Kinetic behavior of cloned mouse acetylcholine receptors

A semi-autonomous, stepwise model of gating

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INTRODUCTION

Our understanding of the activation of acetylcholine receptors (AChR) has been dominated by a kinetic model that posits that gating is a highly concerted process, i.e. after two ligands bind there is a conformational change that results in ionic permeation:

$$C \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AC \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} A_2C \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2O \qquad \text{(concerted model)}$$

This model assumes that any conformational changes consequent to binding one ligand are kinetically undetectable (on the time scale of the instrumentation) and/or irrelevant to the gating process. We have analysed AChR activation kinetics according to an alternative scheme that explicitly incorporates agonist-induced conformational changes. In this stepwise model, each subunit undergoes an isomerization after binding an agonist, with ionic conduction occurring only after both subunits have activated:

$$C \underset{k_{-1}}{\overset{2k_{+1}}{\rightleftharpoons}} AC \underset{\alpha 1}{\overset{\beta 1}{\rightleftharpoons}} AC' \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} A_2C' \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2O \qquad \text{(stepwise model)}$$

C′ indicates a nonconducting channel with only one subunit activated. According to this scheme (which is similar to the Hodgkin-Huxley proposal for voltage-gated Na channels), AChR activation is a stepwise process that requires the semiautonomous activation of distinct structural domains. Both concerted and stepwise models assume that ACh cannot escape from an activated channel/subunit.

I have compared the adequacies of these models to describe the single-channel kinetics of mouse AChR expressed in *Xenopus* oocytes. The cDNA clones coding the AChR subunits were generously provided by Dr. J. Patrick. mRNAs from these clones were individually transcribed using SP6 polymerase. The transcripts were resuspended in water at a concentration of 200 ng/μl (α subunit) or 100 ng/μl (β, δ, or γ subunits) and stored at −80°C. Stage V or VI oocytes were defolliculated, injected with 50 nl of mRNA mixture and incubated for 24–120 h (17°C) in L-15 medium. Single-channel currents were recorded from cell-attached patches (19°C, −100 mV). Kinetic analysis followed the Horn and Lange (1983) full maximum likelihood method, with a first-order correction for missed events (Roux and Sauve, 1985). After selecting a subset of bursts that was apparently homogeneous, the rates of either activation scheme were iteratively adjusted by a Simplex algorithm until the −log likelihood was a minimum. The significance of the log likelihood ratios (LLR) was examined both by resampling and simulation methods (Horn, 1987).

At 1,000 μM, the predominant component of gaps with bursts has a time constant of 64 μs, indicating an effective opening rate at this concentration of 15,600 s^{-1}. The results of the likelihood fit to a patch exposed to 10 μM ACh are shown below (all rates are s^{-1} or s^{-1} μM^{-1}). The association and dissociation rates of the stepwise scheme were assumed to be equal and independent, so that each model had the same number of independent parameters (six).

$$C \underset{385}{\overset{20}{\rightleftharpoons}} AC \underset{4276}{\overset{20}{\rightleftharpoons}} A_2C \underset{282}{\overset{12970}{\rightleftharpoons}} A_2O \qquad \text{(concerted model)}$$

$$C \underset{4474}{\overset{84}{\rightleftharpoons}} AC \underset{2346}{\overset{2910}{\rightleftharpoons}} AC' \underset{4474}{\overset{42}{\rightleftharpoons}} A_2C' \underset{311}{\overset{15253}{\rightleftharpoons}} A_2O \qquad \text{(stepwise model)}$$

According to the stepwise scheme, the association rate is 4.2×10^7 s^{-1} M^{-1}, the dissociation rate is 4,474 s^{-1}, and the equilibrium dissociation constant (K_d) is 107 μM. The concerted scheme rates were quite different for the two binding steps, with the K_d increasing over 10-fold (from 19.5 to 211 μM) because of a low escape rate for the first agonist to bind.

Both models predict opening rates that are in agreement with the 1,000 μM data and with opening rate reported for these channels expressed in BC3H1 cells (Liu and Dilger, 1991). Both models also predict similar channel closing rates. According to the concerted scheme, the channel opening rate is 50 times that of the closing rate. According to the stepwise scheme, the channel opening rate is 98 times faster than its closing rate (note that because the inactivation of one subunit terminates

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conduction, a "channel" closes at twice the rate as does a single subunit).

The stepwise model predicts that the activation and inactivation rates of the first subunit to isomerize are almost equal ($\beta 1/\alpha 1 = 1.2$). The conformational change consequent to binding is therefore neither undetectably rapid nor irrelevant to the activation process. The activation of one subunit substantially lowers the energy barrier for the gating of its counterpart ($\beta/\beta 1 = 5.2$). Moreover, the active conformation of a subunit is greatly stabilized when its counterpart is also in its active conformation ($2\alpha 1/\alpha = 15$). As a consequence, the activation ratio of the isomerization that results in ionic permeation is over 80 times that of a single subunit.

Which model better describes the data? In the 10 μM file (2,070 intervals), the LL of the stepwise model was 8,659.6 and that of the concerted model was 8,655.4, for a LLR of +4.2. The significance of this number was estimated by resampling an equivalent number of intervals (on a burst basis) with replacement, fitting the resampled data to both models and computing new LLRs (Fig. 1). The mean of this distribution (+3.09 ± 0.24; mean ± 90% confidence limits) is significantly greater than zero, indicating that the stepwise model provides the statistically superior description of the current intervals. I have not been able to achieve adequate fits with the stepwise model when the association/dissociation rates were allowed to differ, so it remains unclear whether the binding sites are indeed equivalent.

The following picture of AChR activation emerges from a consideration of the stepwise rates. By inference, a similar scenario may apply to the activation of other multimeric ion channels. Gating results from the random, sequential activation of more than one structurally distinct domain (here, a subunit). After binding an agonist molecule, localized conformational changes occur that are necessary but not sufficient for ion permeation (and, with AChR, that prevent the agonist's escape from the binding site). Although the domains may be equal and independent in the resting channel, after one is activated, the electrical and/or mechanical field of the protein changes altering the isomerization rates of other domains. Thus, channel activation requires the semiautonomous participation of multiple domains. In contrast to the concerted view, the AChR channel appears to be polymorphic, i.e., a collection of interacting structures that each switch between active and inactive conformations.

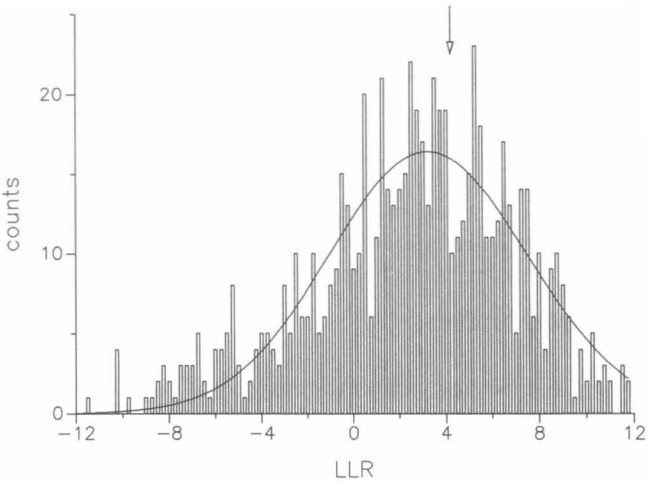

FIGURE 1 Log likelihood ratios (LLR) of resamples intervals. A positive value indicates that the stepwise model fit better than did the concerted model. The arrow indicates the LLR of the original data set.

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