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Regulatory Properties of Acetylcholine Receptor: Evidence for Two Different Inhibitory Sites, One for Acetylcholine and the Other for a Noncompetitive Inhibitor of Receptor Function (Procaine)†

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ABSTRACT: Does the acetylcholine receptor have a specific regulatory (inhibitory) site for the natural receptor ligand acetylcholine? This paper deals with this question. The inhibition of acetylcholine–receptor function by diverse organic cations including local anesthetics such as procaine has been well documented. Evidence indicates that these compounds are noncompetitive inhibitors, enter the open-channel form of the receptor, and block it and that the extent of this blockage depends on the transmembrane voltage of the cell. Recently we reported that in the electroplax of *Electrophorus electricus* the receptor-controlled transmembrane ion flux is inhibited by acetylcholine in a voltage-dependent, noncompetitive manner. We report here that the *Torpedo californica* receptor also has an inhibitory site for acetylcholine. The question of whether acetylcholine, which is an organic cation, binds to the same site as other organic cations such as the noncompetitive inhibitor procaine is important and is addressed. The results reported here of chemical kinetic investigations, with receptor-rich *E. electricus* and *T. californica* membrane vesicles, indicate that the inhibition of receptor function by acetylcholine and by a local anesthetic, procaine, involves two different receptor sites. The existence of a specific inhibitory site for the natural receptor–ligand acetylcholine suggests that this site can play an important role in the modulation of receptor function and in the regulation of transmission of signals between cells.

Noncompetitive inhibitors of the acetylcholine receptor, including positively charged local anesthetics such as procaine, appear to enter the open-channel form of the receptor and block it in a voltage-dependent manner (Neher & Steinbach, 1978; Oswald et al., 1983; Cox et al., 1984). High concentrations of acetylcholine and its analogues also appear to block the receptor channel (Takeyasu et al., 1983; Sine & Steinbach, 1984).

The voltage-dependent inhibition of the receptor-mediated ion flux in *Electrophorus electricus* by acetylcholine and suberyldicholine (Pasquale et al., 1983) has recently been reported (Takeyasu et al., 1983). The inhibitory site was found to be distinct from the sites that are involved in the opening of transmembrane receptor channels and in the inactivation (densensitization) of the receptor. We now report that a voltage-dependent inhibitory site for acetylcholine also exists in *Torpedo californica*. The question of whether positively charged acetylcholine merely acts like the positively charged local anesthetics such as procaine or whether there exists a

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Table I: Equations Used for the Evaluation of the Kinetic Constants

	eq no.
$L_0 + A \overset{K_1}{\rightleftharpoons} AL \overset{K_1}{\rightleftharpoons} AL_2 \overset{\Phi}{\rightleftharpoons} \overline{AL}_2 \xrightarrow[J_A = \bar{J}R_0\overline{AL}_{2_0}]{\text{ion flux}}$ $k_{12}\uparrow\downarrow k_{21} \quad k_{34}\uparrow\downarrow k_{43}$ $IL \overset{K_2}{\rightleftharpoons} IL_2$	1[a]
$M_t/M_\infty = 1 - \exp[-\bar{J}R_0\overline{AL}_{2_0}(1 - e^{-\alpha t})\alpha^{-1}]$	2a[b]
$J_A/J_{A(P)} = [\ln (M_\infty - M_t)(M_\infty)^{-1}]/[\ln (M_\infty - M_t)(M_\infty)^{-1}]_{(P)}$	2b[c]
$\ln [\ln [(M_\infty - M_t)(M_\infty)^{-1}]_T/\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{T=0}] = -\alpha T$	3a[d]
$\ln [(J_A)_T/(J_A)_{T=0}] = -\alpha T$	3b[e]
$R_0 = A + AL + \overline{AL}_2 + AL_2 = \overline{AL}_2\overline{AL}_{2_0}^{-1}$	4a
$\overline{AL}_{2_0} = L_0^2/[L_0^2(1 + \Phi) + 2K_1L_0\Phi + K_1^2\Phi]$	4b
$K_R = [A_T][L_0]/[A_TL]$	5a
$K_P = [A_T][P_0]/[A_TP] = [A_TL][P_0]/[A_TPL]$	5b

