Acetylcholine-induced cation translocation across cell membranes and inactivation of the acetylcholine receptor: Chemical kinetic measurements in the millisecond time region

(*Electrophorus electricus*/carbamoylcholine/desensitization/ion translocation/chemical kinetics)

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ABSTRACT Acetylcholine-induced flux of inorganic ions across membranes and inactivation of the acetylcholine receptor were measured at pH 7.0, 1°C, over a 5000-fold concentration range of acetylcholine. Receptor-containing electroplax membrane vesicles prepared from *Electrophorus electricus* and a quench-flow technique were used, allowing flux to be measured in the 2-msec to 1-min time region. Five different measurements were made: (i) rate of ion translocation with the active state of the receptor, (ii) rate of the slower ion translocation after equilibration of active and inactive receptor states, (iii) rate of inactivation, (iv) equilibrium between active and inactive forms of the receptor, and (v) reactivation of inactivated receptor. The kinetics of the steps in the receptor-controlled ion flux follow single-exponential rate laws, and simple analytical expressions for their ligand concentration dependence can be used. Thus, the rate and equilibrium constants in a scheme that relates the ligand binding steps to ion translocation could be evaluated. It was found that the dependence of the receptor-controlled ion translocation over the concentration range investigated obeys the integrated rate equation based on the proposed mechanism. The flux rate before inactivation was $\approx 10^7$ ions sec^{-1} per receptor, which is comparable with that measured electrophysiologically in muscle cells. The half-time of inactivation is ≈ 100 msec when the receptor is saturated with acetylcholine. The specific reaction rate of the ion translocation ($\bar{J}$) is $3 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$. The results support a minimum reaction mechanism previously proposed on the basis of experiments in which carbamylcholine was used.

Nachmansohn (1) first suggested that interaction between acetylcholine and its receptor in nerve and muscle cell membranes induces a conformational change of the protein that results in the formation of ion-conducting channels through the membrane. The consequent translocation of cations results in an electrical signal; the amplitude of which is determined by the relative rates at which the ions move (2). These rates depend on the number of open receptor-formed channels and, therefore, for a cell containing a given number of receptor molecules, on the concentration of acetylcholine. The amplitude of the signal that determines whether a muscle cell will contract, or whether the signal is propagated if it is a nerve cell (3), depends therefore on the acetylcholine concentration. This relationship between acetylcholine concentration and receptor-controlled ion flux has been unknown. We have now made kinetic measurements that allow us to determine the relationship.

We have developed methods to measure the rate of receptor-controlled ion flux in the 2-msec to 1-min time region (4–11) using membrane vesicles prepared (12) from the electroplax of *Electrophorus electricus* (13). We chose to use these vesicles

FIG. 1. Minimum mechanism (5, 6) to account for the rates of acetylcholine receptor-mediated cation translocation and of receptor inactivation and reactivation as a function of acetylcholine concentration measured using *E. electricus* vesicles. The active forms of the receptor (A) and the inactive forms (I) bind ligand (L) in rapidly achieved equilibria denoted by the microscopic equilibrium constants (K). Active receptor with two bound ligands (AL_2) converts rapidly to an open channel ($\overline{AL}_2$) with an equilibrium constant for channel opening ($1/\Phi$). $\overline{AL}_2$ permits ion flux with a first-order rate constant (J_m) in a given vesicle system. The symbols containing A and I represent fractions of the receptor and have been defined previously in terms of ligand concentration and the constants in the minimum model (5).

rather than *Torpedo* sp. vesicles because they contain relatively few receptors (about 500 times fewer per unit weight than *Torpedo* sp. vesicles), and the time resolution of the flow method that we use is sufficient to make measurements in the physiologically relevant time region. The number of ions translocated per receptor per msec (6×10^3), which we determined by chemical kinetic measurements using eel vesicles (7, 8), is in agreement with results obtained in electrical noise measurements using muscle cells (14, 15).

Originally we used carbamoylcholine (4–6, 10), because it is a stable analogue of acetylcholine, and measured the effect of carbamoylcholine concentration on (i) the ion flux rate corresponding to the active state of the receptor, (ii) the inactivated receptor (16), and (iii) the rates of receptor inactivation and reactivation (4–6, 10). The quench-flow measurements, over a 200-fold range of carbamoylcholine concentration that included saturation of the active state, supported a minimum reaction scheme as shown in Fig. 1 (5, 6). The integrated rate equation based on the minimum mechanism is given by Eq. 1.

