Commentary

How to Force Conformity on Transmitter-gated Channels

Joe Henry Steinbach

From the Department of Anesthesiology, Washington University School of Medicine, Saint Louis, Missouri 63110

Neurotransmitter-gated ion channels (LGIC) transduce a presynaptic event (release of transmitter) into a postsynaptic event (opening of channels). Given the vagaries of life for a synapse—the quantal content, the number of transmitter molecules in a vesicle, the exact release site, the statistics of transmitter binding, noise in the postsynaptic cell, and so forth—it would seem to be advantageous for fidelity in signal transmission for some aspects of the process to be tightly controlled. Indeed, the idea that the functional properties of the ion channels are well defined and reasonably homogeneous is necessary for biophysicists to analyze the kinetics from population measurements. All in all, relatively few data have to be swept under the rug in our effort to define "the" characteristic behavior of a given type of LGIC. However, there are recurring observations of variability in the kinetic properties of LGIC. Some reports have noted relatively subtle effects (for example, that the mean open times differ between individual muscle nicotinic receptors; Sine and Steinbach, 1987). Others have found that receptors can function in more than one kinetic mode, with relatively gross differences in kinetics (for nicotinic receptors, Auerbach and Lingle, 1986; Naranjo and Brehm, 1993; for GABA-A receptors, Newland et al., 1991). This variability has been seen under control conditions, so there is no reason to think that modulation of function is occurring, although one could imagine many processes that could be acting on the receptors. These observations have raised a number of questions, including issues of the origin of the variability (or lack of variability) in rate constants within a mode, the structural basis for a switch in modes, and the possibility that mode switches and physiological "modulation" might be related.

The paper by Wang et al. (2000) examines the issue of how it is that the kinetic properties of a receptor are tightly controlled, using the grandfather of this family of receptors, the muscle nicotinic receptor (hereafter called the AChR). The study focuses on the properties of a mutant ϵ subunit identified in patients showing a congenital myasthenic syndrome, which harbors a point mutation in the "amphipathic helix" (HA), a region of the receptor near the carboxy terminus. The study demonstrates that this particular mutation, ϵ A411P (alanine mutated to proline in the ϵ subunit), produces a receptor with increased heterogeneity in gating kinetics.

The HA region was first identified by analysis of amino acid sequences as a likely helix that had one polar and one nonpolar face, and was first suggested as a candidate for the channel-lining regions of the subunits. It is located at the COOH-terminal end of the major intracellular loop of each subunit, just before the fourth membrane-spanning helix (Fig. 1). After it was shown that receptors could function even when major portions of the HA region were deleted in the α subunit (Tobimatsu et al., 1987), interest waned in this part of the subunit. Later studies of chimeric subunits, however, suggested some role for the HA region in affecting channel kinetics. The fetal and adult forms of the AChR differ structurally in that the fetal form contains α, β, δ, and γ subunits, whereas the adult form contains α, β, δ, and ϵ subunits. Fetal receptors have a (two- to fourfold) longer burst duration, resulting from a smaller channel closing rate. Work by Bouzat et al. (1994) tracked down the structural features required for the difference to several residues in the HA region (Fig. 1), and two additional residues in the adjacent M4 helix. The difference is largely in the closing rate constant, while the opening rate constants are similar for adult and fetal receptors (Maconochie and Steinbach, 1998). An observation that was initially thought to be completely distinct was that the kinetics of the fetal AChR change after a patch is excised (Covarrubias and Steinbach, 1990). In a surprising convergence, it turns out that adult receptors do not show this change after excision, and that the sensitivity to excision is conferred by the residues in the HA and M4 regions identified by Bouzat (Akk and Steinbach, 2000). Finally, a previous study of a mutated ϵ subunit found in other patients with congenital myasthenic syndrome identified a six amino acid repeat, again located in the HA region (Milone et al., 1998). In this mutation, the receptor shows altered behavior in that clusters now appear to fall in one of three different kinetic modes. Two of the modes show reduced values for the opening rate constant and increased values for the closing rate. In addition, it was necessary to add a second open state to describe the kinetics of the mutated receptors. These previous studies had indicated that the structure of the