[a] The minimum model in eq 1, which accounts for the concentration dependence of the receptor-controlled ion translocation in *E. electricus* vesicles for three activating ligands (acetylcholine, carbamoylcholine, and suberyldicholine) over at least a 2000-fold concentration range, has been described in detail (Hess et al., 1983). All the assumptions made in deriving the equations have been stated previously (Hess et al., 1983). Three receptor states are involved: An active receptor form, A, which can give rise to an open-channel form, Ā, when two molecules of the activating ligand are bound, and an inactive receptor form, I, which cannot form a transmembrane channel. Inactive receptor forms arise from the interconversion of AL and AL_2 to IL and IL_2 (Cash & Hess, 1980). The rate constants for this interconversion are designated by k_{12}, k_{21}, k_{34}, and k_{43}. The effect of the activating ligand on the rate coefficient for this interconversion is known (Aoshima et al., 1981; see also Figure 2). K_1 and K_2 represent the intrinsic dissociation constants for the activating ligand from the active and inactive receptor forms, respectively, and Φ^{-1} the channel-opening equilibrium constant ($\Phi^{-1} = \overline{AL}_2/AL_2$). The rate coefficient for ion flux mediated by the active state of the receptor A is designated $J_A = \bar{J}R_0\overline{AL}_{2_0}$. $\bar{J}$ is an intrinsic constant and represents the specific reaction rate for the receptor-controlled ion translocation process (Hess et al., 1981). R_0 is a constant for a given vesicle preparation and represents the moles of receptor sites per liter internal volume of the vesicles. Bound and free molecules of the activating ligand are represented by L and L_0, respectively. [b] M_t and M_∞ represent the tracer ion content of the vesicles at time t and at equilibrium (∞), respectively. $\overline{AL}_{2_0}$ represents the fraction of the receptor in the open-channel form and is defined in eq 4b. [c] When α is the same in the presence and absence of procaine (see Figure 2), the quotient of the log forms of eq 2a pertaining to measurements in the absence and presence of procaine (denoted by subscript P), respectively, gives eq 2b. [d] In experiments with *E. electricus* vesicles α was evaluated as described by Aoshima et al. (1981), using eq 3a. The symbol T represents the duration of preincubation and t the time ion flux is allowed to proceed after preincubation. "Percent activity remaining" (Figure 1) is defined by $\{\ln [(M_\infty - M_t)(M_\infty)^{-1}]_T/\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{T=0}\}100$. [e] For evaluation of α in experiments with *T. californica* vesicles, the data were plotted according to Hess et al. (1982) by using eq 3b.

separate regulatory site for acetylcholine is of importance for the understanding of the receptor-controlled transmission of signals between cells. It is also of interest in view of the intensive investigations of noncompetitive inhibitors of the receptor using various techniques and both cells and vesicles [reviewed by Popot & Changeux (1984)] (Oswald et al., 1983; Neher, 1983; Warnick et al., 1984; Heidmann & Changeux, 1984; Cox et al., 1984).

The effect of procaine concentration on two rate coefficients of the ion translocation process is measured: J_A, the influx rate coefficient associated with the receptor before its conversion to the inactive (desensitized) form, and α, the rate coefficient for the interconversion between the active and desensitized forms of the receptor (Hess et al., 1983) (see eq 1 in Table I). Receptor-rich *E. electricus* and *T. californica* vesicles were used.

The results presented here indicate that the receptor has two different regulatory sites, one for the specific receptor–ligand,

