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Perfection of a synaptic receptor: Kinetics and energetics of the acetylcholine receptor

(allosteric proteins/neuromuscular junction/ion channels/evolution)

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ABSTRACT **The energetics and kinetics of activation of the acetylcholine receptor are evaluated in the context of optimizing rapid synaptic transmission. Physiological needs are used as the basis for estimating optimal values for the closed-to-open channel equilibrium constants of the liganded and unliganded receptor. An estimate is made of the maximum energy that can be derived from the binding of acetylcholine to a perfectly designed receptor binding site. Application of the principle of detailed balance shows that with only one ligand binding site the receptor will not be able to derive enough energy from acetylcholine binding to drive a sufficiently large change in the channel conformational equilibrium. This then provides a rationale for the existence of a second binding site, rather than the often invoked advantage of cooperativity. With two binding sites there is a considerable excess of binding energy and consequently considerable flexibility in how binding energy can be utilized. It is shown that the receptor must have at least one binding site that binds acetylcholine weakly when the channel is closed. This is essential to rapid response termination. However, making the other binding site bind more tightly can enhance and accelerate the activation of the receptor. To optimize both response activation and termination the best solution is to make the two binding sites different in their binding affinities. This qualitatively reproduces an experimental observation.**

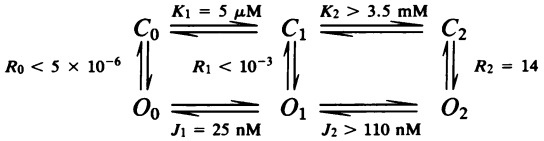
A synaptic receptor is no different from other proteins in the sense that its structure and function are molded, and to some degree perfected, by natural selection. For example, an enzyme is often the product of selective pressure to maximize the rate of catalysis, preserve substrate specificity, and allow appropriate metabolic control (1). However, there are physical factors that constrain the function of an enzyme. Diffusion limits the rate of combination with a substrate. In addition, a constraint is imposed by the limit of how much binding energy is available to be utilized in reducing the activation energy of the catalyzed reaction. Natural selection will direct the evolution of an enzyme toward an optimum defined within these physical constraints. The enzyme triose phosphate isomerase has been used as an example to illustrate these concepts (1).

These ideas are also relevant to synaptic receptors. There may be considerable selective pressure on an animal to react and to move quickly. Such activity is a sequence of many steps including synaptic transmission. Synaptic transmission proceeds through the activation of synaptic receptors by neurotransmitter, with a consequent opening of a transmembrane ion channel. The synaptic receptors are therefore an obvious focus of environmental pressure for speed. With a synaptic receptor, just as with an enzyme, there are physical constraints to function. Some of these constraints, such as diffusion and limited available binding energy, are similar to those that impose themselves on an enzyme. Other constraints, such as the charging time of the postsynaptic membrane, are unique to the synaptic situation.

The present study is an attempt to evaluate the various physical factors that constrain the function of a synaptic receptor dedicated to rapid synaptic transmission. The nicotinic acetylcholine receptor, which mediates transmission of impulses from motoneurons to skeletal muscle, is an appropriate example for such a discussion since a rapid neuromuscular synapse is an obvious selective advantage. The activation of the acetylcholine receptor is understood in great detail and provides a basis for evaluating the impact of the various constraints on the design of a synaptic receptor. Many measurements of the kinetic activity of the acetylcholine receptor can be profitably interpreted in this light. One of the motivations of this study is the analysis of how the binding sites of the acetylcholine receptor utilize binding energy and how this utilization of binding energy by the two agonist binding sites of the receptor is very different (2). The discussion below will evaluate the effectiveness and nonequivalence of the binding sites in the context of selective pressure to optimize receptor function. In a sense what I am doing here is trying to design a perfect synaptic receptor. Where possible, this perfect receptor will be compared with the acetylcholine receptor.

Mechanism of Receptor Activation

There is a consensus among researchers in many laboratories that a mechanism involving the binding of two neurotransmitter molecules provides a good description of the activation of the acetylcholine receptor (2–4). This mechanism is represented by the following scheme, in which C and O represent receptor with closed and open channels, respectively. Values for the equilibrium constants were taken from ref. 2.*



Scheme I

The numbers in this scheme pertain to activation of the receptor by the acetylcholine agonist carbachol; the subscripts indicate the number of bound agonists. The question of whether the binding sites exhibit negative cooperativity or are independent and nonequivalent is not relevant to the present discussion.

