer on the NMDA channel. We speculate that highly mobile protons co-released from glutamatergic synaptic vesicles inhibit NMDA receptors by influencing events preceding the opening of the agonist-bound receptor.

1302-Pos Interplay between Agonist: Protein Interactions and Activation in AMPA Receptors

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Board B278

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, one subtype of ionotropic glutamate receptors, are the primary receptors responsible for excitatory signaling in the central nervous system. Additionally, these receptors also play diverse roles throughout the body, making them important targets of structure-function investigations to understand their mechanism of action. Previous structural investigations of the isolated ligand binding domain of the GluR2 subunit of these receptors have provided important insight into the mechanism of action of these receptors and shown a correlation between the degree of cleft closure in this domain and activation of the channel. However, what question remains unanswered is how specific interactions between the ago-

nist and the protein play a role in receptor activation. To address this, we have used vibrational spectroscopy to analyze the atomic-level agonist:protein interactions and fluorescence resonance energy transfer (FRET) to characterize the large scale cleft closure conformational change, and these results were correlated to the functional consequences in the full receptor. These investigations showed a correlation between the strength of the interaction at the α-amine group of the agonist and the protein and extent of receptor activation, where a stronger interaction leads to a larger activation. This correlation held even in the case of the L650T mutant where the large scale cleft closure does not correlate to the extent of activation. These results show the interaction between the α-amine group of the agonist and the ligand binding domain, a bridging interaction between the two lobes of the ligand binding cleft, to be critical in mediating the allosteric mechanism of activation in this subtype of glutamate receptors.

1303-Pos Conformational Changes Associated with Activation and Desensitization in a Functional Glutamate Receptor

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Board B279

Ionotropic glutamate receptors are important excitatory neurotransmitter receptors in the mammalian central nervous system. Glutamate binding to an extracellular domain initiates a series of conformational changes that culminates in the formation of a cation selective transmembrane channel, which subsequently closes due to desensitization of the receptor. The crystal structures of the isolated ligand binding domain of the AMPA subtype of the receptor have been particularly useful in providing initial insight into the structural changes in the ligand binding domain; however, these structures are in the absence of the transmembrane segments, the primary functional part of the protein. To gain a better understanding of how agonist binding is coupled to channel activation and desensitization, it is necessary to study changes in the ligand binding domain in the presence of the transmembrane segments. Here, LRET was used to determine the conformational changes associated with activation and desensitization in a functional glutamate receptor (ΔN*-AMPA) that contains the ligand binding domain and transmembrane segments and has been modified such that fluorophores can be introduced at specific sites to serve as a readout of cleft closure. These studies of cleft closure show that the conformational changes due to activation of the receptor previously established for the isolated ligand binding domain due to the binding of partial and full agonists are also observed in this functional receptor. Additionally, these investigations show that the cleft closure conformational change is not significantly different between the open channel and desensitized states.

1304-Pos The Activity of a Mutant δ2 Ionotropic Glutamate Receptor is Modulated by Phosphatidylinositol 4,5-bisphospate (PIP_2)

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Board B280

The δ2 glutamate receptor is enriched in the parallel fiber-Purkinje cell (PF-PC) synapse and is thought to play an important role in long-term depression (LTD). It is considered to be an ionotropic glutamate receptor, as it was identified based on homology to other glutamate receptors, but no known ligand has been shown to activate it thus far. On the other hand a naturally-occurring single point mutant (A654T), named Lurcher (gluRδ2^{Lc}), exhibits constitutive activity.

Phosphatidylinositol 4,5-bisphosphate (PIP_2) has been shown to be an important modulator of the activity of most ion channels. Recently we showed that the activity of the NMDA glutamate receptor is modulated by PIP_2 through interactions with α-actinin. Thus, we tested whether the δ2 glutamate receptor activity is also PIP_2-dependent. We utilized different electrophysiological methods to evaluate the behavior of the Lurcher mutant when the concentration of PIP_2 in the membrane was altered.

We report that gluRδ2^{LC} was partially inhibited by PIP_2, resulting in an increase of activity upon depletion of PIP_2 in the membrane. These results place δ2^{Lc} glutamate receptor in the minority of channels that are inhibited by phosphatidylinositol 4,5-bisphosphate (PIP_2).

1305-Pos Agonist-receptor Interactions And Conformational Changes In The Glur6 Ligand Binding Domain

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Board B281

Recent crystal structures of the isolated ligand binding domain of the kainite receptor subunits have shown that it undergoes a graded cleft closure conformational change upon agonist binding to the protein, with the extent of cleft closure correlating to the efficacy of the agonist. At this time, however, there is no structure available for the apo state of the kainate receptor subunit and the structures for the antagonist bound forms show varying degrees for the cleft opening. Hence, the extent to which the cleft is open in the resting state, and therefore the extent of cleft closure conformational change due to agonist binding relative to the apo state is still largely unknown. We have used an luminescence resonance energy transfer (LRET) based

method to determine the distance between domain 1 and domain 2 in the apo, and various agonists bound states of the GluR6 subunit of the receptor and thus established the extent of cleft closure due to agonist binding to this protein relative to the apo state. We have also investigated the agonist:protein interactions using Fourier transform infrared spectroscopy and established that the interactions at the amine group of the agonist is correlated to the extent of activation. These studies show that the kainate receptors not only have similar conformational changes as that observed for the AMPA receptor subtype of the glutamate receptors, they are also similar at the level of chemical interactions that control the extent of activation.