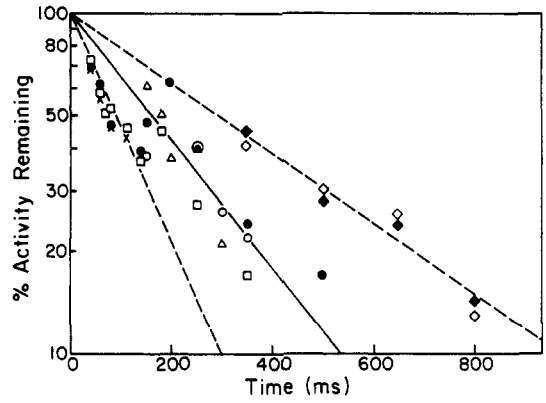

FIGURE 1: Effect of procaine on the rate of receptor inactivation (desensitization) in vesicles that were preincubated with acetylcholine alone (filled symbols) or with acetylcholine and procaine (open symbols) for the times given on the abscissa. Preincubation was followed by addition of $^{86}Rb^+$ and measurement of the $^{86}Rb^+$ content of the vesicles, M_t, after a constant period of time. The data were then plotted according to eq 3. Experiments with *E. electricus* vesicles, pH 7.0, 1 °C, $V_m = 0$ mV (solid line), and $V_m = -45$ mV (lower dashed line), and with *T. californica* vesicles, pH 7.4, 1 °C, $V_m = -25$ mV (upped dashed line). In experiments with *E. electricus* vesicles, 270 μg of membrane protein/mL and 33 μCi of $^{86}Rb^+$/mL were used in the influx measurement. The influx time was 800 ms ($V_m = 0$ mV) or 1.5 s ($V_m = -45$ mV). At $V_m = -45$ mV, M_∞ was obtained as described (Takeyasu et al., 1983); at $V_m = 0$ mV, M_∞ is obtained from a 3-s influx. Acetylcholine (1 mM) was used and the same symbols were used at $V_m = 0$ and −45 mV. (●) Acetylcholine; (×) acetylcholine and 60 μM procaine; (□) acetylcholine and 120 μM procaine; (○) acetylcholine and 240 μM procaine; (△) acetylcholine and 400 μM procaine. All the measurements were used to calculate the coordinates of the lines. An α value of 4.3 ± 0.1 s^{-1} at $V_m = 0$ mV was calculated from the slope of the line and of 8.1 ± 0.4 s^{-1} at $V_m = -45$ mV. In experiments with *T. californica* vesicles, the protein concentration in the solution was the same, 270 μg of membrane protein/mL, during influx. A total of 35 μCi of $^{86}Rb^+$/mL was used in influx measurements. M_∞ and M_t were determined from 66 and 6 ms influx measurements, respectively. Acetylcholine (100 μM) was used. (♦) Acetylcholine; (◇) acetylcholine and 100 μM procaine. The value of α calculated from the slope of the upper dashed line is 2.4 ± 0.2 s^{-1}.

acetylcholine, and the other for the local anesthetic, procaine.

Experimental Procedures

The preparation of receptor-rich vesicles from the electroplax of *E. electricus* (World Wide Scientific Animals, Apopka, FL) has been described (Kasai & Changeux, 1971; Fu et al., 1977). *T. californica* (Pacific Biomarine Lab, Venice, CA) vesicles were prepared as described (Sobel et al., 1977; Delgeane & McNamee, 1980) and equilibrated with 90 mM NaCl and 1.5 mM sodium phosphate, pH 7.4. A transmembrane voltage (V_m) of −25 mV was produced by mixing the latter vesicles with an isotonic arginine solution (66 mM arginine hydrochloride, 21 mM NaCl, 1.5 mM sodium phosphate buffer, pH 7.4), in the quench flow apparatus to give a ratio of $[Na^+]_{in}$ to $[Na^+]_{out}$ of 3.0. All measurements were made at 1 °C. The control experiments, the experimental details of the chemical kinetic measurements made in the absence (Cash & Hess, 1981) and presence (Takeyasu et al., 1983) of a transmembrane voltage, and the procedures used to evaluate J_A and α (Cash & Hess, 1980; Hess et al., 1983) have been described. The ratio of J_A values in the absence and presence of procaine was evaluated from influx measurements using eq 2b (Table I). Use of this equation requires that α is not affected by procaine (footnote *c* in Table I). In agreement with an earlier report (Karpen et al., 1982), procaine does not affect the inactivation rate coefficient, α. This is shown in Figure 1. Experiments with *E. electricus* vesicles

Table II: Equations Used To Differentiate between Possible Binding Modes of Inhibitors