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

[M] is the metal ion concentration inside the vesicles; the subscripts t and ∞ refer to the time of measurement and to 30 sec

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Table 1. Rate and equilibrium constants in the minimum reaction scheme for the two ligands, acetylcholine and carbamoylcholine (5, 8, 10), in eel Ringer's solution at pH 7.0 and 1°C

Parameter	Acetylcholine	Carbamoylcholine (10)
J_m^*, sec^{-1}	37	37
$K_1^* = \frac{2(A)(L)}{(AL)} = \frac{(AL)(L)}{2(AL_2)}, \mu\text{M}$	80	1900
$K_2^* = \frac{(IL)(L)}{2(IL_2)}, \mu\text{M}$	0.70	21
$\Phi^* = \frac{(AL_2)}{(AL)}$	1.5	2.8
$k_{21}^\dagger, \text{sec}^{-1}$	0.23	0.46
$k_{12}^\ddagger, \text{sec}^{-1}$	3.4	4.6
$k_{34}^\parallel, \text{sec}^{-1}$	9.5	11.2
$k_{43}^\nabla, \text{sec}^{-1}$	0.006	0.012
$K_{c1}^{**} = \frac{(AL)}{(IL)}$	0.07	0.10
$K_{c2}^\dagger = \frac{(AL_2)}{(IL_2)}$	0.0006	0.001

$\bar{J} = 3 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$, and inorganic ions translocated per receptor per sec = 6×10^6 . Linear and nonlinear least-squares computer programs were used to obtain an optimal fit of the data to the theory (17). Average error in the rate constants was $\pm 20\%$ and that in the equilibrium constants $\pm 40\%$.

* Calculated from the measurements in Fig. 2 by using Eq. 2.

† Calculated from the data in Fig. 3 A and C by using Eq. 5.

‡ Determined from reactivation experiments (see Fig. 5) and the use of Eq. 3.

§ Calculated from the values of k_{21} and K_{c1} .

|| Calculated from inactivation experiments (see Fig. 3B) by using Eq. 3.

∇ Calculated from the values of k_{34} and K_{c2} .

** Determined by using the values of K_1 , K_2 , and K_{c2} .

influx with a saturating concentration of acetylcholine. The relationship between the rate coefficients, J_A and J_I , and the fraction of the receptor in the open-channel form is given by $J_A = J_m [AL_2]_0$; $J_I = J_m [AL_2]_\infty$, where $J_m = \bar{J} R_0$ and the subscripts 0 and ∞ denote the open-channel form of the receptor before onset of inactivation and after inactivation is complete, respectively. $\bar{J}$ is the specific rate of the receptor-controlled ion translocation, and R_0 is the moles of receptor per liter of internal volume of the vesicle preparation used [$R_0 \approx 1.2 \mu\text{M}$ (7)]. Conversion between the active and inactive receptor states occurs with first-order rate constants k .

We have found that the effects of acetylcholine are predicted by the same minimum mechanism over a 5000-fold concentration range that includes saturation of the active state of the receptor. The rate and equilibrium constants pertaining to this scheme can be determined by using Eqs. 2–5 (5).

$$\left(\frac{J_m}{J_A} - 1 \right)^{1/2} = \frac{K_1 \Phi^{1/2}}{L} + \Phi^{1/2} \quad [2]$$

$$\alpha = \frac{2K_2 k_{21} + k_{43} L}{L + 2K_2} + \Phi \frac{2K_1 k_{12} L + k_{34} L^2}{L^2 (1 + \Phi) + 2K_1 \Phi L + K_1^2 \Phi} \quad [3]$$

When equilibrium between active and inactive forms has been reached,

$$([M]_\infty - [M]_0)/([M]_\infty)^{-1} = \exp - J_I t \quad [4]$$

$$\frac{1}{J_I} - \frac{1}{J_A} = \frac{2K_2 \Phi}{J_m K_{c2} L} + \frac{\Phi}{J_m K_{c2}} \quad [5]$$

These results are compared in Table 1 with those previously obtained with carbamoylcholine.

