ACETYLCHOLINE RECEPTOR

Dynamic Properties

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We have investigated the rates of acetylcholine receptor-mediated transmembrane ion flux using sealed membrane vesicles from the electric organ of the electric eel, *Electrophorus electricus*. The number of receptors per unit internal volume in these vesicles is such that the transmembrane cation exchange equilibration via the receptor channels can be followed up to saturating concentrations of specific ligands, using rapid mixing, flow techniques and radioactive tracer ions. The ligand-induced desensitization of the receptor can be followed by the ion flux activity. The kinetic homogeneity of the specific functional vesicles from this source allows the response of the acetylcholine receptor to be expressed in terms of first-order rate constants for ion flux, desensitization, and recovery from desensitization.

RESULTS AND DISCUSSION

The rate constants for the ion equilibration (J_A) and desensitization of the receptor (α) were determined from the ion influx measurements (1–5). The dependence of J_A and α on ligand concentration with acetylcholine and carbamylcholine is shown in Fig. 1. These results, covering a large concentration range, fit the minimal kinetic scheme shown in Fig. 2. The constant, J_A, is a property of the active species, A, described by the top line in the scheme. Since the initial rates characterizing A could be experimentally separated from the rates corresponding to all species after desensitization ($A + I$), it was possible to determine the constants pertaining to the mechanism in Fig. 2 (Table I) (1–5).

The ion flux follows a sigmoidal shape at the foot of the response curve with a square dependence of J_A on ligand concentration. The experimental points at low ligand concentration are severalfold lower than a hyperbola calculated to fit the experimental points above one-third of the maximum response. At higher concentrations the shape of the curve reflects the displacement of the second binding equilibrium by the channel-opening rearrangement and gives a measure of the channel opening equilibrium constant, ϕ^{-1} (2–5). Thus a ligand with an extremely unfavorable channel opening equilibrium constant approximates a hyperbolic response in this region.

The rate of ion flux is given by Eq. 1. $[M]_t/[M]_\infty$ is the fractional equilibration at time, t. The term containing J_1,

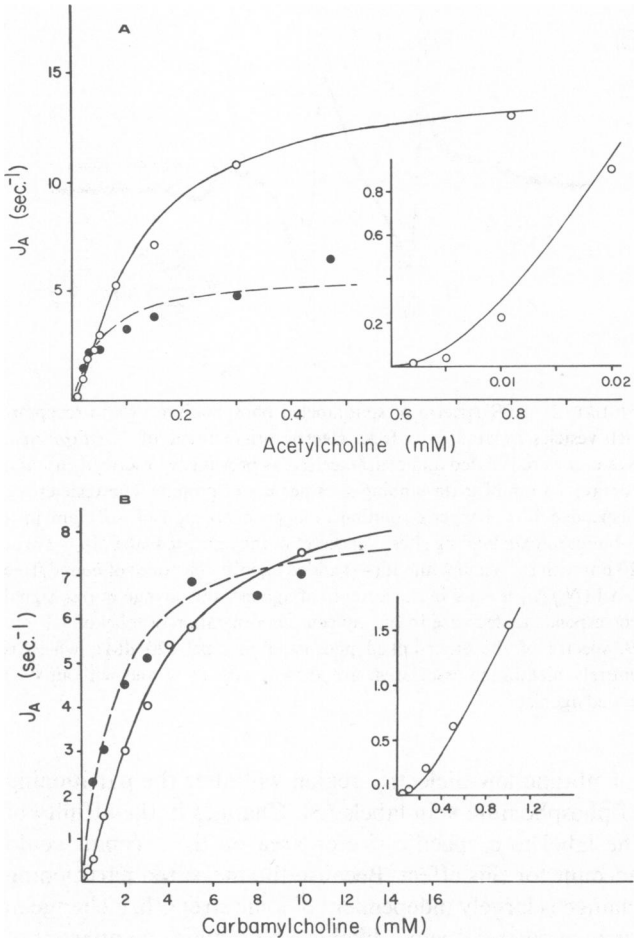

FIGURE 1 First-order rate constants for the transmembrane ion equilibration, J_A (○) and desensitization, α (●) of the receptor as a function of ligand concentration with (*a*) acetylcholine and (*b*) carbamylcholine. *Inset*, Values of J_A at low ligand concentration. Membrane vesicles from *E. electricus* (diam 3,600 ± 600 Å, receptor density 12 ± 3/vesicle) were used in eel Ringer's solution using quench flow techniques with ^{86}Rb tracer ion. The curves for J_A (continuous line) and α (broken line) were computed from the integrated rate equation, Eq. 1, corresponding to the minimal kinetic scheme (Fig. 2).

