2506-Pos Board B476

Molecular Weight and Volume at Position 46 in Transmembrane Domain 1 (TM1) are Important Determinants for Ethanol Sensitivity in P2X4 Receptors

Maya Popova, Kaixun Li, Liana Asatryan, Ronald L. Alkana, **Daryl L. Davies**.
University of Southern California, Los Angeles, CA, USA.

Purinergic P2X receptors (P2XRs) are a family of fast acting, cation-permeable ligand-gated ion channels, which are gated by synaptically released adenosine 5′-triphosphate (ATP). Building evidence supports the notion that P2XRs play a role in mediating and/or modulating behavioral effects of ethanol. However, this work is in the early stages and the sites and mechanisms of ethanol action in P2XRs are poorly understood. Recently, we identified an amino acid located in TM1 that was conserved across P2X3 and P2X4Rs that appeared to represent an important site for the action of ethanol (i.e., Trp41 and Trp46, respectively). The current study tests the hypothesis that physical-chemical properties of the residue at position 46 play a significant role in determining ethanol sensitivity in P2X4Rs. We expressed wildtype and mutated receptors in Xenopus oocytes and determined changes in ethanol sensitivity (200mM) using an 8-channel two-electrode voltage clamp system (OpusXpress 6000; -70mV). Exchanging Trp46 residue with other aromatic residues did not significantly alter ethanol sensitivity whereas replacing Trp46 with aliphatic residues significantly reduced the action of ethanol. Correlation analysis determined that molecular weight and volume of the residue at position 46 were significantly correlated with ethanol sensitivity whereas polarity or hydrophobicity was not. The findings suggest that the size and aromaticity of the residue at position 46 in TM1 play an important role in determining ethanol sensitivity of P2X4Rs. Identifying physical-chemical properties of residues that are important for ethanol sensitivity will increase our knowledge regarding structural requirements that are necessary for ethanol to cause changes in modulation and/or transduction of agonist action in P2X4Rs. Support: NIAAA/NIH F31 AA017029, KO1 AA017243-01A1, AA013922, AA03972 and USC School of Pharmacy.

2507-Pos Board B477

Lack of Ethidium Bromide Uptake during Hypotonic Stress in HEK 293 Cells that Express P2X7 Receptors

Patricia Perez-Cornejo[1], Juan P. Reyes[2], Carmen Y. Hernandez-Carballo[2], Gabriela Perez-Flores[1], Jorge Arreola[2].
[1]School of Medicine, Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico, [2]Physics Institute, Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico.

Recent reports indicate that the long known ethidium bromide uptake resulting from prolong stimulation of cell surface $P2X_7$ receptors is due to Pannexin-1 (Panx-1) hemichannels, which are probably activated by the carboxy terminus of the receptor itself. It has also been proposed that Panx-1 is activated by exposing the cells to hypotonic solutions without the need for $P2X_7$ receptor activation and that this maneuver results in ATP release from cells probably via Panx-1. However, other groups have previously proposed that the hypotonic-induced ATP release is via cell swelling-activated chloride channels. In this work we explored activation of endogenous Panx-1 in HEK 293 cells (untransfected or transfected with $P2X_7$ receptors) exposed to hypotonic solutions, while simultaneously measuring whole cell chloride current and fluorescence signals resulting from ethidium bromide uptake (an index of Panx-1 activation). When HEK cells were exposed to hypotonic solutions that induced activation of cell swelling-activated chloride current ($I_{Cl,swell}$) no ethidium bromide uptake was detected. Treatment of the cells with the Panx-1 inhibitor mimetic peptide ^{10}panx1 resulted in complete inhibition of ethidum bromide uptake induced by ATP activation of $P2X_7$ receptors without affecting $I_{Cl,swell}$. Pretreatment with carbenoxolone (CBX), another blocker of Panx-1, inhibited ethidium bromide uptake induced by stimulation of HEK cells expressing $P2X_7$ receptors and at the same time inhibited $I_{Cl,swell}$ in a dose-dependent manner. We conclude that hypotonic conditions most likely induce ATP release by via activation of CBX-sensitive volume-sensitive chloride channels without participation of Panx-1 hemichannels.

Supported by CONACyT grants 45895 and 79897.

2508-Pos Board B478

A symmetric structural model for Acid sensing ion channel-1: Transmembrane domain dynamics and Implications to Gating

Saher A. Shaikh, Emad Tajkhorshid.
University of Illinois Urbana Champaign, Urbana, IL, USA.