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*R_0 and R_1 were actually determined in ref. 2 as upper bounds. K_2 and J_2 are lower bounds.

It is an essential tenet of theories of allosteric protein activation that agonists bind the inactive state (closed channel) of the receptor less tightly than the active state (open channel) (5). It is this binding energy difference that enables association with agonist to serve as a driving force in the activation of the receptor. The dissociation constants of the open and closed receptor channel configurations in the above scheme illustrate this. Thus, the conformational change of the receptor–channel protein includes changes in the structure of the binding sites that improve the binding interactions (see Fig. 1).

Although both binding sites bind carbachol more tightly when the channel is open, there is a striking difference between the two binding sites in the extent of this difference. One site binds agonist with a relatively high affinity when the channel is closed ($K_1 = 5\ \mu M$) but has a relatively small effect on the open–closed channel equilibrium ($R_1/R_0 = 200$). The other site binds agonist less tightly when the channel is closed ($K_2 > 3.5$ mM) but has a much greater effect on the channel equilibrium ($R_2/R_1 > 14{,}000$).

Now let us consider how the two binding sites utilize binding energy. When the channel is open, the two binding sites have relatively similar binding affinities ($J_1 = 25$ nM and $J_2 > 110$ nM). Consider the free energies of binding to the open state (9.4 and 10.3 kcal/mol; 1 kcal = 4.18 kJ) as a preliminary estimate of the maximum energy that can be derived from binding this agonist. This energy can be utilized in two ways: (*i*) this energy can stabilize binding to the closed channel configuration of the receptor–channel complex; (*ii*) this energy can drive the protein into the open configuration. We then see that the high-affinity site utilizes 70% of the available free energy to stabilize the receptor–carbachol complex when the channel is closed, leaving only 3.1 kcal/mol to drive the conformational transition. In contrast, the low-affinity site utilizes only 35% of the available binding free energy for binding to the receptor when the channel is closed. This leaves much more energy (6.1 kcal/mol) to drive the conformational transition.

These points are illustrated in Fig. 1, which shows how the binding sites of an open channel are perfect complements to the agonist. In contrast, when the channel is closed, the binding sites have different shapes and are to varying degrees poorer fits for the same agonist. The binding site that is the poorest complement to the shape of the agonist will bind most weakly when the channel is closed but has more binding energy to drive activation. The other binding site is opposite

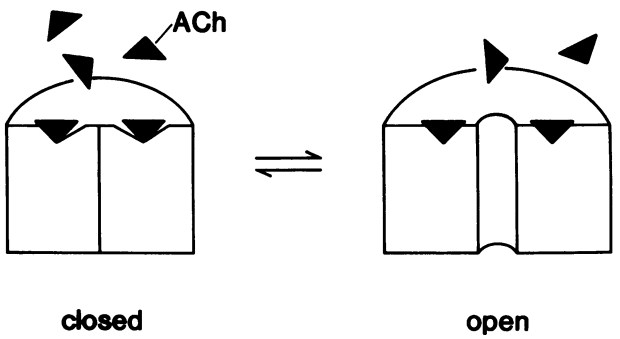


FIG. 1. Conformational change of the acetylcholine (ACh) receptor. In the closed-to-open channel conformational transition, the receptor binding sites also change and in doing so bind acetylcholine (filled triangles) more effectively. The ligand binding sites are represented by triangular indentations on the receptor surface. When the channel opens, the binding sites change to a shape that is a close match with the shape of acetylcholine. Note that in the closed channel configuration the binding sites are not identical. This allows the two binding sites to utilize binding energy differently, as explained in text.

in this respect. It releases more energy upon binding agonist; less energy remains to drive activation.

Optimal Equilibrium Constants

In this section order-of-magnitude estimates will be made of optimal equilibrium constants or of their lower or upper bounds.

(*i*) R_0. The open–closed equilibrium constant of the unliganded receptor must be low enough so that unliganded channel openings cause minimal leakage of ions across the membrane. If the channel is open much in the absence of agonist, a cell will have to expend more energy to maintain ionic gradients. This consideration allows one to estimate a reasonable upper bound to R_0.