	Mechanism I: $L_0 \ll K_R$[a]	
procaine	absent	present
conditions	$R_0 = A_T$[b]	$R_0 = A_T + A_TP$
rate coefficient	(Ia) $J_A = \bar{J}R_0\overline{AL}_{2_0}$	(Ib) $J_{A(P)} = \bar{J}R_0\overline{AL}_{2_0}(1 + P_0/K_P)^{-1}$
$J_A/J_{A(P)}$	(Ic) $J_A/J_{A(P)} = 1 + P_0/K_P$	
	Mechanism II: $L_0 \gg K_R$[c] (Common Inhibitory Site for Acetylcholine and Procaine)	
procaine	absent	present
conditions	$R_0 = A_T + A_TL$	$R_0 = A_T + A_TL + A_TP$
rate coefficient	(IIa) $J_A = \bar{J}R_0\overline{AL}_{2_0}(1 + L_0/K_R)^{-1}$	(IIb) $J_{A(P)} = \bar{J}R_0\overline{AL}_{2_0}(1 + L_0/K_R + P_0/K_P)^{-1}$
$J_A/J_{A(P)}$	(IIc) $J_A/J_{A(P)} = 1 + (P_0/K_P)[K_R/(K_R + L_0)]$	
	Mechanism III: $L_0 \gg K_R$[c] (Separate Inhibitory Sites for Acetylcholine and Procaine)	
procaine	absent	present
conditions	$R_0 = A_T + A_TL$	$R_0 = A_T + A_TL + A_TP + A_TLP$
rate coefficient	(IIIa) $J_A = \bar{J}R_0\overline{AL}_{2_0}(1 + L_0/K_R)^{-1}$	(IIIb) $J_A = \bar{J}R_0\overline{AL}_{2_0}[1 + L_0/K_R + P_0/K_P + L_0P_0/(K_RK_P)]^{-1}$
$J_A/J_{A(P)}$	(IIIc) $J_A/J_{A(P)} = 1 + P_0/K_P$	

[a] When the regulatory site for the activating ligand is not occupied ($L_0 \ll K_R$), the influx rate coefficient in the absence of procaine, J_A, and in the presence of procaine, $J_{A(P)}$, is given by eq Ia and eq Ib, respectively. [b] $A_T = A + AL + AL_2 + \overline{AL}_2$ (see eq 1, Table I). [c] When the regulatory site for the activating ligand is occupied ($L_0 \gg K_R$), J_A is given by eq IIa or IIIa. $J_{A(P)}$ for a common site for acetylcholine and procaine is given by eq IIb and for the case where acetylcholine and procaine occupy two different sites by eq IIIb.

were conducted with a transmembrane voltage, V_m, of 0 mV (the solid line) and −45 mV (lower dashed line) and with *T. californica* vesicles at a V_m of −25 mV (upper dashed line). The percent of activity remaining (defined in the footnotes to Table I, and eq 3) is plotted on a logarithmic scale vs. the time for which the vesicles were incubated with acetylcholine (closed symbols) or with acetylcholine and procaine (open symbols) before the addition of $^{86}Rb^+$ and the measurement of influx (Aoshima et al., 1981; Walker et al., 1981). The slopes of the lines are proportional to α (eq 3). Within experimental error, the slope of the line is found to be the same regardless of whether the preincubation was made in the presence or absence of procaine. This result allows one to use eq 2b for calculation of $J_A/J_{A(P)}$.

Equations

The equations that can be used to distinguish, on the basis of kinetic measurements, between identical or separate inhibitory sites for acetylcholine (or suberyldicholine) and a local anesthetic, procaine, are listed in Table II. The definitions of the constants used are given in Table I. Time intervals for the influx measurements were chosen so that the concentration of the inactive receptor species, IL and IL_2 (eq 1, Table I), could be neglected. The receptor species which have to be considered, A, AL, AL_2, and $\overline{AL}_2$ (eq 1, Table I), and their relation to the total concentration of receptor sites present in the experiments, R_0, is given by eq 4a in Table I and $\overline{AL}_{2_0}$ is defined by eq 4b (Cash & Hess, 1980). The rate coefficient for ion flux mediated by the active state of the receptor, J_A, is defined in terms of $\bar{J}$, R_0 and $\overline{AL}_{2_0}$ (see eq 1) (Hess et al., 1981). The dissociation constant for the inhibitory receptor site for acetylcholine, K_R, is defined by eq 5a, and the dissociation constant for procaine, K_P, is defined by eq 5b where A_T represents $A + AL + AL_2 + \overline{AL}_2$, L_0 and P_0 are the initial concentrations of acetylcholine and procaine, respectively, and $[A_TL]$ and $[A_TP]$ are the concentrations of the complexes of the inhibitory sites with acetylcholine or with procaine, respectively. $[A_TLP]$ represents the concentration of the ternary complex in which acetylcholine and procaine are bound to their specific inhibitory sites. It is assumed that $L_0, P_0 \gg R_0$ so that the initial ligand concentration can be assumed to be equal to the concentration of unbound ligand.

