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.

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

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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.

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

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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

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

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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

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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.

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

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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.

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

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