1306-Pos Zinc Increases NMDA Receptor Sensitivity To Modulation By Stimulus Frequency

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Board B282

NMDA receptors are glutamate-activated ion channels with post-synaptic locations. At many excitatory synapses in brain, zinc is co-released with the neurotransmitter glutamate. Zinc inhibits NMDA receptors with two distinct mechanisms: allosteric, by binding to the amino-terminal domain of the NR2A subunits (IC_{50}, nM); and channel-block, by binding in the pore (IC_{50}, μM). To investigate the consequences of allosteric inhibition on channel activity we measured gating rate constants in the presence and absence of zinc. This was done by kinetic analyses and modeling of single-channel current traces recorded from cell-attached patches of HEK-cells following transient transfection with NR1, NR2A and GFP. Results were compared with those obtained from receptors made resistant to channel-block by introducing a single N596G substitution in the NR2A subunit pore. In the presence of zinc, the two longest closed-time components were prolonged from 2.3 to 5.2 ms and from 7.5 to 17.9 ms, indicating that receptors were delayed in closed-pore conformations. Open-time component durations were shortened to a lesser degree, with a decrease in mean open-time from 8.1 to 5.1 ms. Single-channel records were fully described with a linear state model consisting of three closed states and one open state (3C1O). Results suggest that zinc-bound receptors transition slower toward open-pore conformations, with substantially decreased $C_1 \rightarrow C_2$ and $C_3 \rightarrow O_1$ rate constants. The model accounted for the previously reported zinc-dependent decrease in macroscopic peak open probability and in total charge transfer. Importantly, simulations predicted higher receptor sensitivity to modulation by stimulus frequency. Peak response following high frequency stimulation (100 Hz) increased more when zinc was present, from 1.5-fold to 2.5-fold. These results suggest a new role for zinc at glutamatergic synapses and stimulate further investigation of the effects of zinc on synaptic NMDA receptors.

1307-Pos

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WITHDRAWN

1308-Pos Lithium opens sodium-gated potassium channels

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Board B284

The sodium gated potassium channel subunit Slack (Slo2.2) is expressed in many regions of the brain, and is thought to have an important role in adaptation of neuronal firing rates. We previously developed an HEK 293 cell line stably expressing Slack-B, and which shows large sodium-activated potassium currents. Macroscopic currents were measured in inside-out patches as NMG in the $NMG^+/K^+/Cl^-/Asp^-$ bath solution was replaced with Na^+, Li^+ or NH_4^+. The EC50 for Na^+ activation was 50 mM, and for Li^+ was 250 mM. Ammonium ions activate the channel weakly with an extrapolated EC50 of roughly 10M. The apparent sequence of association constants for the Na^+-sensing site is $Na > Li > NH_4 > K$.

1309-Pos *Expression Levels Of Ca^{2+}-activated K^+ Channels α-subunits In Chicken Skeletal Muscle*

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K channel is expressed in a variety of cells such as neurons, endocrine cells and smooth muscle. It is a tetramer of four α-subunits (Slo), surrounding the pore, and four β-subunits that modulate channel function. This work′s aim was to determinate the presence of BK α-subunits on the chicken anterior *latissimus dorsi* (ALD, slow) and posterior *latissimus dorsi* (PLD, fast) skeletal muscles and to know if the expression levels differ between slow and fast muscle. Expression levels were assessed by RT-PCR, using rat uterine tissue as positive control. In extraction of total RNA the trizol-chloroform, isopropanol technique was used. Primers were designed by intron spanning using the database from NCBI (access NM_204224) being the following sequences: [5′-ATGAG-TAACAATATCAACGCCAAC-3′ (sense) and 5′-AAGTC-TATCTTCCTGCACATAC-3′ (antisense). Data normalization with β-actin was made. Real-time RT-PCR and analysis were carried out by LightCycler (Roche Applied Science) and analysis and its associated Software (ver. 4.0). We found differences in the α-subunits expression between slow and fast muscle; in the fast muscle α-subunits resulted more abundant than in the slow type, with variations depending on animal age, so that the higher level was at 5 days and decreasing with lapse time in the following way: [ALD 5d (0.46 ± 0.18), 30d (0.16 ± 0.10), 60d (0.13 ± 0.07); PLD 5d (7.81

± 5.46), 30 d (1.88 ± 1.84), 60 d (0.21 ± 0.14), however, differences were not significant in any period of age (P=0.24, 0.41, and 0.46 for 5, 30 and 60 days respectively). These results show the presence of BK α-subunits, which could form functional channels in slow and fast skeletal muscle, a statement that need to be tested in further researches.

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1310-Pos Evidence For Unconventional Interactions Between Purinergic $P2X_2$ And $P2X_5$ Subunits

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Purinergic P2X receptors are cationic channels activated by extracellular ATP. Seven subunits have been cloned, that can assemble as trimeric association of homo- or heteromeric subunits. Several potential recombinant heteromeric P2X receptors have been proposed, but only the heteromeric $P2X_{2/3}$ receptor has clearly been identified in neurons. In the case of the $P2X_5$ subunit, homomeric receptors can not be activated by ATP whereas functional recombinant heteromeric $P2X_{5/1}$ and $P2X_{5/4}$ receptors have been described.

In this study, we show that $P2X_5$ subunits, when expressed alone, are retained in intracellularly but that their co-expression with either $P2X_1$, $P2X_2$ or $P2X_4$ subunits allows their surface expression. Association of $P2X_5$ with $P2X_2$ or $P2X_4$ was confirmed by immuno-staining, co-immunoprecipitation of biotynilated membrane proteins as well as from brain extracts, suggesting that $P2X_5$ subunits only exist in heteromeric receptor.