Acid sensing ion channels (ASICs) are cation-selective neuronal membrane channels, activated by H+ binding upon decrease in extracellular pH. The mechanistic and structural details of channel activation and ion permeation in ASICs are only partially understood. The only known crystal structure for ASICs is in the desensitized, non-conducting state, and the open and closed structures remain unsolved. The crystal structure reveals ASIC1 to be a trimer with 3-fold symmetry, but reports significant asymmetry in the transmembrane (TM) region, suggested to be induced by crystal lattice contacts, hence restricting further hypotheses about TM behavior. In order to enable a study of TM domain dynamics, we sought to derive models from the crystal structure, where this asymmetry was corrected using a simulation based approach. We designed a model target based on normal mode analysis, and employed targeted molecular dynamics to generate the initial model from the crystal structure followed by a series of simulations to equilibrate the structure. The resulting model, though starting with nearly straight TM helices, exhibits kinks as the simulations proceed, suggesting dynamic hinges in the TM pore, which could be participants in gating. Observations about channel behavior from these models show consistence with experimental data. An interesting revelation from the model is the identification of a possible gating region near the cytoplasmic end of the TM pore constituted of highly hydrophobic residues. Overall the study reveals several details about the putative cation permeation pathway in ASICs and also provides a symmetric structural model to test further hypotheses on ASIC channel function.

2509-Pos Board B479

Analysis of IP_3 Receptor Activation Using Novel Partial Agonists

Ana M. Rossi[1], Andrew Riley[2], Stephen C. Tovey[1], Olivier Dellis[1], Barry V.L. Potter[2], Colin W. Taylor[1].
[1]Cambridge University, Cambridge, United Kingdom, [2]University of Bath, Bath, United Kingdom.

Inositol 1,4,5-trisphosphate receptors (IP_3R) are ubiquitous intracellular Ca^{2+} channels. Each subunit of the tetrameric IP_3R comprises a N-terminal IP_3-binding site, a cytosolic domain and six transmembrane domains (TMD) near the C-terminal. TMD5 and 6 form the pore. IP_3 binding to the IP_3-binding core (IBC, residues 224-604) initiates conformational changes that lead to opening of the pore. The mechanisms are unresolved but require the suppressor domain (SD, residues 1-223) and interactions between N and C termini. We have synthesized a family of 2-*O*-modified IP_3 analogues that are partial agonists of the IP_3R. In defining their properties, we identify a novel form of partial agonism that allows us to define key steps in IP_3R activation. By combining analysis of ΔG for ligand binding, single-channel recordings and molecular modelling we show that these partial agonists are IP_3-like in their interactions with the IBC, but they less effectively rearrange its relationship with the SD. The partial agonists open the channel at slower rates than full agonists. IP_3R with a point mutation in the SD that occupies a position similar to the 2-*O*-substituent of the partial agonists has reduced open probability that is similar for full and partial agonists. This suggests that bulky or charged substituents at the IBC-SD interface, whether provided by the ligand or SD, frustrate an obligatory coupling between IBC and SD. Our analysis of ΔG for ligand binding to truncated IP_3R shows that conformational changes initiated by IP_3 binding propagate entirely via the SD to the pore. We suggest that IP_3 first closes the clamp-like structure of the IBC, then the IBC interacts with the SD to give a compact structure that allows the SD alone to signal onwards to the pore.

2510-Pos Board B480

Single-Channel Kinetic Analysis for Activation and Desensitization of Homomeric 5-HT_{3A} Receptors

Jeremias Corradi, Fernanda Gumilar, Cecilia B. Bouzat.
Instituto de Investigaciones Bioquimicas, Bahia Blanca, Argentina.

The 5-HT_{3A} receptor is a member of the Cys-loop family of ligand-gated ion channels. Due to its low conductance, kinetic analysis of this receptor has been restricted to the macroscopic level. We introduced mutations in the 5-HT_{3A} subunit to obtain a high-conductance form so that single-channel currents can be detected. At all 5-HT concentrations (>0.1 μM) channel activity appears as opening events in quick succession forming bursts, which, in turn, coalesce into clusters. By combining single-channel and macroscopic data we generated a detailed kinetic model that perfectly describes activation, deactivation and desensitization. The model shows that full activation arises from receptors with three molecules of agonist bound. It also reveals an earlier conformational change of the fully-liganded receptor (flipping) that occurs while the channel is still closed. From this pre-open state the receptor enters into an open-closed cycle involving three open states, which conforms the cluster whose duration parallels the time constant of desensitization. This suggests that at a synapse the lifetime of the elementary response of 5-HT_{3A} receptors is determined mainly by desensitization. Since the desensitized state is a stable state, the inter-response latency is expected to be prolonged. The present kinetic model provides a foundation for studying molecular mechanisms of drug action. We show that mutations at valine 10′ of M4 affect opening and closing rates

within the open-closed cycle. This reveals that the outermost transmembrane domain is important for appropriate gating and shows a high conservation of M4 function among members of this superfamily.