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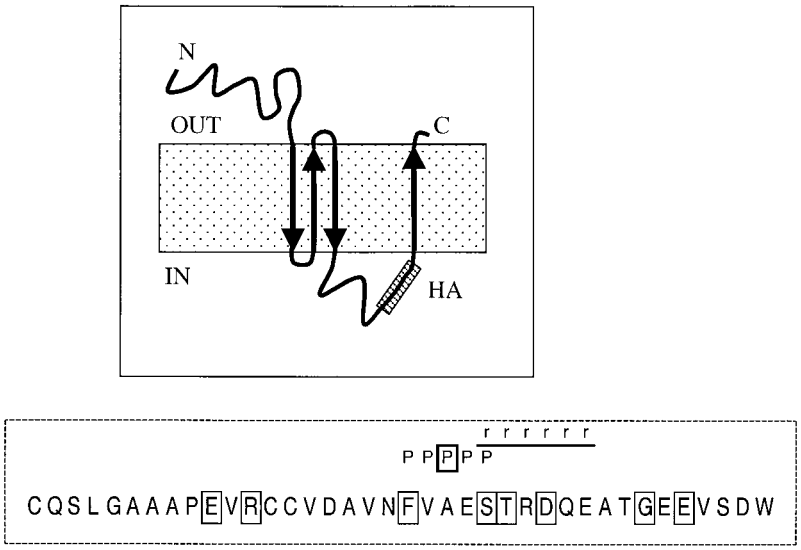

Figure 1. (Top) Cartoon indicating the approximate position of the HA region. (Bottom) Sequence of the ϵ subunit in the HA region. The specific mutation (alanine 411 to proline) studied is indicated by the boxed P above the sequence. Other residues that, when mutated to proline, showed similar effects are shown by unboxed Ps above the sequence. The six amino acids indicated by overlining with r's are the six-residue repeat, which results in multimodal behavior, while the boxed residues are those indicated as important in determining the difference in kinetics between fetal and adult AChR (see text).

HA region in the ϵ subunit can affect the gating behavior of the AChR. In general, the results are consistent with the idea that the HA region, in some fashion, can affect the modal kinetic behavior of the receptor.

Wang et al. (2000) describe the properties of a point mutation in the HA region of the ϵ subunit, which does not produce clear changes in modal behavior, but increases the variability in channel gating kinetics. The gating properties of the AChR containing the mutated subunit are, in some ways, very frustrating to anyone who wants to quantitatively analyze the kinetics. The experiments therefore were conducted using a relatively high concentration of ACh so that, most of the time, all the receptors in a patch were desensitized and inactive. Occasionally, a single receptor recovered from desensitization and underwent a paroxysm of activity—opening and closing repeatedly—until it finally desensitized again and the patch became silent. The advantage of studying the activity in such a "cluster" is that it results from the activation of a single receptor. Even then, frustration arose because different clusters from AChR containing mutated ϵ subunits showed obviously different kinetics. Even a straightforward parameter, the probability that the channel is open during the cluster, showed more variability—for the wild-type AChR, the mode under the experimental conditions was ∼0.65, with a range from 0.45 to 0.9, while the AChR with the mutated receptor showed a mode at ∼0.5, with a range from 0.1 to 0.95. Wang et al. (2000) then performed a cluster-by-cluster analysis of channel kinetics, using a kinetic model with four free parameters: the channel opening and closing rate constants and the ACh association and dissociation rates. They found that the variability across clusters of the estimates for the association and dissociation rates were essentially identical for wild-type AChR and AChR containing mutated ϵ subunits. However, the variability of the estimates for the channel opening and closing rate constants was greater for the receptors with the mutated subunit. The increase in the heterogeneity of the rates was relatively large in terms of channel behavior, about twofold. However, this reflects a relatively small difference in terms of the energetics of gating. As a rough indication, suppose the limits of the variability in the rate constants are set by ±3 SD from the mean values. In this case, the spread in the case of wild-type receptors would correspond to ∼1.2 RT units (0.7 kcal/mol), while the spread in the mutated receptors would be ∼1.9 RT units (1.1 kcal/mol). Since both the opening and closing rates became more variable, it might be that the energy of the transition state was affected by the mutation (applying the simplest two well, one barrier model to the open/closed transition). However, this does not seem to be the case, since there was not a significant correlation between the estimated open and closing rate constants across clusters (S.M. Sine, personal communication). Therefore, it seems that the depth of the energy wells for the liganded-closed and -open states became more variable. The variability in kinetics was not manifest by the appearance of additional kinetic modes. Wang et al. (2000) observed that, although switches between distinct kinetic modes were observed in receptors containing the mutated ϵ subunit, the mode transitions appeared to occur at a rate similar to that seen in the wild type.