The three different mechanisms considered are given in Table II. Mechanism I is obtained at low acetylcholine concentrations ($L_0 \ll K_R$), conditions under which acetylcholine does not occupy the inhibitory site. Mechanisms II and III are obtained at high acetylcholine concentrations ($L_0 \gg K_R$), conditions under which acetylcholine binds to its inhibitory site. In mechanism II it is assumed that acetylcholine and procaine occupy the same inhibitory site, and in mechanism III the existence of two inhibitor sites is assumed, one for acetylcholine and another for procaine. As can be seen from Table II, a plot of the ratio of $J_A/J_{A(P)}$ vs. procaine concentrations at constant low ($L_0 \ll K_R$, eq Ic) and constant high ($L_0 \gg K_R$, eq IIc and IIIc) acetylcholine concentrations allows one to compare the two mechanisms. For a common site for the activating ligand and procaine, K_P will have different values at low and high concentrations of the activating ligand (compare eq Ic and IIc). For separate sites, K_P will have the same value at low and high concentrations of the activating ligand (compare eq Ic and IIIc). The equations in the table were explicitly written for the case where both the activating ligand and procaine bind to all forms of the receptor (A, AL, AL_2, and $\overline{AL}_2$; eq 1, Table I). The observation that the observed K_P value is not larger when $L_0 \gg K_R$ than when $L_0 \ll K_R$ indicates the existence of separate inhibitory sites, regardless of whether the activating ligand and procaine bind only to the open-channel form of the receptor ($\overline{AL}_2$, eq 1 of Table I) or whether one compound binds to all forms of the receptor and the other only to the open-channel form.

Results

The data in Figure 2 indicate that the regulatory sites for acetylcholine or suberyldicholine, characterized by the dissociation constant K_R, are different from the inhibitory site to which procaine binds. The ratio of the influx rate coefficients in the absence and presence of procaine, $J_A/J_{A(P)}$, is plotted vs. the procaine concentration according to eq Ic, IIc, or IIIc, with the slopes of the lines being inversely proportional to the dissociation constant of procaine, K_P. The experiments in Figure 2 show that the same value for K_P is obtained (the same slope) whether the channel-activating ligand is acetylcholine or suberyldicholine (Figure 2a) or whether $L_0 \ll K_R$ or $L_0 \gg K_R$. This indicates that the inhibitory sites for suberyldicholine (Figure 2a) or acetylcholine (Figure 2a,b) and procaine are different in both the *E. electricus* receptor (Figure 2a,b) and the *T. californica* (Figure 2c) receptor. The dashed lines in Figure 2 indicate the slopes of the lines that are to be expected if the channel-activating ligand and the local anesthetic bind to the same site. The coordinates of each dashed line were