The density of receptors in the most receptor-dense region of the postsynaptic membrane of the neuromuscular junction is 15,000 receptors per μm^2 (6). The background resistivity of typical excitable membranes is 1000 $\Omega \cdot cm^2$ (7). Since we are looking for an order-of-magnitude bound to R_0, we can arbitrarily say that the conductance of the R_0 pathway should not exceed 10% of the background conductance. With a 40-pS channel conductance we find that $R_0 < 1.7 \times 10^{-6}$. This is close to the experimental estimate (2).

(*ii*) R_2. The open–closed equilibrium constant of the fully liganded receptor also has an optimum. Once the channel is open >90% of the time, it is not economical to expend additional binding energy for stabilization of the open channel. Thus, an order of magnitude estimate of R_2 is 10. Colquhoun and Sakmann (4) obtained a value of 43 for R_2. Consideration of the need to terminate a response rapidly (discussed below) also proscribes a value for R_2 in this range.

(*iii*) K_1 *and* K_2. With a diffusion-limited binding rate of the order of $10^8\ M^{-1} \cdot s^{-1}$ (8) the concentration of ligand must be >10 μM in order for binding to occur in <1 ms (the significance of this time scale to be discussed below). If the concentration of transmitter must be this high then the dissociation constants would be expected not to be much lower, because that would waste binding energy that could be used to drive the conformational transition. This reasoning suggests a lower bound to K_1 and K_2 of $\approx 1\ \mu M$.

(*iv*) J_1 *and* J_2. The structure of a ligand limits the amount of free energy that can be released in binding to a protein binding site. This limit can be realized if the binding site is perfectly complementary to the ligand structure, and all opportunities for attractive interactions are fully exploited. We expect, as a manifestation of receptor perfection, that binding to the receptor with an open channel should approach this limit. Binding must be stabilized by noncovalent bonds because they can be rapidly formed and broken. The energy of formation of such a complex, in which all noncovalent interactions are optimal, would then be similar to the intrinsic binding energy discussed by Jencks (9).

The intrinsic binding energy can be estimated by considering all possible contacts between neurotransmitter and protein. Each contact would have a maximum energy when the interacting groups are separated by the optimal distance. For a system described by a harmonic potential one can rigorously show that the sum of all of the energies of the optimal contacts is the maximum energy of stabilization (10). Even without the restriction of a harmonic potential, additivity is a reasonable assumption and may be likened to the approximation of ignoring three-body and higher-order terms in the potential energy of a liquid.

For the neurotransmitter acetylcholine, there are three types of interactions to consider. These are hydrogen bonds with oxygen atoms, an electrostatic interaction with the charged choline, and van der Waals interactions with the carbon atoms. The energies available from these interactions will now be estimated.

Hydrogen bonds. Estimating the energy available from hydrogen bonding is very difficult, primarily because in the dissociated state hydrogen bonds can form with water. However, recent experiments with genetically engineered proteins have provided useful estimates for the stabilizing contributions of individual hydrogen bonds in a protein–ligand complex (11). The range of energies is 1.5–3.0 kcal/mol for hydrogen bonds between uncharged groups and 3–6 kcal/mol when one of the groups is charged. Though 3 kcal/mol can be used as an upper bound for a hydrogen bond between uncharged groups, 6 kcal/mol is a poor choice for hydrogen bonds involving a charged group, because these measurements can seriously overestimate this energy when a charged group is involved (12). Four kilocalories/mole will be used as the upper bound to the energy of a hydrogen bond involving a charged group, with the assumption that a hydrogen bond with a charged group can be stronger than with an uncharged group.

Although neither of the oxygen atoms of acetylcholine is charged, one of the hydrogen donors of the protein should be charged to exploit this means of strengthening binding. It is unlikely that both hydrogen donors of the protein are charged, since this would involve positioning two similarly charged groups close together. One hydrogen bond would then involve a neutral donor and the other would involve a charged donor. These two hydrogen bonds could then contribute a total of 7 kcal/mol to stabilizing the bound state in an optimal receptor binding site.