the rate constant for ion flux after desensitization, arises because a small but significant proportion of open channel form, $\overline{AL_2}$ remains in the equilibrium mixture after desensitization, giving rise to a residual ion flux rate. Ion flux after preincubation with ligand follows Eq. 2. The situation

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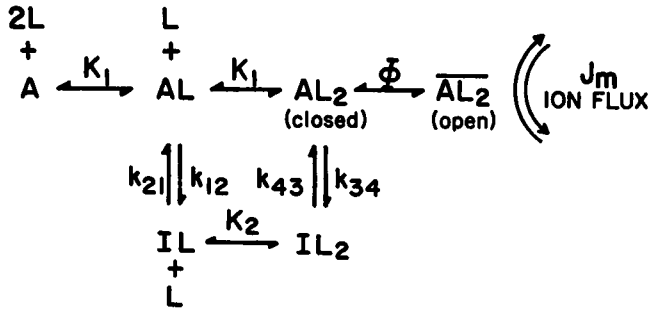

FIGURE 2 Minimal mechanism accounting for the rates of transmembrane ion flux and receptor desensitization. The binding of ligand (L) to the active (A) and inactive (I) states of the receptor, and the channel opening equilibrium, are rapid relative to the transmembrane ion flux equilibration and the conversions between the A and I states. The open channel form, ($\overline{AL_2}$) permits ion equilibration with a first-order rate constant $J_m(=\bar{J}R_o)$. Definitions and values for the constants are given in Table I.

is more complicated in preparations from *Torpedo sp.* electroplax in which the first desensitization is followed by a second desensitization on a slower time scale, leaving no detectable ion flux activity (6, 7).

$$[M]_t/[M]_\infty = 1 - \exp - \left(\frac{J_A}{\alpha}(1 - e^{-\alpha t}) + J_I\, t\right) \quad (1)$$

$$[M]_t/[M]_\infty = 1 - \exp - J_I\, t \quad (2)$$

$$[\overline{M}]/[M]_\infty = 1 - \exp - J_A/\alpha \quad (3)$$

The ligand concentration dependencies of desensitization and ion flux are different. This is accounted for by the scheme in which ion flux is significant only with two bound ligand molecules while desensitization occurs with one or two ligand molecules bound to the receptor. This results in a variation of the amplitude of the fast phase of ion flux ($\overline{M}$), given by Eq. 3, with ligand concentration.

TABLE I
EQUILIBRIUM AND RATE CONSTANTS IN THE MINIMAL REACTION SCHEME (FIG. 2) FOR ACETYLCHOLINE AND CARBAMYLCHOLINE, pH 7.0, 0°C, $J_m = \bar{J}\,R_0 = 37\ s^{-1}$

Parameter	Acetylcholine	Carbamylcholine
$K_1 = \frac{2[A][L]}{[AL]} = \frac{[AL][L]}{2[AL_2]}$, μM	80	1900
$K_2 = \frac{[IL][L]}{2[IL_2]}$, μM	0.7	21
$\Phi = \frac{[AL_2]}{[\overline{AL_2}]}$	1.5	2.8
k_{12}, s^{-1}	3.4	4.6
k_{21}, s^{-1}	0.2	0.5
k_{34}, s^{-1}	9.5	11.2
k_{43}, s^{-1}	0.006	0.01
$\bar{J}$, $M^{-1}\,s^{-1}$	3×10^7	3×10^7

Fig. 2 is a minimal kinetic scheme showing only those protein species in the mixture with a significant contribution to the observations. Thus forms such as I, $\overline{AL}$ are not indicated, although their mechanisms of formation are not precluded. The constants pertaining to the scheme are indicated for two different ligands in Table I.