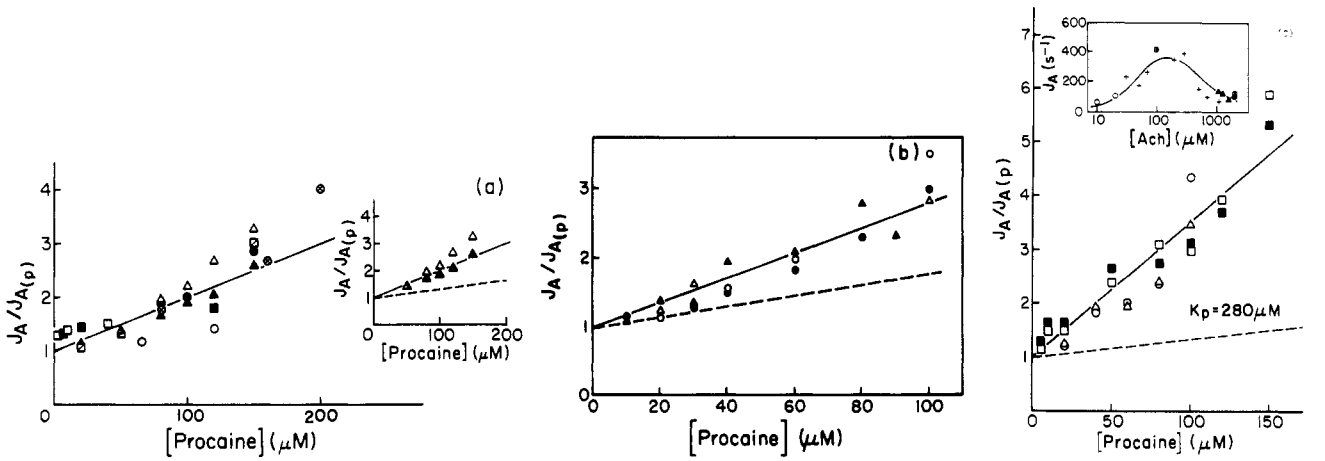

FIGURE 2: Effect of procaine on the rate of ion translocation in membrane vesicles. The ratio of the rate constant for ion flux initiated by a constant concentration of acetylcholine or suberyldicholine, J_A, and the rate constant obtained in the presence of procaine, $J_{A(P)}$, are plotted vs. procaine concentration according to eq Ic, IIc, or IIIc (Table II). The ratio $J_A/J_{A(P)}$ was calculated by measuring the $^{86}Rb^+$ influx and evaluating the influx rate coefficient J_A by using eq 2a (Cash & Hess, 1980) (a, ⊗ and ○) or from measurement of the $^{86}Rb^+$ content of the vesicles after a constant period of influx, M_t, and use of eq 2b. Six *E. electricus* with which nine experiments were done and two *T. californica* preparations were used. (a) Experiments with *E. electricus* vesicles, pH 7.0, 1 °C, V_m = 0 mV. A total of 400 μg of membrane protein and 50 μCi of $^{86}Rb^+$/mL was used in the influx measurements. M_∞ was obtained after a 3-s influx in the presence of 1 mM acetylcholine. Results obtained with 3 μM and 1 mM suberyldicholine, which were done with the same membrane preparation, are reported in the inset. *Suberyldicholine*: (△) 3 μM (M_t at 200 and 500 ms); (●) 3 μM (M_t at 200 and 700 ms); (○) 10 μM [from determination of J_A (eq 2)]; (▲) 1 mM (M_t at 200 and 500 ms). *Acetylcholine*: (▨) 100 μM acetylcholine (M_t at 200 and 700 ms); (□) 300 μM (M_t at 200 ms); (⊗) 1 mM [from determination of J_A (eq 2)]; (■) 1 mM (M_t at 800 ms). The coordinates of the solid line were calculated by using all the measurements. A value for K_P of 100 ± 9 μM was calculated from the slope of the line. The coordinates of the dashed line (inset) pertain to the data with 1 mM suberyldicholine alone and were calculated on the assumption of a common inhibitory site by using eq IIc, a K_P value of 100 μM, and a K_R value of 500 μM (Pasquale et al., 1983). The slope of the dashed line gives an apparent value for K_P of 300 μM. (b) Experiments with *E. electricus* vesicles, pH 7.0, 1 °C, V_m = −45 mV. The establishment of the membrane potential (V_m) and associated calculations have been described (Takeyasu et al., 1983). A total of 400 μg of membrane protein and 40 μCi of $^{86}Rb^+$/mL was used in the influx measurement. *Acetylcholine*: (●) 60 μM (M_t at 0.5 and 1.5 s); (△) 300 μM (M_t at 0.35 and 0.8 s); (○) 800 μM (M_t at 0.35 and 0.8 s); (▲) 1 mM (M_t at 0.35, 0.5, and 1.5 s). The coordinates of the solid line were calculated by using all the measurements. A value of K_P of 56 ± 4 μM was calculated from the slope of the line. The dashed line was drawn on the assumption of a common inhibitory site by using eq IIc and with a K_P value of 56 μM and a K_R value of 800 μM at V_m = −45 mV (Takeyasu et al., 1983). The slope of the dashed line gives an apparent K_P of 130 μM. (c) Experiments with *T. californica* vesicles, pH 7.4, 1 °C, V_m = −25 mV. A total of 270 μg of protein/mL and 35 μCi of ^{86}Rb/mL was used in the influx experiments. *Acetylcholine*: (△) 10 μM (M_t at 100 ms, M_∞ at 200 ms); (□) 25 μM (M_t at 47 ms, M_∞ at 104 ms); (○) 60 μM (M_t at 10 ms, M_∞ at 200 ms); (■) 1000 μM (M_t at 47 ms, M_∞ at 104 ms). The coordinates of the solid line were calculated by using all the measurements and a K_P value of 40 ± 3 μM. The dashed line was drawn on the assumption of a common inhibitory site by using eq IIc, a K_P value of 40 μM, and a K_R value of 160 μM. With 1 mM acetylcholine the slope of the dashed line gives an apparent K_P value of 280 μM. (Inset) A plot of J_A vs. acetylcholine concentration. The preparations were normalized to one another by using the J_A values at 100 μM acetylcholine. The influx time for determining M_∞ was 160 ms. The influx time for the M_t values were (⊗) 6, (+) 10, (●) 33, (○) 47, and (▲) 60 ms. A K_R value of 160 μM was evaluated from these data as described previously (Pasquale et al., 1983; Takeyasu et al., 1983).