Salt bridge. This energy can be computed from Coulomb's law. The distance of closest approach of the choline to a negatively charged oxygen atom is 4.7 Å (C—N bond length, 1.5 Å; CH_3 radius, 1.8 Å; O^- radius, 1.4 Å). With a dielectric constant of 80 (the binding site must be at the surface of the protein to allow rapid association) the energy is 0.87 kcal/mol.

van der Waals contacts. The strategy employed here to estimate the maximum energy available from van der Waals contacts is that of Eisenberg and McLachlan (13). The surface of all of the carbon atoms is estimated and then multiplied by the parameter 18 cal/Å^2 (D. Eisenberg, personal communication). It is not necessary to consider the oxygen atoms since the hydrogen bond estimates were based on measurements that would have included these interactions. The nitrogen atom can also be neglected, because it has no exposed surface area.

The accessible surface area of the carbon atoms was computed from the crystal coordinates of acetylcholine chloride (14). The computation was carried out with computer programs of the UCLA structure group as described by Eisenberg and McLachlan (13), using a 1.4-Å spherical probe. The hydrogen atoms were treated as extensions of the carbon atoms, so that the areas are really for methyl groups, methylene groups, etc. The total accessible carbon area determined in this way was 307 Å^2. Estimates from the structures of two other acetylcholine salts are similar.

If acetylcholine were completely surrounded by protein in the binding site, then it would be appropriate to count this area twice, since an equal and complementary area of carbon atoms on the protein would also be transferred out of water upon binding. However, if the protein enveloped the agonist completely, then association could not be diffusion limited. To allow maximal rates of association and dissociation, we must allow approximately half of the ligand to remain exposed to water. We are thus left with half of the surface area of acetylcholine and an equal surface area on the protein to give 307 Å^2/mol × 18 cal/Å^2 = 5.5 kcal/mol.

Adding the energies from these various sources together gives 13.4 kcal/mol. This means that if the binding site has evolved to exploit the limit of energy available, binding would yield 13.4 kcal/mol to give a dissociation constant of 0.14 nM.

Though the arguments that produced this estimate are not quantitative, the use of maximal values produces a useful upper limit to the free energy available from acetylcholine binding. It is important to realize that such an upper limit to the available binding energy must exist and that it is a function of the specific ligand under consideration.

Optimal Rate Constants

In this section order-of-magnitude estimates are made of optimal rate constants. There are a number of time-consuming steps in synaptic transmission in addition to receptor activation. These steps include excitation of the presynaptic terminal, release of transmitter, diffusion of transmitter across the synaptic cleft, and charging of the postsynaptic membrane capacitance. Although it is beyond the scope of this work to evaluate all of these steps, a general familiarity with these processes suggests that in total they require about 1 ms. Thus, making the synaptic receptor much faster than 1 ms offers little advantage and would be deleterious if it were achieved through sacrifices in other functions.

(*i*) *Binding rates.* The receptor should bind acetylcholine in <1 ms. Diffusion sets an order-of-magnitude limit of 10^{-8} $M^{-1}\cdot s^{-1}$ to a first-order association rate constant in water (8). This value will be used here for all association rate constants.

(*ii*) *Opening rate.* In order that the rate of the closed-to-open conformational transition of a fully liganded receptor not be rate limiting, it must have a rate constant of at least 1 ms^{-1}. A comfortably fast rate of 10 ms^{-1} will be used as an order-of-magnitude estimate, which is close to experimental values (4). We will see in the discussion of response termination below that a value of 10 ms^{-1} is compatible with another requirement.

(*iii*) *Dissociation rates.* The rate of response termination must also be rapid. This will allow rapid repetitious postsynaptic action potential generation and the use of complex temporal patterns of impulses to encode information. A response must have a duration approaching 1 ms to charge the postsynaptic capacitance and evoke an action potential. Prolonging the response would have the disadvantage of lengthening the time before another action potential could be evoked. We will assume that selective pressure exists to have the duration of the response be in the range of 1–2 ms.

