usually consisting of combinations of alpha, beta, and gamma subunits. A photoreactive structural analog of etomdiate ([3H]azietomidate) labels amino acids on transmembrane domains in both alpha (M236) and beta (M286) subunits. This suggests the presence of two interfacial anesthetic binding sites per $GABA_A$ receptor, consistent with receptor structural homology models based on *Torpedo* nicotinic acetylcholine receptor. To further characterize the etomidate binding site, we have created a number of Cys substitutions within the α/β intersubunit region and have synthesized a novel etomidate derivative, 2-(methylsulfonyl) thio-etomidate (MTS-etomidate), designed to covalently modify cysteines. Human $GABA_A$ receptors (α1, β2, γ2L) were expressed in *Xenopus* oocytes and current responses were measured using two-electrode voltage clamp. Using pCMBS and MTSEA, we found that cysteine substitutions at both α1M236 and β2M286, were accessible to modification. Cysteine modification was also evident with pCMBS at α1L232C, one helical turn above α1M236 in TM1. In contrast, modification by MTS-etomidate was evident only at α1M236C, but not at β2M286C or, α1L232C. These results suggest that MTS-etomidate orients itself in a precise conformation within its binding pocket and acts as a highly selective structural probe, yielding information not only about the residues with which it interacts but also about the orientation of etomidate within the biding pocket consistent with its mechanism of action.

2517-Pos Board B487

Investigations of Ligand-gated Ion Channels Using Caged Neurotransmitters Reveal Mechanism-based Approaches to Modulating Receptor Function

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Recognizing the problem of rapidly equilibrating membrane-bound proteins on cell surfaces with activating ligands due to the water layer that tightly adheres to the surface of spherical objects (1), we began to develop caged (2) neurotransmitters (3,4). A caged neurotransmitter can be equilibrated with receptors on a cell surface before photolysis releases the neurotransmitter in the microsecond time region (3,4). Using caged neurotransmitters in whole-cell current recordings enables the rate constants for the opening (k_{op}) and closing (k_{cl}) of the receptor channel, and thus the channel-opening equilibrium constant (Phe^{-1}), to be measured without interference from diffusional delays or receptor desensitization.

The rate constants and the channel-opening equilibrium constant of the excitatory nicotinic acetylcholine receptor (5) are altered in the presence of noncompetitive inhibitors, such as cocaine, as are those of a mutated inhibitory gamma-aminobutyric acid (GABA) receptor (6) linked to epilepsy (7). Based on the mechanisms of the two receptors, we developed compounds that bind with higher affinity to the open-channel than to the closed-channel conformation of each receptor, alleviating (8,9) the inhibition of the acetylcholine receptor and potentiating (10) the function of a mutated $GABA_A$ receptor linked to epilepsy.

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2518-Pos Board B488

Toward CFTR Structural Dynamics During Ion Channel Gating

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While CFTR catalyses the irreversible hydrolysis of its physiological ligand (ATP), there is disagreement about whether CFTR channel gating is a thermally driven process (Aleksandrov & Riordan 1998 FEBS Lett. 431:97, Csanady et al., 2006 JGP128:509). Knowing the answer to this question is of fundamental importance to understand problems leading to CFTR functional failure in cystic fibrosis, an inherited disease of high morbidity and mortality. Although CFTR single channel recordings do not provide a direct measure of enzymatic activity, they do provide an opportunity to establish the relationship between ATP binding and hydrolysis at the nucleotide binding domains (NBDs) and channel gating. The use of Rate Equilibrium Free Energy Relationship (REFER) is a possible way to probe structural dynamics in an ion channel gating (Auerbach, 2005 PNAS 102:1408). We use this conceptual framework to reveal the role of ATP hydrolysis in CFTR ion channel function. The REFER graphs for the set of nucleotide ligands have slope $\Phi \approx 1$ for the openings and $\Phi \approx 0$ for the closings. This means that the nucleotide triphosphate interaction with the binding sites is required for channel opening. In contrast, the ion channel transition from the open to the closed state occurs independently of any events in the binding sites. This could not be interpreted by conventional mechanical models of molecular devices where the mobile species are levered from the initial to the final position by force generated in the binding sites. However this behavior fits very well to Brownian ratchet models where internal thermal diffusion is a key element of the process, and specific interactions induce no mechanical movements directly but instead are used to trap favorable fluctuations.

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Use Of Isolated Vesicles From *Xenopus* Oocytes For Planar Patch Clamp Recordings

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Oocytes from the *Xenopus laevis* toad represent an efficient expression system. The two-electrode voltage-clamp technique (TEVC) as well as patch clamp recordings from excised patches are powerful tools to study ion channels following expression in *Xenopus* oocytes. The TEVC technique allows the measurement of macroscopic currents from whole cells. It is widely used in basic research and also applied for compound screening in pharmaceutical industry. Recordings from excised patches enable the analysis of ion channels under cell free conditions and even single channel analysis. In contrast to the TEVC technique, its application requires highly skilled electrophysiologists.

A publication by Yong Zhang et al. * describes a technique to prepare isolated membrane vesicles from *Xenopus* oocytes by incubating them, after removal of the vitelline membrane, in a hypertonic ''blebbing'' solution. This treatment induces the formation of plasma membrane blebs that eventually detach from the oocyte surface to form isolated plasma membrane vesicles (PMVs).

Here we used these PMVs to create macro patches onto a planar borosilicate glass surface using the Port-a-Patch (Nanion) automated patch clamp device. The PMVs formed readily gigaseals and showed small capacities as expected for macro patches. Currents could be recorded from voltage-gated as well as ligand-gated ion channels.

In conclusion, we present an application that has the potential to overcome the practical limitations associated with the generation of excised patches and might enable the automated analysis of such patches.

*Mechanically gated channel activity in cytoskeleton-deficient plasma membrane blebs and vesicles from Xenopus oocytes.
Yong Zhang, Feng Gao, Vsevolod L. Popov,* Julie W. Wen,* and Owen P. Hamill J Physiol. 2000 February 15; 523(Pt 1): 117-130.

2520-Pos Board B490

Ultrafast Applications For Determining the Kinetics Of Ligand-gated Channels

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Determining kinetic models that describe and predict the behavior of ligand-gated channels is a daunting task. It can be done by the analysis of a considerable amount of single channel recordings or, as it has recently shown for P2X2 receptors, by the analysis of macrocurrents after the application of very short pulses of different concentrations of the agonist. Here we present some advances towards a general strategy for determining a kinetic model for any particular ligand-gated channel using ultrashort pulses. First is the problem of generating the agonist pulses. For that purpose we optimize the movement of the liquid interface between agonist solution and saline. This movement is generated by driving a PZT actuator, whose movement is measured by the deflection of a laser that hits a four quadrant movement detector. The movement of the piezo was optimized to produce pulses as large as 20 micrometers and as brief as 20 microseconds of duration. The piezo drives the movement of a theta tube that eject the agonist and the saline, the movement of whom is measured using stroboscopic microscopy. On the other hand, in order to achieve high speed of solution exchange, a high velocity of fluid is necessary at the right times, so a valve-controlled pressurized system was developed. On patch controls have to be made by switching from saline to a low cation solution using the drop in current as an indicator of the time profile of agonist concentration as seen by the patch. Finally, the gathered data is analyzed by contrasting the likelihood of different allosteric models. For that purpose, an extension of the Macroscopic Recursive method is presented that allow its application to time averaged recordings.