Stoechiometry of subunits in the $P2X_{2/5}$ receptor was tested using biochemical and electrophysiological approaches. However, no clear evidence was obtained for the existence of "conventional" heteromeric receptors between $P2X_2$ and $P2X_5$ (i.e. heteromeric $P2X_{2/3}$ receptors). Furthermore, extracellular chemical cross-linking of monomeric or concatenated P2X subunits suggest that $P2X_5$ and $P2X_2$ interact in both heteromeric and oligomeric receptors.

Finally, using bioluminescent resonance energy transfert (BRET), we observed that specific BRET signal could be obtained between coexpressed $P2X_2$ and $P2X_5$ subunits suggesting a closed proximity between the two subunits. The dynamic of this interaction was tested by measuring variation BRET efficiency following ATP stimulation. Whereas ATP stimulations induce a decrease of BRET efficiency between homomeric $P2X_2$ subunits, no such change could be obtained when $P2X_2$ and $P2X_5$ were coexpressed. Our results show that $P2X_2$ and $P2X_5$ subunits interact at the plasma membrane in unconventional way. These interactions modulate the $P2X_2$ conformational changes induced by ATP.

1311-Pos Probing the pore of Open, Closed and Desensitized ATP-gated P2X2 receptors

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P2X receptors are ATP-gated ion channels comprising seven distinct subunits ($P2X_{1-7}$). Each P2X subunit has a large extracellular loop that binds ATP, intracellular N-and C termini and two transmembrane (TM) domains(1). A functional channel consists of three P2X subunits. P2X receptors undergo conformation changes that open an intrinsic pore within milliseconds of binding ATP. However, the precise details of how the ion channel pore forms, and the conformational changes it undergoes in response to ATP binding are not fully understood. $P2X_2$ receptors have three major states; closed, open and a desensitized state during prolonged ATP applications. We have carried out state- and activation-dependent substituted cysteine accessibility mutagenesis (SCAM) on TM1 and TM2 with Cd^{2+} to determine how the P2X pore forms and how it undergoes changes during open, closed and desensitized states of P2X channels. Wild type $P2X_2$ was not blocked by Cd^{2+} in the open, closed or desensitized states but was slightly potentiated. In contrast single cysteine mutants showed block in the closed, open and desensitized states with a strong dependence on amino acid position and channel state. Our experiments on TM2 are most complete, and suggest that this domain lines the permeation pathway of $P2X_2$ channels. The pattern of Cd^{2+} block was consistent with this domain adopting an alpha helical structure in the open state. We will present data that determines Cd^{2+} block for all single cysteine mutants in TM1 and TM2, determine how accessibility varies between closed, open and desensitized states, as well as rationalize our findings with the similarities and discrepancies between previous work(2) aimed at understanding the $P2X_2$ pore.

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1312-Pos Voltage And [ATP]- Dependent Gating Of The ATP Receptor Channel $P2X_2$

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P2X receptors are ligand-gated cation channels activated by extracellular ATP. The $P2X_2$ channel current at the steady-state after ATP application is known to have voltage-dependence in spite of the absence of voltage-sensor domain, i.e. upon hyperpolarization it shows a gradual increase in the inward current on top of an instantaneous increase presumably due to its pore property. We analyzed this "activation" phase quantitatively under two-electrode

voltage clamp using *Xenopus* oocytes expression system. We analyzed the conductance-voltage relationship in the presence of various [ATP] by measuring the tail current, and observed it shifted toward the depolarizing potential with the increase in [ATP]. By analyzing the rate constants for the channel's transition between a closed and an open state, we showed the gating of $P2X_2$ is determined by complex factors of the membrane voltage and [ATP]. In R290K mutant of the ATP binding site known to have a decreased ATP sensitivity, the [ATP] - dependency of $V_{1/2}$ of conductance-voltage relationship was less clear than w.t. The results suggest a possibility that the ATP binding step is critically involved in the voltage dependency. We also approached the structural background of the final step of the gating by mutating a glycine residue (G344) in the 2nd-transmembrane helix (2nd-TM), a putative kink for the "gating". We observed that the inward current of G344A mutant increased instantaneously upon hyperpolarization without a gradual increase as if it were a constitutively active channel. In G344P mutant the "activation" phase remained, and it became slower than that of WT. The G-V relationship of the G344P mutant was shifted to more hyperpolarized potential than that of WT. The results show that the flexibility of G344 in the 2nd-TM contributes to the voltage dependent "opening" of $P2X_2$ channel.

1313-Pos Interaction between $P2X_4$ and $P2X_7$ Receptors Modulates Ivermectin Effect