calculated by using eq IIc, the values of K_P determined in the experiments shown, and the values of K_R for suberyldicholine and acetylcholine from previously published results (Pasquale et al., 1983; Takeyasu et al., 1983) for *E. electricus* receptor or the values of K_R for *T. californica* receptor determined from the data in Figure 2c (inset) by using the procedures published in detail previously (Pasquale et al., 1983; Takeyasu et al., 1983). Experiments with *T. californica* were included (Figure 2c, inset) to demonstrate that the inhibitory site for acetylcholine is not restricted to the *E. electricus* receptor. Also, because the rates of receptor-controlled ion translocation are much higher in *T. californica* than in *E. electricus* vesicles (Neubig & Cohen, 1980; Walker et al., 1981), this allows the use of higher ratios of L_0 to K_R and gives greater differences between the observed and calculated slopes (compare parts a and b with part c of Figure 2).

The results in Figure 2a,b indicate that K_P is about twice as large when V_m = 0 mV (K_P = 100 μM) than when V_m = −45 mV (K_P = 56 μM), in agreement with the voltage dependence of the dissociation constant of local anesthetics that was observed in single-channel measurements with frog muscle cells (Neher & Steinbach, 1978).

The inset in Figure 2c shows that, in experiments with *T. californica* vesicles at V_m = −25 mV, J_A first increases with increasing acetylcholine concentration but then decreases again as the acetylcholine concentration is further increased. This progressive decrease of J_A after the ligand concentration is increased beyond a certain value has been observed with suberyldicholine (Pasquale et al., 1983) and with high concentrations of acetylcholine (Takeyasu et al., 1983) in experiments with *E. electricus* vesicles. The K_I and K_R values were calculated from the data in the inset by published procedures (Pasquale et al., 1983; Takeyasu et al., 1983) and were found to be 82 and 160 μM, respectively.

Discussion

The evidence that suberyldicholine and acetylcholine bind to a preexisting inhibitory site of the receptor has been given (Pasquale et al., 1983; Takeyasu et al., 1983). The results described here are consistent with this interpretation: the activating ligands investigated do not bind to the local anesthetic (procaine) site, which presumably exists in the opened receptor channel and which, when occupied, blocks the channel (Neher & Steinbach, 1978; Adams, 1981; Oswald et al., 1983; Heidmann & Changeux, 1984; Cox et al., 1984).

There are potentially important biological consequences of this regulatory site for acetylcholine. For a given concentration of acetylcholine the number of receptor channels that open depends not only on the dissociation constant of the sites responsible for channel opening (K_I, eq 1), which appears to be independent of V_m, but also on the dissociation constant of the regulatory site, which is dependent on V_m (Takeyasu

et al., 1983). The receptor-controlled transmission of signals between cells can, therefore, be varied and depends not only on the amount of acetylcholine released (Kandel & Schwartz, 1982) but also on the transmembrane voltage of the cell.

Registry No. Acetylcholine, 51-84-3; procaine, 59-46-1.

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