MATERIALS AND METHODS

Electric eels (*E. electricus*) were supplied by World Wide Scientific Animals (Ardsley, NY). The vesicles were prepared by a modification (unpublished results; ref. 18) of the original procedure (13). The acetylcholinesterase inhibitor, Tetram [*O,O*-diethyl-S-(β -diethylaminoethyl)thiophosphate] (19), was added to 0.05 mM in the final vesicle solution. Acetylcholine bromide was supplied by Eastman, *d*-tubocurarine chloride was from Sigma, and $^{86}\text{RbCl}$ was from New England Nuclear. All other chemicals were reagent grade.

The ligand incubation and ion flux measurements were performed with a quench-flow apparatus as described (4, 9). Experiments with 1 mM carbamoylcholine in the presence and absence of Tetram indicated that this acetylcholinesterase inhibitor did not affect the ion flux rate or the rate of inactivation of the receptor. Kinetic measurements of the rates of $^{86}\text{Rb}^+$ and K^+ influx in presence of 1 mM carbamoylcholine gave results that were indistinguishable (11). All determinations were made in triplicate in eel Ringer's solution (169 mM NaCl/5 mM KCl/3 mM CaCl_2 /1.5 mM MgCl_2 /1.5 mM sodium phosphate buffer, pH 7.0) at 1°C.

The methods of measuring the inactivation of receptor and its reactivation after dilution of the ligand using a quench-flow technique have been described (6, 10).

RESULTS

With 300 μM acetylcholine, the receptor was saturated and ion flux was substantially complete before inactivation became sig-

FIG. 2. Receptor-mediated flux of $^{86}\text{Rb}^+$ into vesicles at various concentrations of ligand in eel Ringer's solution. (M_i/M_∞) is the fraction of complete influx that has occurred at the time indicated. (A) Comparison of acetylcholine at saturation (300 μM) (○) with a lower concentration (60 μM) (□). Points for 800 μM acetylcholine (not shown) were identical with those for 300 μM . The lines were computed by using Eq. 1, in which $J_A = 13 \text{ sec}^{-1}$, $\alpha = 5 \text{ sec}^{-1}$ for 300 μM acetylcholine and $J_A = 3.3 \text{ sec}^{-1}$, $\alpha = 2.3 \text{ sec}^{-1}$ for 60 μM acetylcholine. (B) Measurements at 4–100 msec for 300 μM acetylcholine demonstrate the true resolution of the quench-flow apparatus. The line was taken from the experiment in A. (C) Measurements made at a range of acetylcholine concentrations. The lines were computed from Eq. 1: Δ , 20 μM , $J_A = 0.7 \text{ sec}^{-1}$, $\alpha = 1.2 \text{ sec}^{-1}$; $\circ$, 50 μM , $J_A = 2.2 \text{ sec}^{-1}$, $\alpha = 1.6 \text{ sec}^{-1}$; $\square$, 150 μM , $J_A = 7 \text{ sec}^{-1}$, $\alpha = 3.8 \text{ sec}^{-1}$.

nificant (Fig. 2A). At lower concentrations of acetylcholine (20–150 μM ; Fig. 2C), inactivation was observed and the vesicles were filled at a rate that is characteristic of the inactivated receptor. This occurs in a time range longer than that covered by the experiments in Fig. 2A. At $<5 \mu\text{M}$ acetylcholine, ion flux before inactivation was negligible. The influx curves are predicted by Eq. 1, and the parameters were determined with a curve-fitting procedure (5, 7). The effect of acetylcholine concentration on J_A , the rate coefficient for ion translocation before receptor inactivation, is shown in Fig. 3A. The ligand response curve is sigmoidal; the results cannot be fitted to a hyperbolic curve but fit well to the curve calculated from the scheme in Fig. 1. Different vesicle preparations gave somewhat different J_A values. Thus, with 0.05 mM acetylcholine, 10 preparations gave a mean J_A of 2.7 ± 0.7 (SD) sec^{-1} . Corresponding variations of α were not detected. When the data in Fig. 3A were plotted according to Eq. 2, they gave a linear plot corresponding to the values of J_M , K_I , and Φ (see Table 1) in the reaction scheme in Fig. 1. The linearity of this plot, demonstrated previously (7, 8), confirms the square dependence of ion flux on acetylcholine concentration, showing that two ligand molecules bound to the receptor are required for channel opening and that the process is cooperative.

The results obtained with acetylcholine and carbamoylcholine were compared by using the same vesicle preparation in the same experiment. At saturation with carbamoylcholine (10 and 30 mM), the ion flux rate, J_A , was about half that measured with saturating concentrations of acetylcholine.