Constraining R_2 to be near 10 and the rate of opening to be near 10 ms^{-1} gives a rate of channel closing of 1 ms^{-1}. However, this rate alone does not determine the duration of a synaptic response. If the closed channel conformation of the fully liganded receptor is stable, then the channel can open repeatedly, producing a long burst of openings. The response duration is given by the mean duration of these bursts, which is $1/k_{-2} + R_2/k_{-2} + 1/\alpha$ (15). k_{-2} is the rate of acetylcholine dissociation from the closed receptor channel and α is the rate of channel closing. When $R_2 = 10$, $\alpha = 1$, and $k_{-2} = 10$ ms^{-1}, the mean burst duration is 2.1 ms. With R_2 near 10, increasing k_{-2} above 10 ms^{-1} or α above 1 ms^{-1} offers little advantage; 10 ms^{-1} will therefore be taken as an order-of-magnitude estimate for k_{-2}. [Dissociation of acetylcholine from the receptor when the channel is open is not a viable pathway for response termination since when the channel is open, binding is much tighter (2).]

With a rate of dissociation constrained to be >10 ms^{-1} and diffusion-limited binding, the dissociation constant of at least one binding site of the receptor with a closed channel must be at least 100 μM. This is well above the lower limit estimated above for the dissociation constants of the closed receptor channel.

Activation by One Agonist

The above estimates allow us to evaluate the efficacy of a synaptic receptor with a single agonist binding site. In order for the binding of a single molecule of acetylcholine to change the open-closed conformational equilibrium constant from 1.7×10^{-6} to 10 (values of R_0 and R_2 estimated above), detailed balance requires that the dissociation constants to the open and closed conformations differ by a factor of 0.6×10^7. Using the above estimate of the intrinsic binding energy for J, the dissociation constant of the receptor with a closed channel would then be 0.85 mM. This is clearly high enough to allow rapid response termination, as discussed immediately above, but it is somewhat higher than estimates of the peak acetylcholine concentration following release in a synapse (16, 17). Thus, with such a receptor additional metabolic energy would have to be used to concentrate acetylcholine.

Furthermore, if a synaptic receptor did have only a single binding site, then essentially all of the binding energy would have to be used for activation. There would be no binding energy left over for other functions such as desensitization. Furthermore, the above estimate of the intrinsic binding energy was a rather generous upper bound. If the actual limit were 1 or 2 kcal/mol lower, then the single-binding-site receptor would not be viable.

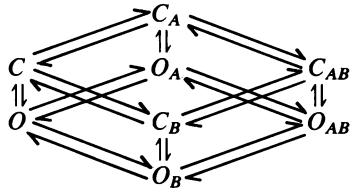
A widely accepted explanation for multiple binding sites of an allosteric protein is to provide a cooperative response. In this way the receptor can be activated over a narrow range of agonist concentration. This is a well-established function of the multiple binding sites of hemoglobin where the advantage to the rate of gas transport is clear; the oxygen tension varies by only a factor of about 2 between lungs and tissue. However, the concentration of acetylcholine changes from 10^{-8} M in the absence of stimulus (18) to roughly 0.5×10^{-3} M at the height of synaptic transmission (16, 17). With such a large change in concentration there is little need for cooperative activation. Thus, the rationale for multiple binding sites on a synaptic receptor may be different from that for hemoglobin. With a synaptic receptor the challenge is that the conformational equilibrium must undergo a very large change, and the binding of only one small molecule does not provide enough energy.

Activation by Two Agonists

For a receptor to undergo such a large change in its conformational equilibrium, it requires more or bigger transmitter. With acetylcholine as the transmitter, one binding site per receptor may not be enough. Two binding sites per receptor provide a considerable excess of binding energy. However, with two binding sites the energetics of the system are less well defined. We can relax the perfection of the binding site and still achieve vigorous activation. We can allow R_0 to be lower than its upper bound. We can also allow K_1 or K_2 to be below 1 μM and waste some binding energy in that way. The two binding sites can have different binding affinities, providing another dimension for flexibility. Detailed balance must be satisfied, but within this constraint thermodynamic considerations alone define the system poorly.

The situation is improved somewhat by considering the requirement of rapid response termination, which prescribes a dissociation constant of at least 100 μM for at least one of the binding sites of the receptor when the channel is closed. With both binding sites equivalent the system is defined with $J_1 = J_2 = (K^2R_0/R_2)^{1/2} = 40$ nM. This is well above the dissociation constant corresponding to the intrinsic binding energy, and there would appear to be little selective pressure for J_1 and J_2 to approach that value. However, only one of the K values needs to be near 100 μM to provide sufficiently rapid response termination. The other K could be much lower without making the response last too long. With K_2 fixed at 100 μM, we can then examine how variation of K_1 affects receptor function.