2511-Pos Board B481

Targeted Delivery of Glycine Receptors to Peripheral Neurons as Treatment for Pain

Michael Cascio[1], James R. Goss[1], Shaohua Huang[1], David M. Krisky[1], Richard J. Clarke[2], Jon W. Johnson[2], Joseph C. Glorioso[1].
[1]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA,
[2]University of Pittsburgh, Pittsburgh, PA, USA.

Neurotransmitter-gated receptors play a vital role in the regulation of nociception. In the adult CNS, the ionotropic glycine and GABA receptors are typically inhibitory and act to silence neurons by transiently permitting inward Cl^- currents upon activation. However, depending on the Cl^- equilibrium potential, these receptors may instead depolarize neurons. We hypothesized that targeted expression of the α1 subunit of glycine receptor (GlyR) in peripheral sensory neurons using a non-replicating herpes simplex virus (HSV)-based vector might reduce nociceptive behavior upon subsequent GlyR activation by exogenously-applied glycine. HSV-directed expression of the human α1 subunit of GlyR alone is sufficient to produce glycine-gated chloride currents in whole-cell patch clamp studies of infected mammalian cells. In cultured dorsal root ganglion neurons, expressed GlyRs are diffusely localized in the neuronal plasma membrane. In both a formalin footpad model of inflammatory pain or an osteosarcoma pain model, rats inoculated with the GlyR-expressing vector (vHGlyRα1) exhibited significantly reduced nociceptive behavior following subcutaneous injection of glycine into the footpad. This reduction in pain-related behavior was reversed following injection of the GlyR antagonist strychnine. Targeted expression of GlyRs and their modulation via mutagenesis and/or exogenous application of agonists, antagonists, and allosteric modulators offer a novel and potentially powerful method to alter neuronal activity. Here we show that selective activation of HSV-directed α1 GlyR expression in peripheral neurons eliminates pain-related behavior in two pain models, and has the potential to be used therapeutically for pain management.

2512-Pos Board B482

Simultaneous Recording Of Ligand-binding And Channel-gating On Individual Nicotinic-acetylcholine Receptors In Living Cells

Ralf Schmauder, Davor Kosanic, Ruud Hovius, Horst Vogel.
Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

Simultaneously measuring both the ligand-binding and the gating of ligand-gated ion channels will provide important novel insight in receptor and channel function (see e.g. Edelstein et. al.). Such measurements require a fluorescently labeled agonist as ligand, single molecule sensitive optical and electrical detection, micro-second time-resolution, and optimal alignment of the optical and electrophysiological single-channel acquisition parts.

Here we present first results from simultaneously measurements of ligand-binding and channel gating of individual prototypical ligand-gated ion channels in living cells, using APD-based fast confocal detection and conventional patch-clamping in single channel mode. In order to reach a reasonable throughput of experiments, it was essential that the sample, patch-pipette and the confocal volume could be moved independently and be aligned with micrometer precision. We show data from nicotinic-acetylcholine receptors, expressed in HEK293 cells and fluorescently labeled epibatidine as agonist, to elucidate details of the channel gating.

S.J. Edelstein, O. Schaad & J.P. Changeux: Biochmistry;1997; 36(45), 13755

2513-Pos Board B483

Functional Characteristics of alpha3beta3gamma2 and alpha1beta2-gamma2 GABA-A receptors

Angelo Keramidas, Neil Harrison.
Weill Medical College of Cornell University, New York, NY, USA.

The $GABA_A$ receptor is a member of the cys-loop family of ligand gated ion channels and the major mediator of neuronal inhibition. Each receptor is a pentameric protein complex composed of homologous subunits, the combination of which gives rise to numerous $GABA_A$ receptor subtypes. Within the thalamus, two synaptic $GABA_A$ receptor isoforms predominate; the $\alpha_1\beta_2\gamma_2$ channels, which are found at the synapses of relay neurons, and the $\alpha_3\beta_3\gamma_2$ channels that are located at synapses in the reticular nucleus. We endeavoured to characterize the kinetic properties to these two $GABA_A$ receptors. Our preliminary data suggest that both receptors open to a single channel conductance level of 26 pS, irrespective of the GABA concentration used to elicit activity. $\alpha_3\beta_3\gamma_2$ $GABA_A$ receptors, however, exhibit longer active periods (bursts) at saturating (5 mM) and low (2 μM) concentrations of GABA compared to $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors. The mean burst length and intra-burst open probability (P_o) for the $\alpha_3\beta_3\gamma_2$ channels was 136.7 ± 16.8 ms and 0.84 ± 0.05 (n = 4), respectively and for the $\alpha_1\beta_2\gamma_2$ channels was 87.4 ± 12.0 ms and 0.82 ± 0.02 (n = 3), respectively, when exposed to 5 mM GABA. At 2 μM GABA, the mean burst length and P_o for $\alpha_3\beta_3\gamma_2$ channels was 25.8 ± 4.7 ms and 0.78 ± 0.03 (n = 3), respectively and for $\alpha_1\beta_2\gamma_2$ channels was 8.4 ± 1.9 ms and 0.73 ± 0.02 (n = 3), respectively. These measurements are consistent with the reported slower deactivation phase of ensemble, $\alpha_3\beta_3\gamma_2$ mediated synaptic currents and suggest that GABA has a longer occupancy at $\alpha_3\beta_3\gamma_2$ channels than at $\alpha_1\beta_2\gamma_2$ channels.