The rate of ligand-induced inactivation of receptor was measured by determining the ion influx in a short fixed time after incubating the vesicles with acetylcholine for various periods (6, 10). Thus, α can be determined over a wide range of ligand concentration as described (6, 10). Acetylcholine-induced (100 μM) inactivation of the receptor follows a first-order rate law for more than 85% of the reaction (see Fig. 3B *Inset*). The value of α obtained from the slope of the line is 5.6 sec^{-1} . As with carbamoylcholine (5, 10), the dependence of α on ligand concentration (see Fig. 3A and B) differs from that of J_A . In a comparison of carbamoylcholine (25 mM) and acetylcholine (1 mM), both at saturation, the inactivation of the receptor was 1.3 times faster with carbamoylcholine than with acetylcholine. From Eq. 3, the rate constants k_{34} and k_{21} were evaluated. It should be noted (Eq. 3) that, at saturating concentrations of ligand, $\alpha_{\text{max}} = k_{34}\Phi(1 + \Phi)^{-1}$ and, at low ligand concentrations, $\alpha_{\text{min}} = k_{21}$. α can also be determined from analysis of the ion influx (Eq. 1) over a period of time (see Fig. 2), but only within a certain range of ligand concentration (≈ 0.1 – 0.001 mM acetylcholine with a preparation of typical activity). Within this range, the values of α determined by the two methods are in reasonable agreement. Above that range, ion flux was essentially complete before much inactivation occurred and, below it, inactivation was complete before much ion flux had taken place.

The percentage of inactivated receptor at equilibrium with ligand increased with increasing ligand concentration (Fig. 4A). With 1.0 μM acetylcholine, the receptor was 50% inactivated and, with 50 μM acetylcholine, the activity was reduced to 0.4% (Fig. 4A *Inset*). This was measured by a rapid ion flux assay of the active receptor remaining after the vesicles were incubated with ligand for at least 1 hr as described (6, 10). At ligand concentrations in which receptor inactivation was almost complete (50 μM acetylcholine), the fast ion flux characteristic of J_A was not detected and the total transmembrane ion equilibration occurred with first-order kinetics in a much longer time period (Fig. 4B). The rate constant, J_I , that is characteristic of the equilibrium mixture of active and inactive receptor states at a particular ligand concentration, was determined from first-or-

FIG. 3. First-order rate constants for (A) influx of $^{86}\text{Rb}^+$ with receptor in the active state, (B) inactivation of the receptor, and (C) influx of $^{86}\text{Rb}^+$ after inactivation as a function of acetylcholine concentration. The lines were calculated by using the integrated rate equation pertaining to the scheme in Fig. 1 (Eq. 1) and the values of the parameters given in Table 1. The points were determined as described in the text. (B, *Inset*) Inactivation of the receptor by 100 μM acetylcholine. The relationship (10) between $[\text{AL}_2]$ before onset of inactivation (O), after equilibrium is reached (∞), and after exposure to acetylcholine for a period of time (t) is given (5, 10) by $[\text{AL}_2]_t - [\text{AL}_2]_\infty = ([\text{AL}_2]_0 - [\text{AL}_2]_\infty)e^{-\alpha t}$. Activity (the ordinate of the graph) corresponds to $([\text{AL}_2]_t - [\text{AL}_2]_\infty)/([\text{AL}_2]_0 - [\text{AL}_2]_\infty)$. The concentrations of the various species in this equation, determined by measuring acetylcholine-induced influx of $^{86}\text{Rb}^+$ for a constant period of time after variable periods of incubation with acetylcholine, have been described (10). The slope of the semilogarithmic plot gives $\alpha = 5.6 \text{ sec}^{-1}$.

FIG. 4. Ion flux after incubation of receptor with acetylcholine. (A) Percentage inactivation of the receptor at equilibrium after incubation with acetylcholine at various concentrations. After incubating the vesicles for 1 hr with acetylcholine at the concentrations indicated, the influx was performed with $^{86}\text{Rb}^+$ and 0.3 M acetylcholine for 1.2 sec. The results are expressed as a percentage of the influx after incubation without ligand. The percentage is a measure of the percent active receptor at equilibrium. Superscripts $t = 0$ and $t = \infty$ indicate that the receptor had not been exposed to acetylcholine or had been exposed for 1 hr before influx measurements were made (10). (Inset) Percentage of active receptor calculated from the observed influx assay. The line was calculated by using the equations derived from the minimum mechanism (see Fig. 1) and the values of the parameters given in Table 1 (10). (B) Ion influx with inactivated receptor. The vesicles were incubated with ligand for 1 hr and then influx of $^{86}\text{Rb}^+$ was followed at the same ligand concentration: $\circ$, 5 mM carbamoylcholine, $J_I = 0.012 \text{ sec}^{-1}$; $\triangle$, 0.25 mM acetylcholine, $J_I = 0.010 \text{ sec}^{-1}$. The line was calculated by using Eq. 4 and the mean value of the measurements made with carbamoylcholine and acetylcholine. The points were determined by using the Millipore filtration assay after dilution into a solution of *d*-tubocurarine (20).