The present and previous structure studies have in common that the single-channel conductance is not altered (suggesting that the open channel is similar), that the changes in rates are relatively modest (<10-fold), that the opening and closing rates change independently, and that there seems to be more change in channel gating than in ACh binding. It is intriguing

that in some cases the occurrence of distinct modes is enhanced, while in others the heterogeneity of kinetic behavior (in the absence of clear modes) in increased. Many other mutations have been identified that alter channel kinetics or ACh binding. In general, the kinetics change, but have not been reported to be markedly more variable than for wild type AChR, nor have the mutations been reported to accentuate multiple kinetic modes.

What does this mean in terms of understanding AChR function (and, by extrapolation, learning more about the properties of other LGIC)? It is reassuring that a recent analysis of AChR gating in terms of linear free energy relationships supports the idea, at least for the wild-type receptor, that channel gating proceeds along a single reaction path (Grosman et al., 2000). As Wang et al. (2000) suggest, it may be that the HA region is critically important in determining some aspects of protein structure that shift the energy levels of the open or closed states (or possibly of the transition state between them). The data would be consistent with the idea that some alterations in HA produce the ability to adopt several conformations that do not differ much in energy (broadening the distribution of rate constants), while other alterations result in conformations that are different enough to produce distinct kinetic modes. For us to observe and analyze the alterations, the different conformations have to be relatively long lived (a second or so), so the energy barriers between them need to be high. As mentioned before, the changes in the kinetics are relatively modest, implying that the alterations in energy levels for the liganded-closed and -open (or the transition) states are relatively small. (Of course, if the channel never opened, we would not know what was going on, so the requirement for getting some data to analyze may bias our sample.) There is independent evidence that the HA region is important in some fashion in defining the overall structure of the AChR. When receptors are expressed containing the α subunit HA deletions or either of the ϵ HA mutations, there are significant reductions in the number of receptors on the surface of the cell. It is not known whether subunit assembly or interactions with transport processes are affected.

Unfortunately, the actual location of the HA region with respect to the membrane or the rest of the cytoplasmic loop is not known. After a possible role in forming the channel was dismissed, it was thought that it might lie parallel to the membrane with one face in lipid and the other exposed to the cytoplasm. Most recently, a projection from the cytoplasmic side of each subunit has been identified that has helical content, so it may be that the HA region projects into the cytoplasm and interacts with the intracellular loops from other subunits as well (Miyazawa et al., 1999). In either case, the HA region is situated in a good place to couple alterations in the cytoplasmic loop (e.g., phosphorylation or noncovalent interactions with cytoplasmic components) to the rest of the receptor. Of course, this begs the question of how the changes in HA itself are coupled to receptor function.

Two additional points are of interest. First, the present study reports that homologous mutations in the HA regions of the β and δ subunits do not produce these effects on kinetics (no currents were recorded from AChR-containing α subunits with the homologous mutation, and the γ subunit was not examined). Does this mean that there is a unique capacity of the HA region in the ϵ subunit, or that there are minor structural differences in other subunits and that a mutation nearby would show effects? Finally, these results yet again emphasize the novelty of the insights that are provided from studies of subunits identified in patients with congenital myasthenia. There was no reason a priori to examine the HA region for a role in controlling kinetic variability in the AChR. It is still not clear whether the kinetic alterations contribute directly to development of symptoms, since both of the mutations studied by Sine's group have shown up as recessive—patients present with symptoms when the other allele of the ϵ subunit contains a null mutation. Accordingly, it is possible that the clinical symptoms reflect the lower expression levels for receptors in the patients.

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