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Recently we reported a functional interaction between murine $P2X_4$ and $P2X_7$ receptor channels ($mP2X_4R$ and $mP2X_7R$) expressed in HEK cells. In this work we show that co-expression of $mP2X_4R$ and $mP2X_7R$ in HEK cells modulates the degree of current enhancement caused by 3 μM Ivermectin (IVM), a drug that potentiates $P2X_4R$ but no $P2X_7R$. Bath and pipette solutions containing 140 mM NaCl were used to record Na^+ currents (I_{Na}) elicited with Tris-ATP using the patch-clamp technique. Current enhancement was computed as I_{Na} in presence of IVM divided by control I_{Na}. Our results show that IVM enhanced current through $mP2X_4R$ activated with either 0.03 or 5 mM ATP (3.17±0.99 and 2.99±0.40, respectively). IVM failed to enhance I_{Na} through $mP2X_7R$ activated with either 0.03 mM or 5 mM ATP (1.35±0.12). Surprisingly, IVM failed to enhance I_{Na} activated by 5 mM ATP in cells co-expressing $mP2X_4R$+$mP2X_7R$ in 1:1 and 2:1 ratios (1.21±0.07 and 1.08±0.05, respectively). However, IVM enhanced I_{Na} activated with 0.03 mM ATP in cells co-expressing both receptors in a 2:1 ratio (6.22±1.53). Moreover, concentration-response experiments did not revealed changes in affinity ($EC_{50} \sim 0.035$ mM) of $mP2X_7R$ or $mP2X_4R$+$mP2X_7R$ (2:1 ratio). Consistent with the HEK data, IVM also failed to enhance I_{Na} activated by 5 mM ATP in parotid acinar cells (0.98±0.05). Furthermore, the kinetics of $mP2X_4R$ + $mP2X_7R$ (2:1)-generated currents were similar to those recorded from acinar cells. These results support the idea that $mP2X_4R$ and $mP2X_7R$ could form heterotrimeric channels in HEK as well as in parotid acinar cells.

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1314-Pos P_2X_7-mediated Cellular Uptake of FM 1-43 Fluorescent Molecule

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The ATP-gated cation channel P2X7 plays important roles in immune cell function such as cytokine release, apoptosis, and microbial killing. The channel exhibits unique biophysical properties, notably low affinity for ATP but high affinity for 3′-o-(4-benzoylbenzoyl) ATP (BzATP) and the conduction of a non-desensitizing current. Prolonged exposure to ATP results in a gradual opening of a pore highly permeable to large molecules (up to 900 Da) such as YO-PRO-1 and N-methyl-D-glucamine. Other properties include actin filament reorganization and membrane blebbing in macrophages and stimulation of plasma membrane trafficking in thyrocytes. Following an earlier report that the amphipathic styryl dye FM 1–43 was capable of gaining access to cellular cytoplasm by directly permeating non-selective cation channels [1], we investigated the uptake of FM 1–43 by P2X7 channels. When human embryonic kidney cells stably expressing P2X7 were exposed to 10 – 300 μM BzATP in the presence of 2 μM FM 1–43, we observed an intense fluorescent staining (IFS) which was absent in untransfected cells under similar conditions. IFS did not occur in the absence of BzATP and 3 μM BzATP induced only slight fluorescence. IFS persisted at 4°C but could not be induced in the presence of 20 μM P2X7 antagonists AZD9056 and A-740003 or by the non-P2X7 agonist UTP. IFS could also be induced in differentiated but not undifferentiated THP-1 monocytes. The present study suggests that activation of P2X7 can result in cellular uptake of FM 1–43 and intense fluorescence which can be exploited in novel P2X7 assays for the discovery of new agonists and antagonists.

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1315-Pos Cloning and Pharmacological Characterization of the Rhesus $P2X_3$ Receptor

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Board B291

The purinergic receptor, $P2X_3R$, has emerged within the last decade as an attractive pain target. To date $P2X_3R$ has been cloned and characterized from several mammals including human, rat, and mouse. We have cloned the rhesus ortholog of the $P2X_3R$. The rhesus receptor exhibited 97.4% and 98.99% identity to the human receptor on the nucleotide and amino acid level, respectively. Only 4 of 397 amino acids differ between rhesus and human: F67S, F127L, F144L, and M162T. Rhesus $P2X_3R$ was expressed in HEK-293 cells and automated patch clamp electrophysiology was used to study its pharmacology. Agonist, 30 μM αβ-meATP, elicited a rapidly activating and desensitizing inward current from rhesus $P2X_3R$-HEK cells, demonstrating gating kinetics similar to human $P2X_3R$. The rank order potency of agonists for activation of rhesus $P2X_3R$ was ATP (EC_{50}: 278 nM)>αβ-meATP (1 μM)>CTP (16.5 μM). The potency of antagonists for inhibition of rhesus $P2X_3R$ was 2.7 nM (IC_{50}) for TNP-ATP, 53 nM for A-317491, and 2.2 μM for PPADS. These pharmacological results are in agreement with published potencies on human $P2X_3R$. In conclusion, the rhesus $P2X_3R$ represents a valid alternative to the human receptor for the pharmacological evaluation of $P2X_3$ modulators.

1316-Pos Physical-Chemical Properties of Tryptophan (Trp) 46 in Transmembrane Domain 1 (TM1) Play an Important Role in Ethanol Sensitivity of P2X4 Receptors

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Board B292

P2X receptors (P2XRs) are a family of ligand-gated ion channels widely distributed throughout the central nervous system and activated by extracellular ATP. Recent studies suggest that P2XRs play a role in mediating and/or modulating some of the cellular and behavioral effects of ethanol. We have shown that ethanol inhibits ATP-gated currents in P2X2Rs and P2X4Rs, whereas, it potentiates the function of P2X3Rs. However, sites of ethanol action in P2XRs are unknown. Utilizing a chimeric strategy, combined with site-directed mutagenesis, we recently found that the TM1 domain plays a role in ethanol modulation of P2XRs. The present study begins to investigate the importance of amino acids within TM1 in regards to ethanol sensitivity of P2XRs. TM1 residues of P2X3Rs were individually mutated to alanine, expressed in Xenopus oocytes and tested for changes in ethanol (10–200 mM) sensitivity using two-electrode voltage clamp (−70mV). Mutating Trp at position 41 to Ala (W41A) in P2X3Rs reversed the action of ethanol (potentiation to inhibition). Sequence alignment of P2X3Rs and P2X4Rs revealed a Trp at position 46 in P2X4Rs homologous to Trp 41 in P2X3Rs. We thus tested the mutant (W46A) P2X4R and found that the action of ethanol in this mutant receptor was also reversed (inhibition to potentiation). Further, ethanol studies focusing on the role of position 46 found that replacing Trp 46 in P2X4Rs with an aromatic residue increased the inhibitory action of ethanol while replacing position 46 with an aliphatic amino acid decreased the action of ethanol. Taken together, these findings suggest that physical/chemical properties of Trp at position 46 in TM1 play an important role in ethanol sensitivity of P2X4Rs.