2514-Pos Board B484

DPNI-caged Gaba As A Tool For Investigating The Kinetic Properties And Distribution Of Gaba Receptors And For Silencing Neurons In Situ

David Ogden.
Univ Paris Descartes, Paris, France.

The nitroindoline caged amino acids are hydrolytically stable and have photochemical and pharmacological properties suitable for characterising neurotransmitter receptors. Although the NI and MNI - caged glutamates show no interference in the activation of glutamate receptors at 10 mM concentration range, the corresponding GABA and glycine NI derivatives showed evidence of binding and interference in the amplitude and kinetics of activation at their respective receptors at 0.1 mM concentrations. Similarly interference with GABAergic transmission by 50 μM CNB-caged GABA has also been reported. We describe here a nitroindoline caged GABA modified by addition of a biphosphate on the caging group with the idea that the high negative charge will impede binding of the cage to the GABA receptor. Experiments to test the inhibition of miniature GABA mediated synaptic events in cerebellar molecular layer interneurons showed much reduced inhibition, approximately 66% inhibition of amplitude at 1 mM DPNI-GABA. However, with laser pulses of 0.1 ms at 1 mM DPNI-GABA the 10-90% risetimes were comparable with synaptically evoked currents, indicating that equilibration of the cage with the receptor is fast and interferes little with kinetic measurements. The decline of laser evoked currents was prolonged compared with spontaneous events suggesting that the effective volume of GABA release in the laser spot is larger than synaptic release. The precision of locating GABA 'hot spots' in the focal plane was estimated to decline 50% in 1.75 microns, permitting detailed mapping of the distribution of receptors over neuronal compartments. Furthermore, at 100 μM DPNI-GABA, where there is no interference with receptor activation, photolysis localised to the soma of Purkinje neurons or interneurones suppressed spiking with millisecond precision, providing a useful tool for network analysis.

2515-Pos Board B485

A Single Steroid-binding Site is Sufficient for Potentiation of GABA-A Receptors

Joe Henry Steinbach, John R. Bracamontes.
Washington University, Saint Louis MO, MO, USA.

Neuroactive steroids are efficacious potentiators of GABA-A receptors. Recent work identified a site in the α1 subunit of the GABA-A receptor which is essential for potentiation by steroids. However, each receptor contains two copies of the α1 subunit, leading to questions of whether both sites are required and whether the sites mediate distinguishable effects. We generated subunit concatemers so the α1 subunits could be mutated separately, and examined consequences of a mutation which removes potentiation by most neurosteroids (α1 Q241L) and a mutation which mimics the presence of bound steroid (α1 Q241W). Concatemers were expressed in *Xenopus* oocytes, and activation by GABA, potentiation by neuroactive steroids and the agonist activity of the partial agonist P4S were determined. When the α1 Q241L mutation is made in both α1 subunits the EC_{50} for activation by GABA is shifted to higher concentration and the ability of neurosteroids to potentiate responses to a low concentration of GABA is lost. Conversely, when the α1 Q241W mutation is made in both subunits the EC_{50} for GABA is shifted to lower concentration, the relative ability of P4S to activate the receptor is increased and potentiation by neurosteroids is lost. For both, mutation of only one α1 subunit did not produce the full effect of mutating both sites. Hence, the presence of a single wild-type site is sufficient to mediate all effects seen in macroscopic responses. Overall, mutation of the α1 subunit between the γ and β subunits had a larger effect, which might reflect a subtle difference between sites or a consequence of subunit position. The data demonstrate that at a macroscopic level the presence of a single wild-type steroid-binding site is sufficient to mediate the responses to steroid.

Supported by grant P01 GM047969.

2516-Pos Board B486

MTS-Etomidate Selectively Reacts Within the $GABA_A$ Receptor Etomidate Binding Site

Deirdre S. Stewart, Shaukat S. Husain, Keith W. Miller, Stuart A. Forman.
Mass General Hospital, Boston, MA, USA.

The general anesthetic etomidate exerts its major clinical actions through potentiation of $GABA_A$ receptor activation. $GABA_A$ receptors are pentameric,