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Ion Channel Structure and Function

Christopher Miller

The business of nerve cells is to generate, propagate, and integrate electrical signals, and ion channels are the proteins that support this cellular task. They directly catalyze the ion fluxes that bring about voltage changes across neuronal membranes and simultaneously act as sensors of those physiological signals—changes in ligand concentrations and in transmembrane voltage itself—that orchestrate the neuron's electrical symphony. Since their discovery nearly half a century ago, ion channels have been studied by exceptionally sophisticated assays of their electrical function, but until recently they have been molecular black boxes. Now, however, we are faced with a large number of ion channels for which the genes have been cloned and expressed in electrophysiologically accessible systems; consequently, the question "how do ion channels work?"—a question posed for decades but answered only in the formal language of state diagrams and kinetic models—is taking on a more palpable life. The field is beginning to smell blood as it approaches the molecular bases of channel gating and ion selectivity.

This Perspective is entitled "Ion channel structure and function" rather too hopefully, I think, since answers to these questions ultimately depend on structural information at the atomic level and since we have never seen a neuronal ion channel structure at high resolution. But the field's optimists—and there are many of them—argue that the unique strengths of electrophysiological assays manifested in fast voltage-clamping, single-channel recording, and gating current detection, when combined with genetic manipulation, can circumvent the structural vacuum. At this early stage, when the molecular questions are not yet too detailed, this enthusiastic attitude has produced important, novel conclusions about ion channel structure and its relation to function: pictures of the gross molecular architecture of these proteins, views of membrane topology of their constituent subunits, and identification of local regions of the protein sequence that participate in specific channel functions.

We now know that the two broad classes of channels involved in neuronal excitability—the ligand-gated and the voltage-activated channels—are formed as more or less cylindrically symmetric aggregates of multiple subunits (or of tandem-repeated homologous domains within a single polypeptide), with the ion conduction pore located on the axis of symmetry. Although esthetically pleasing and strongly implied by earlier low-resolution structural work (*1*), this symmetrical-pore picture was not a foregone conclusion; in the days of channel biophysics before cloning, when the pore's existence was clear but its shape was not, there were many unpublished arguments about what its "tortuosity" might be. Now we know that residues at equivalent positions on the five nicotinic acetylcholine receptor channel subunits have roughly equal electrostatic influence on ion conductance (*2*); similarly, in voltage-gated K^+ channels, a pore-blocking site for tetraethylammonium may be constructed out of four aromatic residues, one from each subunit, such that all four interact simultaneously with this hydrophobic cation with precisely equal energetic contributions (*3*). These mechanistic results nail down the rotational symmetry of the specific pores under study, and they argue for similar architectures for channels related to these by molecular homology—fivefold symmetry for the multitude of channels activated by the neurotransmitters acetylcholine, glutamate, glycine, and γ-aminobutyric acid (GABA), and fourfold symmetry for the voltage-gated K^+, Na^+, and Ca^{2+} channels, and perhaps for the distantly related cyclic nucleotide–activated channels as well.

Another broad result emerging from the recent work is that ion channels are constructed like other proteins, along modular lines, in which local regions of sequence can sometimes be identified with specific functions. Point mutation leading to alterations of ion selectivity, conductance, and affinity for blocking agents has led to wide, although happily not unanimous, acceptance of the idea that the linings of the conduction pores of both ligand-gated and voltage-dependent channels are formed by localized stretches of sequence of only 20 to 25 residues repeated in each subunit. It is surprising that even channel gating appears to be due to movements of local domains, at least in the case of ball-and-chain inactivation of A-type K^+ channels (*4*). Likewise, the ability of voltage-gated channels to sense the intramembrane electric field has been widely laid at the feet of a 20-residue positively charged transmembrane stretch of sequence (the infamous S4 region), although to be fair to any remaining skeptics, I must admit that no experimental evidence supports this notion in a rigorous (as opposed to a merely suggestive) fashion. In the nicotinic acetylcholine receptor, careful measurements of ligand-binding curves in cell lines expressing receptor complexes missing single subunits argue that the two nonequivalent ligand-binding sites located at the α subunit interfaces with γ and δ (*5*); the involvement of critical aromatic residues in forming this binding pocket is becoming clear (*6*), but the long-sought prize, the anionic group that attracts the choline headgroup, is still hiding in the bushes. However, not all channel functions are neatly modular; in K^+ channels, voltage-dependent activation and C-type inactivation are influenced by residues widely separated along the linear sequence (*7*), just as the quaternary interaction surfaces of hemoglobin are formed out of residues similarly dispersed.