der plots of this slow ion flux (6, 10) by using Eq. 4. Rates of ion influx induced by carbamoylcholine and acetylcholine, both at concentrations that saturate the inactivated receptor, can be compared (Fig. 4B). The dependence of J_I on acetylcholine concentration is shown in Fig. 3C. From the linear plot (not shown) of $(J_I^{-1} - J_A^{-1})$ versus L^{-1} according to Eq. 5, K_2 was determined and, knowing J_m and Φ , we could calculate K_{α} .

A solution of vesicles exposed to acetylcholine for 2 hr was diluted seven-fold in the flow-quench apparatus. In the experiment shown in Fig. 5, we measured the relaxation of the equilibrium concentration of inactive receptor characteristic of 3.5 μM acetylcholine to that of 0.5 μM ligand by using an ion flux assay (10). The reactivation follows a single exponential rate law (Fig. 5B). Measurements of reactivation gave values of α (0.25 sec^{-1} in the case shown) in agreement with those calculated from the other measurements described. Thus, at the concentration of acetylcholine after dilution (0.5 μM), the receptor was not completely reactivated. This agrees with data (Fig. 4A Inset) indicating that 25% of the receptor remains in the inactivated state at this concentration of acetylcholine. The receptor inactivation obtained by prolonged incubation with 3.5 μM acetylcholine appears, therefore, to be reversible. From the prin-

FIG. 5. Reactivation of inactivated receptor. (A) The receptor was inactivated by incubating the vesicles with 3.5 μM acetylcholine for 2 hr. The vesicle suspension was diluted to 0.5 μM acetylcholine, and incubation was continued for the times indicated; then influx (1.2 sec) was initiated by addition of $^{86}\text{Rb}^+$ and 0.3 mM acetylcholine and followed by Millipore assay. Δ , Control experiment (M_{∞}): The receptor was not inactivated. No ligand was present before the addition of $^{86}\text{Rb}^+$. $\triangle$, Control experiment for nonspecific influx (M_I). As in control but no acetylcholine was present in the assay (influx) incubation. $\circ$, $^{86}\text{Rb}^+$ influx. The measurement at zero time ($\otimes$) was obtained by keeping the acetylcholine concentration the same (3.5 μM) in the incubation and in the diluted solution. (B) Evaluation of α by measurement of the rate of reactivation. The data shown in A are plotted (10) according to the equation in Fig. 3 legend rearranged for reactivation measurements to give a linear plot in which $\alpha = 0.25 \text{ sec}^{-1}$. "Fraction inactive" corresponds to $([AL_2]_{\infty} - [AL_2]_t) / ([AL_2]_{\infty} - [AL_2]_0)$. Another experiment with a final concentration of 0.2 μM acetylcholine gave $\alpha = 0.15 \text{ sec}^{-1}$. These results compare well with the value ($\alpha = 0.21 \text{ sec}^{-1}$) calculated from the constants listed in Table 1.

ciple of reversibility, the remaining parameters in the reaction scheme (see Fig. 1), K_{c1} , k_{12} , and k_{43} , are determined (Table 1).

DISCUSSION

The methods for isolating (15, 18, 21) and characterizing vesicle preparations containing active receptors (15, 22–24), and the rapid reaction techniques (time resolution of 2 msec) using sequential mixing of solutions that we have developed (4–6, 9–11), allowed us to make several types of measurement as a function of acetylcholine concentration: (i) rapid ion translocation mediated by the receptor in the active state, (ii) slow ion translocation that occurs after inactivation of the receptor, (iii) rate of receptor inactivation, (iv) equilibrium between active and inactive receptor states, and (v) reactivation of the inactive receptor. The concentrations of acetylcholine used ranged from that in which only the equilibrium mixture of active and inactive receptor states is observed (0.2 μM) to that required to establish saturation of the active state of the receptor (1 mM).