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1317-Pos Key Residues Involved in Zinc and Copper Inhibition of $P2x_7$ Receptors

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Board B293

Submicromolar concentrations of zinc and copper strongly potentiate ATP-gated currents at all P2X receptor subtypes except the $P2X_7$ receptor, at which currents are markedly inhibited (North, 2002). The molecular basis for the strong functional inhibition by divalent cations at this purinergic ionotropic receptor is controversial.

There are fourteen potential residues in the extracellular domain that are conserved among the $P2X_7$ but other P2X receptor subunits. Alanines were singly substituted by site-directed mutagenesis into each position of the rat $P2X_7$ receptor subunit. At WT receptor, zinc (0.1 μM to 100 μM) inhibited the currents evoked by 30 μM 2′–3′-(*O*)-(4-benzoyl) benzoyl ATP (BzATP) in a dose-dependent manner with an IC_{50} value of 4.1 ± 0.6 μM and Hill coefficient (n_H) of 0.8 ± 0.1 (n = 5). Interestingly, strong rebound currents appeared when simultaneously washing zinc and BzATP, suggesting that zinc directly binds $P2X_7$ receptor and that zinc dissociates from $P2X_7$ receptor much faster than BzATP. The H62A/D197A double mutant $P2X_7$ receptor was completely resistant to inhibition by zinc at concentrations up to 300 μM which were not different from the control (n = 5; paired Student's *t*-test). In addition, the rebound currents were significantly reduced or completely abolished at D197A, H62A and H62A/D197A mutants. Copper also inhibits $P2X_7$ receptors with even greater potency than zinc. Similar results were also found when BzATP was replaced by ATP as the agonist.

The results provide direct evidence in support of the view that the potent functional inhibition of $P2X_7$ receptors by zinc and copper results primarily from interaction with the receptors, in which His^{62} and Asp^{197} residues are critical.

This work is supported by the BBSRC.

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1318-Pos Agonist and antagonist induced conformational changes of the Loop F in the σ1 GABA receptor: insights into the role of Loop F in channel function

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Board B294

γ-aminobutyric acid (GABA)-gated ion channels belong to cys-loop receptor family of ligand-gated ion channels. Binding of GABA to its receptor induces a conformational change to open a chloride conducting pore with a not fully understood mechanism. In this study, using site-specific fluorescence with a relatively small fluorophore, we scanned 18 residues (210–227) of loop F in the N-terminal domain of the ρ1 $GABA_C$ receptor for conformational rearrangements related to channel function. Since loop F is not directly involved in ligand binding, fluorescence change detected can directly reflect the conformational change rather than binding effect. From the 18 scanned residues, we detected conformational changes at 6 positions. At these positions, GABA-induced dose-dependent fluorescence changes were highly correlated to channel function. However, directions of the GABA-induced fluorescence change were not the same. At positions of 210 and 211, fluorescence increased during channel activation, indicating these residues become more buried. In contrast, at positions of 216, 217, 218, and 222, fluorescence intensity decreased, suggesting more exposed to aqueous solution. Moreover, $GABA_C$ receptor competitive antagonist, 3-APMPA induced a fluorescence change that is in opposite direction as GABA-induced change at two positions but in the same direction at other four positions. Finally, GABA-induced conformational change can be partially blocked by non-competitive antagonist picrotoxin. Thus, we have detected conformational changes in the loop F that is related to channel activation and antagonism. Potential mechanism relating the conformational change to channel gating is further discussed with the context of the homology model of the N-terminal domain of ρ1 $GABA_C$ receptor.

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1319-Pos Ligand-Specific Temperature-Dependent Shifts in EC_{50} Values for the $GABA_A$ Receptor

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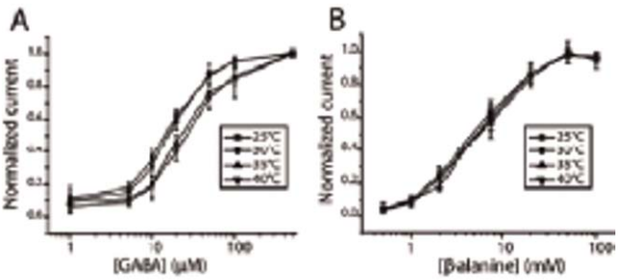
Board B295

We demonstrate how addition of local temperature control to a rapid solution exchanger device, in combination with patch-clamp, enables fast acquisition of dose-response data at different temperatures. Temperature control was achieved by adding a thin-film structure to the under side of the device. By applying a voltage to this structure, resistive heating was enabled.

