T-AM-SymI-1 MOLECULAR DESIGN AND ACTION OF CELL COMMUNICATION CHANNELS. Nigel Unwin, Stanford University School of Medicine, Stanford, CA 94305

T-AM-SymI-2 **FUNCTIONAL SITES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR.** **Arthur Karlin, Mario DiPaola, Peter Kao, Li Wang, and Amitabh Chak. Departments of Biochemistry and Molecular Biophysics, Neurology, and Medicine, College of Physicians and Surgeons, Columbia University, New York, NY 10032.**

The nicotinic acetylcholine receptor is a multisubunit, membrane-spanning protein which contains a gated, cation-conducting channel. The receptor exists in at least four major functional states. To understand the function of the receptor in terms of its molecular structure, we will require high resolution structures of the receptor in its different functional states. We will also need to identify in these structures the major interacting functional sites, such as the acetylcholine binding sites, noncompetitive inhibitor binding sites, and channel sites. To this end, among others, amino acid residues contributing to these functional sites are being identified in the sequences of the receptor subunits. For example, by affinity labeling and subsequent peptide mapping and sequencing, we found that Cys 192 and Cys 193 of the alpha subunit form a disulfide at the acetylcholine binding site. Furthermore, by time-resolved photoaffinity labeling, we found that residues in one of the putative membrane-spanning segments of alpha, M1 (alpha 211 to 237), contribute to a noncompetitive inhibitor binding site that is revealed only in the open-channel and rapid-onset desensitized states, both transient. The strong interaction of the acetylcholine binding site and the noncompetitive inhibitor binding site and their relative proximity in the alpha sequence is consistent with their presence within a single structural and functional domain of the alpha subunit. **Supported by research grants from NIH (NS07065) and MDA and training grants from NIH (NS07258 and AM01336).**

T-AM-SymI-3 STRUCTURE AND FUNCTION IN VOLTAGE-DEPENDENT SODIUM CHANNELS. R.L. Barchi, University of Pennsylvania Medical School, Philadelphia, PA.

With the successful purification of voltage-dependent sodium channels from several sources, attention is turning toward the elucidation of channel structure and its relationship to function. All sodium channels so far isolated contain one 260 kDa alpha subunit that is heavily glycosylated (26-28%). Mammalian channels may be associated with smaller glycoprotein subunits (~30% CHO) of 30-40 kDa with an $\alpha:\beta$ stoichiometry of 1:1 in rat skeletal muscle and 1:2 in rat brains. Functional reconstitution into lipid vesicles and planar bilayers has been demonstrated for purified channels from eel, and from rat skeletal muscle and brain. Sodium channels from eel and rat brain have been cloned and sequenced; each contains four extensive domains of striking internal homology. Strong homology also exists between species. Analysis of 1^{o} sequences has led to a number of models for channel 2^{o} and 3^{o} structure. Actual channel topography can be probed with antibodies raised against synthetic oligopeptides that mimic discriminant segments of the channel sequence in these models. Using this approach the cytoplasmic location of the C-terminus and of AA residues 927-941 has been confirmed. Nine other segments are presently under investigation. Monoclonal antibodies are being used to define the interrelationship between epitopes adjacent in the channel's 3^{o} structure but separated in it's 1^{o} sequence. Some monoclonals have proven specific for various subtypes of sodium channel in adult muscle and will be useful for identifying structural elements that differentiate these subtypes.

T-AM-SymI-4 AMINO ACID SEQUENCE ALTERATIONS AND STRUCTURE-FUNCTION STUDIES IN A MEMBRANE CHANNEL. Olaf S. Andersen, John T. Durkin, Dept. Physiol. Biophys., Cornell Univ. Med. Coll., New York, NY 10021, and Roger E. Koeppe II, Dept. Chem. Biochem., Univ. Arkansas, Fayetteville, AR 72701.

The linear gramicidins were used to examine how the function of a membrane channel is affected by alterations in the amino acid sequences of the channel-forming peptides. To this end we exploited the fortunate fact that there is a generally accepted structure for the transmembrane gramicidin channel, a symmetrical dimer formed by $\beta^{6.3}$-helical monomers. This is important for two reasons: one can establish functional criteria for structural invariance among channels based on the formation of asymmetric dimers by chemically dissimilar monomers; and one can use the constraints imposed by knowing the general channel conformation to guide the interpretation of functional data. We can thus determine whether mutant channel conductance changes result from side chain-dependent modulations of the permeation path, or from alterations of the channel structure. In the latter case the mutant channel structure must be established de novo. Studies on channels formed by natural and semisynthetic gramicidin analogues illustrate how single side chain substitutions can result in greater than ten-fold conductance changes without affecting the channel structure. The side chains project from the exterior surface of the channels, and do not contact the permeating ions, emphasizing that there need not be close proximity between the region where a chemical alteration is produced and the region where the consequent functional perturbation is induced.

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