The results are consistent with a minimum kinetic mechanism (see Fig. 1) proposed on the basis of experiments using carbamoylcholine (5, 6). Notable features of the scheme are (i) the requirement that two (only) ligand molecules be bound for channel opening to occur, thus giving rise to a sigmoidal ligand response curve; (ii) that a cooperative effect of ion flux and ligand binding is due to channel opening and increases with the channel opening equilibrium constant, $1/\Phi$; (iii) the formation of inactive receptor species that do not themselves give rise to open channels but decrease the amount of active receptor in a reversible equilibrium between active and inactive forms at a rate much slower than ligand binding and channel opening and measurable with the ion flux probe; (iv) the occurrence of receptor inactivation with one or two bound ligand molecules, so that inactivation has a different dependence on ligand concen-

tration than does ion translocation. If inactivated receptor in absence of ligand exists, its concentration is too small to make a significant contribution to our measurements. By using the integrated rate equation (Eq. 1) pertaining to the mechanism that relates acetylcholine binding to ion translocation (see Fig. 1), we elucidated the pertinent kinetic and equilibrium constants for acetylcholine (see Table 1).

The finding that two bound ligand molecules are required for channel opening is consistent with the finding that, in acetylcholine receptor from *Torpedo californica*, there are two apparently identical α -subunits (25, 26) that are specifically covalently labeled by affinity reagents directed at the acetylcholine binding site (27, 28). On the basis that the acetylcholine receptor from *E. electricus* resembles that from *Torpedo* sp. (29), this suggests that the two binding sites that must be occupied for channel opening to occur are situated one on each α -subunit and that channel opening involves a concerted change in conformation of both subunits.

The effects of carbamoylcholine and acetylcholine were compared by using the same vesicle preparation in the same experimental session (see Table 1). Notable differences (5, 6, 10) are the lower dissociation constants and the increased channel opening constant, $1/\Phi$, for acetylcholine. Although the rate constants for inactivation are comparable for the two ligands, the equilibrium constants are more in favor of inactivation for acetylcholine. The rate constant for ion translocation, J_m , pertains to the open-channel form of the receptor and is independent of ligand (7, 8).

The constants listed in Fig. 1 and Table 1 are, with the exception of J_m , each a measured property of the receptor with a given ligand. However, J_m is a property of the vesicles and the receptor and increases with the number of receptors per unit of internal volume. In contrast, the rate coefficient for receptor inactivation is expected to be characteristic of the receptor and independent of these other properties of the vesicles. To express the ion flux rate as a property of the receptor, it must be divided by R_0 : $\bar{J} = J_m [R_0]^{-1}$ where $\bar{J}$ is the specific reaction rate of the receptor-controlled ion translocation and has a value of $3 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$, regardless of whether acetylcholine or carbamoylcholine is used (7, 8). If we multiply $\bar{J}$ by M' , the molar concentration of inorganic ions per liter of internal volume of the vesicles, we find that 6×10^6 (8) ions are translocated per receptor sec^{-1} at pH 7.0, 1°C in this and previous experiments with carbamoylcholine (7, 8). This value agrees with the one estimated from electrophysiological conductance measurements using frog muscle cells (14), 1×10^7 ions sec^{-1} . In stopped-flow measurements of carbamoylcholine-induced ion translocation in *Torpedo* sp. vesicles, 10^6 (30) ions were found to be translocated per α -bungarotoxin binding site, in essential agreement with our measurements and those using muscle cells. Flow-quench measurements with *Torpedo* sp. vesicles gave the much lower value of 3.5×10^3 (31).

The use of rapid mixing methods and membrane vesicles allows the study of a measurable number of receptors exposed to known ligand concentrations that can be rapidly changed at will during an experiment. The concentrations of inorganic ions can be varied widely both inside and outside the vesicles (7, 8). The movement of different radioactive and nonradioactive (11) inorganic ions can be measured and may be used to follow changes in the receptor that occur at rates comparable with or slower than the transport process. By using *E. electricus* vesicles the rates of receptor-controlled ion translocation can be

measured in the physiologically relevant time region with a precision comparable with that of enzyme-catalyzed reactions. Many different receptors are known, and the methods described may have general application to the chemistry of receptors.

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