We obtained dose-response curves for the $GABA_A$ receptor, a ligand-gated ion channel, for two different agonists (GABA and β-alanine) at temperatures between 25°C and 40°C. For GABA, the dose-response curves shifted towards higher EC_{50} values as the temperature increased, whereas for β-alanine the EC_{50} values were constant. This shows that temperature is an important factor for obtaining accurate estimations of EC_{50} values, and also that such temperature effects can be ligand-specific. Furthermore, the technology introduced here is generally applicable to all patch-clamp studies where temperature control is desirable, *e.g.* studies of kinetics and thermodynamics, drug screening, compliant ADME/Tox testing, and in studies of temperature-gated ion channels.



1320-Pos Gaba, Naturally Immunosuppressant of T Lymphocytes

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Board B296

Neurons have recently been shown to have a crucial role in governing central nervous system inflammation (Liu et al., 2006). Here we report that the main inhibitory neurotransmitter in the brain gamma aminobutyric acid (GABA), decreases proliferation of T lymphocytes at concentrations that can be found around neurons in the extracellular space in the brain (Tossmann et al., 1986) and normally evoke tonic inhibition in neurons (Lindquist and Birnir, 2006). Our results indicate that not only does the extracellular GABA help set the neuronal tone but that it also has a protective role in the brain. The low physiological concentrations of GABA (nM) do activate extrasynaptic-like GABAA channels on the lymphocytes. The channels are activated after a delay from the GABA application and the conductance increases with time. The GABAA subunits detected were: alpha1, alpha4, beta2, beta3, gamma1, delta. Perhaps the most striking results in the T-cells are that only the alpha1 and alpha4 subunits of the potential 6 different alpha GABAA subunits are expressed in the lymphocytes. These two subunits impose very different pharmacological and functional profile on the expressed receptors but importantly they have been

shown to form extrasynaptic receptors in neurons. The mechanism of the immunoprotective effect of GABA may be as follows: GABA opens T-cell GABA channels (GABAA receptors) that depolarize the lymphocytes resulting in decreased calcium entry and thereby inhibition of the cytotoxic T-cells. The results have implication for diseases such as MS and type-1 diabetes.

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1321-Pos Effect of a Mutation on the Channel-opening Equilibrium of a Malfunctioning GABA(A) Receptor Linked to Epilepsy

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Board B297

γ-Aminobutyric acid A ($GABA_A$) receptors are a family of neurotransmitter receptors that participate in regulating signal transmission between the $\sim 10^{12}$ cells of the mammalian nervous system by controlling the amount of chloride ion (Cl^-) flux across the cell membrane. The $GABA_A$ receptor-mediated Cl^- influx into a neuronal cell inhibits signal transmission between cells. Differential activity of excitatory *versus* inhibitory neurotransmitter receptors is believed to be linked to epileptogenesis by an abnormal down-regulation of inhibitory neuronal transmission. Mutations in various subunits of the $GABA_A$ receptor, including two independent mutations in the δ subunit - a glutamate-to-alanine mutation at residue 177 or an arginine-to-histidine mutation at residue 220 - are believed to interfere with normal signal transmission between cells. Previous work showed that the primary effect of the δ subunit mutations is to reduce the mean channel-open time of the receptor [Feng et al. (2006) *J. Neurosci.26*, 1499–1506]. However, the value of the channel-opening rate constant (k_{op}), and consequently the value of the channel-opening equilibrium constant ($\Phi^{-1} = k_{op}/k_{cl}$), of the receptor was unknown. Using two transient kinetic techniques, the cell-flow method and the laser-pulse photolysis technique [reviewed Hess, G. P. and Grewer, C. (1998) *Methods Enzymol. 291*, 443–473], we have determined the value of the dissociation constant of GABA and the values of the channel-opening (k_{op}) and -closing (k_{cl}) rate constants and, therefore, the value of the channel-opening equilibrium constant (Φ^{-1}) for both the wild-type and mutated forms of the receptor.

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1322-Pos An intrasubunit salt-bridge linking Loop A and Loop 2 is critical for $GABA_A$ receptor activation

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Board B298

For Cys-loop receptors, the structural machinery that links the ligand binding site to the channel gate is just beginning to emerge. Current models propose that neurotransmitter binding triggers channel gating via a "conformational wave" involving sequential movements of nm-sized protein blocks from the binding site to the channel. How movements are relayed from one "block" to another is not clear. Salt bridges can form and break within nanoseconds making them ideal structures for dynamically linking the blocks. In $GABA_A$ receptor ($GABA_AR$) models, a pair of oppositely charged residues in Loop 2 (β_2D56) and Loop A (β_2K102) are in close proximity and potentially link the GABA binding site (Loop A) to the gating interface (Loop 2) via a salt bridge. We examined the consequences of reversing and swapping these charges by expressing wild-type and mutant $GABA_ARs$ in *Xenopus* oocytes and using two-electrode voltage clamp. Charge reversals (D56K, K102D) significantly decreased GABA efficacy and increased GABA EC_{50} ~900- and ~1200-fold respectively, as compared to wild-type (13.3 ± 1.5μM). The charge swap (D56K-K102D) restored GABA efficacy to near wild-type values and increased GABA EC_{50} (900-fold), much less than the shift predicted ($\sim 10^6$-fold) in the absence of any interaction. Mutant cycle analysis yielded a substantial free energy of coupling between the two residues of ~4.0 kcal/mol. Notably, mutant cycle analysis also yielded a significant interaction energy (~1.6 kcal/mol) between the residues for the allosteric modulator, pentobarbital to activate the $GABA_AR$. In summary, the data indicate that β_2D56-β_2K102 form a salt-bridge that links the GABA binding site to Loop 2. Breaking this salt bridge hinders receptor activation, identifying a potentially new structural path that links GABA binding to channel gating.