Consider an expanded version of Scheme I, which allows binding to the two binding sites in either order.



Scheme II

Here the subscripts A and B denote the occupation of different binding sites. The theoretical dose–response curve of this model has been plotted in Fig. 2 for sets of parameters in which K_1 is varied and with $J_1 = J_2 = (K_1K_2R_0/R_2)^{1/2}$. R_0 and R_2 are taken as the optimal values determined above. It is seen that reducing K_1 makes the receptor more sensitive to acetylcholine and less cooperative. Reducing K_1 can increase the sensitivity by a maximum of about 4-fold, and this improvement is realized after K_1 is reduced by somewhat more than 10-fold. Based on considerations presented above, it is unlikely that the loss of cooperativity that results from reducing K_1 is a significant disadvantage.

The time course of activation for this model has also been simulated by numerical integration of the rate equations using the fourth-order Runge–Kutta method (Fig. 3). The same sets of parameters were used as in Fig. 2, with all association rates at the diffusion limit of 10^8 $M^{-1} \cdot s^{-1}$ and with closing rates of 1 ms^{-1} for all states of receptor occupancy. Acetylcholine concentrations of 30 and 100 μM were used. This simulation shows that as K_1 is reduced, the rate of activation is enhanced. The enhancement reaches a limit after K_1 has been reduced by a factor of slightly more than 10. The enhancement is more impressive at 30 μM than 100 μM acetylcholine. With higher acetylcholine concentrations, response rise times as short as those of synaptic potentials can be simulated.

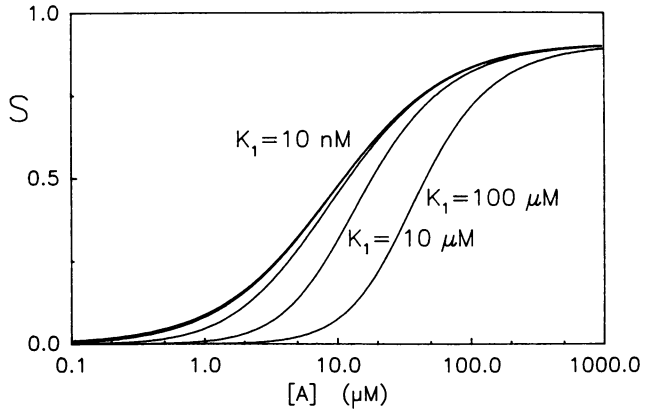


FIG. 2. Theoretical dose–response curves for different degrees of nonequivalence of binding sites. The fraction of open channels, S, is plotted versus the logarithm of acetylcholine concentration. Scheme II was used to derive the following relation for response versus concentration

$$S = \frac{(1 + A/J)^2}{(1 + A/J)^2 + (1 + A/K_1)(1 + A/K_2)/R_0}.$$

For these calculations, $K_2 = 100$ μM, $R_0 = 1.7 \times 10^{-6}$, and $R_2 = 10$ (optimal values taken from text). J was determined from a condition of detailed balance as given in the text. K_1 was varied between 10 nM and 100 μM at one-decade intervals, with values indicated in the figure, except that since the curves computed with 10 nM, 100 nM, and 1 μM are so similar, only 10 nM is indicated.