Most of these early results have served mainly to locate hot spots in channel sequences, to list the cast of characters, but

The author is at the Howard Hughes Medical Institute, Brandeis University, Waltham, MA 02254.

the big payoffs will come in an understanding of basic mechanisms of channel operation. For instance, close examination of ionic interactions with the K^+ channel inactivation ball shows that this type of gating represents the physical plugging of the cytoplasmic face of the pore and that nonspecific electrostatic forces focus this positively charged domain into its pore-blocking site (8). The slowing of inactivation by adenosine 3′,5′-monophosphate–dependent protein kinase phosphorylation of an equivalent domain on the Na^+ channel (9) leads to testable speculations about electrostatic steering as the basis for functional modulation by phosphorylation. Recently, point mutations were described that lead to a profound change in Na^+ channel selectivity, a phenomenon that requires the creation of sites for simultaneous binding of two Ca^{2+} ions (10).

Most of the questions addressed up to the present have been "minimalistic," in that they have focused on fundamental issues of mechanism and structure studied on simplified channels in heterologous expression systems, largely uncontaminated by biological reality. One of the most difficult challenges for more neurobiologically minded scientists will be to understand the structure and function of channels in their native cellular environments. The recent cloning of huge numbers of ligand-gated channel subunit isoforms, for instance, points to what an awesome task it will be merely to identify the subunit composition of a given channel in a given cell. A combination of antisense techniques, pharmacological manipulation, and single-channel analysis has recently begun to sort out this combinatorial nightmare in neuronal acetylcholine receptors (11).

The approach of genetic manipulation of ion channels as an analytical tool is still very young. It holds great promise in two directions, downward to the level of macromolecular structure and upward to the level of cellular function. The field's youth ensures that even primitive answers to the questions posed will remain exciting for some time yet, and that the inevitable frustration arising from the dearth of hard structural data will not flummox its practitioners in the near future.

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Bench to Bedside: The Glutamate Connection

Dennis W. Choi

Like so many other areas of neuroscience, the field of excitatory amino acid transmitter research has seen a rush of new information during the past year. Glutamate or related amino acids mediate fast synaptic transmission at the majority of excitatory synapses throughout the brain and spinal cord. An exhilarating appreciation of the role of this signaling process in health and disease is now unfolding.

Building on the pioneering efforts of Heinemann and colleagues, who cloned the first glutamate receptor subunit 3 years ago, ionotropic receptor subunits have been cloned faster than they can be definitively named (1). GluR1, GluR2, GluR3, and GluR4 subunits (alternatively referred to as GluRA through GluRD or α1 through α4 by other laboratories) exhibit functional and binding properties akin to those of the native α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)–preferring glutamate receptors defined by earlier pharmacological studies. GluR5, GluR6, and GluR7 as well as KA-1 and KA-2 may be components of high-affinity kainate receptors. NMDAR1 together with its growing family of siblings (the ζ series) and cousins NMDAR2A, NMDAR2B, NMDAR2C, and NMDAR2D (the ε series) are all probably components of heteromeric NMDA receptors.

The author is the chairman of the Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Correlations between molecular structure and function of these receptors are emerging. The AMPA receptor subunits GluR1 through GluR4 can each be expressed by alternative mRNA splicing in two different versions, flip and flop. Although these two versions have only minimally different sequences in a 38–amino acid transmembrane region, flip forms show less desensitization and hence carry larger currents. Consistent with current work on voltage-gated channels, critical amino acid residues determine the Ca^{2+} permeability of AMPA receptor channels and the sensitivity of the NMDA receptor channel to Mg^{2+} block (2).

Much has also been learned about the intracellular events set in motion by glutamate receptor stimulation. These are proving to be complex: a remarkably intricate and interacting network of enzyme cascades, messenger compounds, and changes in gene expression capable of exerting lasting influence over excitatory synaptic behavior. In addition to ionotropic receptors, glutamate activates multiple metabotropic receptors coupled through G proteins to inositol phosphate or adenosine 3′,5′-monophosphate (cAMP) formation (3). Furthermore, glutamate stimulates an increase in intracellular free Ca^{2+}, which triggers the production of potential intercellular messengers such as fatty acid metabolites or nitric oxide (4).

Consistent with the spirit of the "Decade of the Brain," this basic information may also lead with surprising directness to advances in the clinical arena. Neurology, neurosurgery, and psychiatry are all concerned with numerous diseases with known phenotypes but unknown pathogeneses. The current knowledge explosion in the glutamate receptor field points to new molecular sites where a specific disturbance might cause disease or where a therapeutic intervention might be targeted.

One mechanism capable of connecting abnormalities in the glutamate system to disease is excitotoxicity—the ability of glutamate or related compounds to induce neuronal death, often after receptor overstimulation. Degeneration of human motor neurons in neurolathyrism is caused by ingestion of the chick pea excitotoxin, β-oxalylaminoalanine (5). A newly recognized clinical syndrome characterized by seizures and brain damage has been linked to the digestion of a kainate receptor agonist, domoate, found in contaminated mussels (6). Excitotoxicity is also strongly implicated in the brain or spinal cord damage induced by insults such as hypoxia-ischemia or trauma. NMDA antagonists can protect against damage in numerous experimental models of stroke (focal brain ischemia) consistent with in vitro studies indicating that NMDA receptor overactivation is a critical step in neocortical or hippocampal