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1323-Pos 5-HT_{3A} and GABA σ_1 Channels are Functional with a Heptapeptide in Place of the M3M4 Loop

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Board B299

Cys-loop neurotransmitter receptors form ion channels by the pentameric assembly of homologous subunits. All subunits share the same 3 domain topology:

1. a ~200 amino acid, extracellular, N-terminal domain harbors the ligand binding site,
2. a transmembrane domain with four α-helical segments (M1-M4), and

3. an intracellular domain formed by the ~100 residue cytosolic loop between M3 and M4.

Most of the M3M4 loop was unresolved in the nicotinic acetylcholine receptor cryo-EM structure only an α-helix preceding M4 was resolved. M3M4 loop residues are determinants for single channel conductance (QDA mutant in the 5-Hydroxytryptamine type 3A (5-HT_{3A}) receptor). M3M4 loop involvement in receptor clustering, channel kinetics, trafficking and localization has been investigated. The archaebacterial, proton-activated Cys-loop receptor homologue from *Gloeobacter violaceus* (Glvi) and all other bacterial homologues lack a large M3M4 loop. We hypothesized that the large M3M4 loop in metazoan homologues is neither required for assembly nor function of Cys-loop receptors. We replaced the 5-HT_{3A} M3M4 loop (115 AA) with the Glvi heptapeptide M3M4 loop to obtain 5-HT_{3A}-glvM3M4. The electrophysiological characteristics of 5-HT_{3A}-glvM3M4 and wild type 5-HT_{3A} were comparable, as determined in *Xenopus* oocyte two-electrode voltage clamp experiments: 5-HT EC_{50}, picrotoxin IC_{50} [6 μM (5-HT_{3A}-glvM3M4) and 60 μM (5-HT_{3A})], inhibition by diltiazem, QX-222 and ondansetron. Coexpression of hRIC-3 in oocytes significantly attenuated serotonin-induced current amplitudes in 5-HT_{3A} but not 5-HT_{3A}-glvM3M4. Patch-clamp experiments (HEK293) demonstrated that the truncated channel remained cation-selective. The single channel conductance of 5-HT_{3A}-glvM3M4 was 70-fold increased compared to wild type. Similar macroscopic results have been obtained with the anion-selective GABA ρ_1 receptor. Our results indicate that the M3M4 loop is not essential for Cys-loop receptor assembly and function.

Support: NS030808 and GM077660 (to MHA) and K99NS059841 (to MJ).

1324-Pos A Partial Agonist of the Glycine Receptor: beta Alanine

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Board B300

Glycine-gated channels belong to the Cys-loop superfamily and in their α1β heteromeric form mediate fast synaptic inhibition in the caudal CNS. We recently reported (Burzomato *et al.*, 2004) that the activation of this receptor can be described by a mechanism that identifies a distinct "flipped" conformational change that follows agonist binding but precedes opening. The maximum open probability with an agonist depends on both the flipping and the opening steps of the bound channel. Glycine is highly efficacious at both levels, but we don't know which step is impaired when the channel is activated by a partial agonist, such as β-alanine.

Cell-attached recordings of β-alanine-activated single-channel currents from rat recombinant α1β glycine receptors were idealised by time-course fitting. Records at several agonist concentrations were then fitted simultaneously with the "flip" mechanism.

With β-alanine, maximum single-channel open probability was 0.93, only slightly smaller than that observed with glycine (0.98). However, β-alanine was very poor at stabilising the fully-bound "flipped" pre-opening conformation, with an equilibrium constant of 0.48 (cf. 27 for glycine). Interestingly, once "flipping" has occurred, both agonists are effective in opening the channel, the gating equilibrium constants being 46 and 20 for β-alanine and glycine, implying that flipped channels are open for 98 and 95% of the time, respectively.

Another way of looking at the results is to note that glycine has a 65-fold greater affinity for the flipped conformation than for the resting one (8 and 520 μM, respectively), but that β-alanine does not (130 and 200 μM)

Our findings suggest that the main reason β-alanine is a partial agonist on α1β GlyRs is that it is not very effective at eliciting the conformational changes that precede channel opening.

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1325-Pos Strychnine Inhibition of Human α1 Glycine Receptors

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Board B301

The strychnine-sensitive glycine receptor is a member of the cys-loop ligand-gated ion receptor superfamily. We investigated channel activation by glycine and competitive inhibition by strychnine. cDNA for the α1 subunit of human glycine receptors, a gift from Dr. Heinrich Betz, was transiently expressed as a homopentamer in BOSC23 cells. Outside-out patches excised from cells were placed in front of a 2- or 3-tube rapid perfusion device (solution exchange within 100μs). Glycine activated currents with EC_{50}=290±20 μM, n_H=1.36±0.09. Application of 3 mM glycine activated ~95% of the channel activity as determined by fluctuation analysis. Receptors equilibrated with the competitive antagonist strychnine were rapidly perfused with 3 mM glycine to determine the fraction of activatable channels. Equilibrium inhibition by strychnine was characterized by IC_{50}=18±2 nM, n_H=1.85±0.25. Channel activation onset time was unaffected by the presence of 230 nM strychnine (99% inhibition). Onset kinetics of strychnine inhibition were determined by exposing receptors to strychnine for various time intervals, then perfusing with 3 mM glycine. Offset kinetics were determined similarly. Onset time constants varied from 7.5±1.8s (7 nM) to 0.23±0.11s (200 nM). The rate of inhibition was a linear function of [strychnine] with a slope of 0.032±0.004/s/nM. Offset times were [strychnine]-independent, 6.9±2.0s. Interpretation of these data requires consideration of models with up to 5 agonist/antagonist binding sites. The steepness of the strychnine inhibition curve suggests that

(a) 3–5 molecules of strychnine may bind,
(b) channels with 1 or 2 molecules of strychnine bound may be activatable by high [glycine] and
(c) partially strychnine-liganded channels may be opened as efficaciously as unliganded receptors.