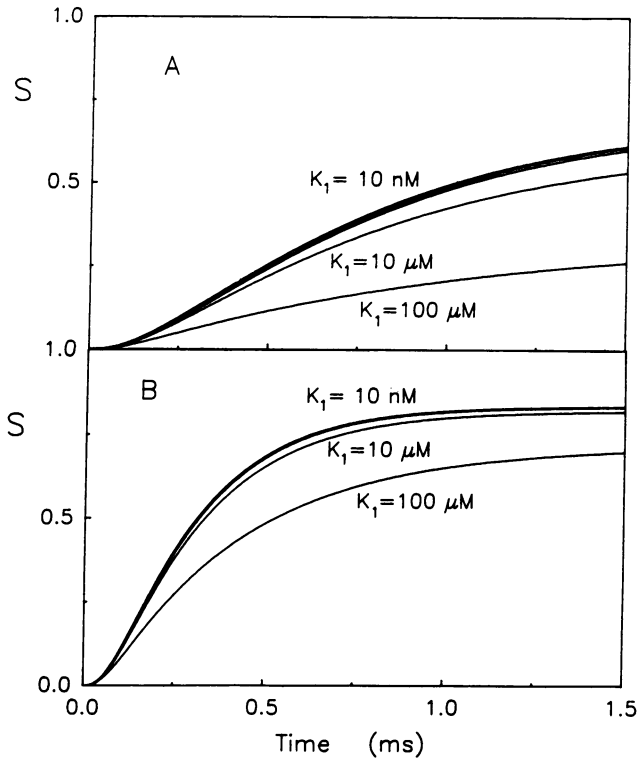


FIG. 3. Time course of receptor activation simulated for different degrees of nonequivalence of binding sites. The fraction of open channels, S, is plotted versus time after the ligand concentration is increased from 0 to 30 μM (A) or 100 μM (B). The equilibrium constants are selected as in Fig. 2. All binding rates are $10^8\ M^{-1}s^{-1}$. All closing rates are 1 ms^{-1}. This completely defines the kinetics of the system. The eight coupled linear differential equations derived from Scheme II are then integrated numerically. Values of K_1 were varied as in Fig. 2 and are indicated in the figure. As in Fig. 2, the curves computed with 10 nM, 100 nM, and 1 μM are similar, so that only 10 nM is indicated.

It would appear that tighter binding to one of the binding sites enhances the rate of receptor activation by making the singly liganded closed state a more stable intermediate kinetic species. The effect is concentration dependent, and under some circumstances nonequivalent binding sites offer a means of accelerating receptor activation without retarding response termination.

Discussion

Perfection of the acetylcholine receptor. The values of many of the equilibrium and rate constants of the acetylcholine receptor can be shown to be within the limits prescribed by physical constraints and near order-of-magnitude estimates of optimal values. In these respects it appears that the receptor has reached a certain degree of perfection, presumably through evolution by natural selection. The presence of two agonist binding sites can be seen as a contribution to receptor perfection, since the binding of only one molecule of acetylcholine cannot supply sufficient energy.

The addition of a second binding site provides more than enough energy for optimal function and also increases the flexibility in choice of parameters. The requirement of rapid response termination specifies one binding site and leaves the other free to vary. Reducing the other dissociation constant has two significant consequences: the receptor is activated by lower concentrations of acetylcholine, and it is activated more rapidly.

With peak acetylcholine concentrations of roughly 500 μM (16, 17), the advantages of a lower dissociation constant of one of the binding sites may seem unclear. However, the peak acetylcholine concentration is seen only by receptor directly under the site of release. If we consider a disk-like region around the release site within which transmitter is saturating (17), then the size of this disk will be larger if lower concentrations of transmitter are still saturating. With a 4-fold reduction in the saturating concentration achieved by reducing K_1, the area of the saturating disk would be 4-fold larger and so would the response. The receptors at the edge of the disk would also be activated rapidly enough to add to the contributions of receptors near the center of the disk. Thus, there is a significant advantage to lowering the dissociation constant of one of the binding sites.

Perfection of other receptors. There are many neurotransmitter substances with properties very different from those of acetylcholine. There are also functions other than rapid excitation that are served by synaptic receptors. These considerations would influence the design of different synaptic receptors. A few implications can be noted as follows.

Larger neurotransmitter molecules, peptides in particular, could have much higher intrinsic binding energies. With a larger molecule the energy derived from binding to a single site would be sufficient to drive a very large change in a conformational equilibrium. The receptors for these substances may then function well with only a single binding site. The excess in intrinsic binding energy would also allow very low concentrations of the neurotransmitter to activate the receptor. However, with concentrations below 10 μM diffusion-limited binding would prevent activation on a millisecond time scale.

Since the need for rapid response termination may be the critical requirement that leads to nonequivalent binding sites, receptors in synapses that do not require rapid response termination may have equivalent binding sites. The requirement of rapid termination may be more severe in rapid excitatory synapses but less severe in inhibitory synapses. In fact, inhibitory synaptic potentials decay more slowly (19), and bursts of channel openings of receptors for the inhibitory transmitter γ-aminobutyric acid last longer (20). Such receptors may have binding sites that are more similar to one another than they are in the receptor for acetylcholine.

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