The number of receptors per unit internal volume, $[R_0]$, was calculated from the measured internal volume of the specific vesicle fraction and the number of α-bungarotoxin binding sites (8, 9). The rate of ion translocation is given by Eq. 4

$$\frac{-d[M']}{dt} = \bar{J}\,[M]\,[R_0]\,[\overline{AL_2}]_0 \quad (4)$$

where

$$\bar{J}[R_0]/(1 + \Phi) = J_A(\max).$$

$[\overline{AL_2}]_0$ is the fraction of the receptor in the open channel form before any desensitization, $[M']$ is the concentration gradient of tracer cation, and $\bar{J}$ is the rate constant (specific reaction rate) for ion translocation. The determined value $\bar{J} = 3 \times 10^7\ M^{-1}\ s^{-1}$ (9) at 1°C pH 7.0 corresponds to a single channel conductance value Λ of 32 pS. The value of Λ obtained from single channel current measurements (10) with electroplax cells (whose cell membranes form the vesicles used in our measurements) is 53 pS (14°C pH 7.0).[1] The values of Λ indicate that the two types of measurements and their interpretations give the same results within experimental error, indicating that the minimum mechanism of receptor function (Fig. 2) is consistent with the properties of the receptor in cells that give rise to the vesicles.

Measurements with rapid mixing techniques have yielded information on the rates of desensitization, on ion translocation per open channel, and on the equilibria of ligand binding and channel opening. The rates of channel opening and closing are now being investigated by a chemical kinetic technique with membrane vesicles.

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ACETYLCHOLINE-ACTIVATED CHANNEL CURRENT-VOLTAGE RELATIONS IN SYMMETRICAL Na^+ SOLUTIONS

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A primary function of the acetylcholine receptor-channel complex (AChR) is transporting ions. The channel is impermeable to anions but passes divalent cations and a wide variety of monovalent cations with diameters less than ~7–8 Å (1, 2). A simple two-barrier, one-site (2B1S) model has been used to describe the transport of the simplest and biologically most important monovalent cations (3–6). In each case certain restrictions were applied to the model, such as arbitrarily setting the position or affinity of the site. In addition, because of experimental limitations the model was applied to data obtained at one symmetrical concentration or by changing only the external concentration.

Certainly permeation through ion channels is very complex. A simple barrier model may be viewed as a crude approximation of reality most useful as a phenomenological description of transport. The model can serve a functional role in the analysis of new data; any extensions or revisions necessary to describe transport of other permeants can give clues regarding the different interactions between the ions and channel. The model is also useful when considering the effect of electrolyte composition on other properties of the channel such as gating (4). Exciting hypotheses have recently been proposed relating the primary amino acid sequences to the structure of the AChR subunits, and relating subunit organization to the formation of the ion channel (7, 8). A simple barrier model can provide the first step toward integrating the details of permeation to the overall structure and function.

Here we test if a 2B1S model, applied without restricting the location or energies of the barriers and site, can describe current-voltage (*I-V*) relations obtained over a wide concentration range of symmetrical Na^+ solutions. The shortcomings of such an absolute rate theory model, like those that arise when the energy barriers are small (9), are not addressed. Although we shall conclude that a general 2B1S model without additional provisions is not completely adequate to describe the data, it does provide a surprisingly good first approximation and gives information about the energy profile that may be useful in further investigation of AChR permeation, structure, and function.

RESULTS

Single AChR events from rat myotubes were studied by voltage clamping membrane patches in symmetrical Na^+ concentrations (10). To obtain *I-V* relations, voltage ramps were applied to the membrane (11). The legend of Fig. 1 describes the solutions, methods, and procedure for obtaining *I-V* relations from single-channel events. Fig. 1 *C* shows a leak-corrected single-channel response to a voltage ramp. Fig. 2 is a plot of the zero-voltage conductance (G_0) vs. activity. A Michaelis-Menten curve having a dissociation constant of 74 mM (activity) is drawn through the high concentration data. The insert is an Eadie-Hofstee plot (G_0 vs. G_0/activity) that shows the deviation of the low concentration data from an expected straight line. Fig. 3 shows the *I-V* relations obtained in symmetrical Na^+ solutions of 45 mM, 150 mM, and 450 mM. The smooth curves result from the best theoretical fit of the 2B1S model. Although the fit to the *I-V* relations is quite good,

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