We are currently assessing models consistent with our kinetic data. We will discuss our findings compared with previously published data.

Supported by NIH NS045095

1326-Pos Identifying the Lipid-Protein Interface of the 5-HT_{3A}R: Hydrophobic Photolabeling Studies with Affinity-Purified 5-HT_{3A}Rs

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Board B302

The 5-HT_3R is a member of the Cys-loop superfamily of ligand-gated ion channels (LGICs) and mediates excitatory fast synaptic transmission in the CNS. Despite the clear physiological importance of the 5-HT_3R, only a small number of published studies have directly examined the structure of the receptor. The principal objective of this work is to express and affinity-purify 5-HT_3Rs and initiate structural studies. A mouse 5-HT_{3A}R containing a C-terminal α-bungarotoxin (αBgTx) pharmatope tag (1) was constructed and stably transfected into HEK 293 cells. To obtain sufficient quantities of receptor protein for affinity-purification, αBgTx-5-HT_{3A}R-HEK cells were cultured either in 140 mm dishes (∼1000 dishes) or in a 5 L spinner flask containing microcarrier glass beads (Cytodex-3). Typically, cells were treated with 100 μM serotonin 24 h prior to harvesting resulting in a ∼2.5 fold increase in receptor expression (upregulation). αBgTx-5-HT_{3A}Rs ([^{125}I]αBgTx Kd ∼10 nM) were affinity-purified (αBgTx-derivatized Sepharose 4B affinity column) from detergent (1% CHAPS) solubilized membranes. In this first study, the lipid-protein interface of the 5-HT_{3A}R was examined by hydrophobic photolabeling with [^{125}I]TID. Our data demonstrate that [^{125}I]TID photoincorporates into the 5-HT_{3A}R and the labeling maps to two proteolytic fragments, designated V8-17K and V8-8K. N-terminal sequencing of each rpHPLC purified fragment revealed that V8-17K starts at Val^{195} and based on its apparent molecular weight extends through the M1–M3 transmembrane segments. V8-8K starts at Val^{424} and contains the M4 segment. Approximately 60% of the total subunit labeling is localized to V8-8K suggesting that the M4 segment has the greatest exposure to lipid. Additional experiments are in progress to further identify lipid-exposed segments/residues in the 5-HT_{3A}R and the results will be compared with those previously determined for the *Torpedo* nAChR.

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1327-Pos Acid-Sensing Ionic Channels in Rat Anterior Pituitary Cells

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Board B303

Acid-sensing ionic channels (ASICs) are members of ENaC/degenerin family of channels. To date, four different isoforms have been cloned. Three of them, termed ASIC1-3, form functional homo- and/or heteromeric channels transiently opening in response to a drop in extracellular pH. The ligand for ASIC4 and functional role of this isoform remains elusive. They are widely distributed in central and peripheral nervous system and they have been implicated in neuronal ischemia, mechanoreception, and nociception. At present time, little is known about the expression and role of these channels and the pH sensitivity of anterior pituitary cells in general. Here we show that mRNA transcripts for ASIC1, 2 and 4 are expressed in anterior pituitary cells. In agreement with this data, drop in extracellular pH below 7.0 evoked transient inward current in one third of cells tested. The current was characterized by fast desensitization and diminution in response to repeated low pH application. ASIC-like current was inhibited by amiloride (50 μM) but was not affected by the TPRV1 antagonist ruthenium red (10 μM). Channels underlying ASIC-like current were permeable to sodium and potassium with no permeability for calcium. These data suggest expression of ASIC channels in pituitary cells, with possibility for their heteromeric composition. Further experiments are needed to clarify their exact structure and roles in pituitary cells, including their possible modulation by peptidergic hormones.

Voltage-gated K Channels - III

1328-Pos Differential Effects of Amidoarone and Dronedarone on hERG and KCNQ1/KCNE1 Channels

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Board B304

Purpose. To address the question whether dronedarone, a new noniodinated methanesulfonanilide derivative of amiodarone, may differ from amiodarone, we comparatively studied the effects of DR and AM on HERG and KCNQ1/KCNE1 channels and determined the binding sites of both drugs within HERG channel.

Methods. HERG (wild and mutant) and KCNQ1/KCNE1 (wild) channels were expressed in Xenopus Oocytes and currents were measured using 2-electrodes voltage clamp technique. To determine binding sites, ala-scanning mutagenesis of S6 (L646-Y667) and pore helix (L622-V625) in HERG channels were performed.

Results.

(i) DR potently blocked both HERG channel ($IC_{50\%}$: 1.7±0.3 μM, n=5) and KCNQ1/KCNE1 channels ($IC_{50\%}$: 13.4±3.3 μM, n=5), whereas AM preferentially blocked HERG channel ($IC_{50\%}$: 5.6±1.4 μM, n=5) rather than KCNQ1/KCNE1 channels ($IC_{50\%}$: >100 μM, n=5).

(ii) DR rapidly binds (time constant (tau), 264±47 ms, n=5, 40mV) to and unbinds (tau=360±24 ms, n=5, −80mV) from