Chemical Kinetic Measurements of the Effect of *trans*- and *cis*-3,3′-Bis[(trimethylammonio)methyl]azobenzene Bromide on Acetylcholine Receptor Mediated Ion Translocation in *Electrophorus electricus* and *Torpedo californica*†

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ABSTRACT: A quench–flow technique was used to study the effect of *trans*- and *cis*-3,3′-bis[(trimethylammonio)methyl]azobenzene bromide (*trans*- and *cis*-Bis-Q), photoisomerizable ligands, on the acetylcholine receptor in vesicles prepared from the electric organ of *Electrophorus electricus* and of *Torpedo californica*. In *E. electricus*, two rate coefficients of the receptor-mediated translocation of $^{86}Rb^+$ induced with *trans*-Bis-Q were measured: J_A, the rate coefficient for ion flux, and α, the rate coefficient for receptor inactivation (desensitization). Both rate coefficients increase with increasing concentrations of Bis-Q up to 50 μM. At higher concentrations J_A decreases in a concentration-dependent manner while α remains unchanged. This effect was previously observed with suberyldicholine [Pasquale, E. B., Takeyasu, K., Udgaonkar, J., Cash, D. J., Severski, M. C., & Hess, G. P. (1983) *Biochemistry 22*, 5967–5973] and with acetylcholine [Takeyasu, K., Udgaonkar, J., & Hess, G. P. (1983) *Biochemistry 22*, 5973–5978] and was analyzed in terms of a minimum mechanism that accounts for the properties of activation, desensitization, and inhibition of the receptor. Two molecules of *trans*-Bis-Q must be bound for the channel to open, but at concentrations greater than 50 μM the population of open channels decreases because of the additional binding of one molecule of *trans*-Bis-Q to a regulatory inhibitory site, independent of the activating sites. *cis*-Bis-Q does not induce transmembrane ion flux, but it does inhibit the response of the receptor to acetylcholine and induces inactivation (desensitization) in the micromolar range. In *T. californica* no activity of the receptor could be observed in the presence of *trans*-Bis-Q, but *trans*-Bis-Q did inhibit acetylcholine-induced flux. The implication of the results to the use of Bis-Q as a photoactivatable ligand in kinetic studies is discussed.

Photosensitive compounds have been designed and used to investigate the kinetics of protein-mediated reactions in the millisecond time region by the generation of concentration jumps of substrates (Kaplan et al., 1978; Morad et al., 1983; Lester & Nerbonne, 1982; Nerbonne et al., 1984). Cis–trans isomerizations of azobenzene derivatives are particularly useful because they occur within microseconds with a high quantum yield and do not lead to the production of reactive intermediates (Zimmerman et al., 1958; Ross & Blanc, 1971; Duchek & Huebner, 1979). In 1971, an azobenzene derivative, 3,3′-bis[(trimethylammonio)methyl]azobenzene bromide (Bis-Q), was synthesized (Bartels et al., 1971). The cis and trans geometrical isomers differ in their physical and pharmacological properties. *trans*-Bis-Q was reported to be a potent agonist of the *Electrophorus electricus* acetylcholine receptor (Bartels et al., 1971), while the cis form is inactive. The compound is of interest because in principle it allows investigations of elementary steps in the channel-opening process, which may occur in the submillisecond time region (Lester et al., 1980; Sakmann et al., 1980; Land et al., 1984). It has been extensively used by Lester and co-workers [reviewed in Lester & Nerbonne (1982)] in electrophysiological experiments on isolated *E. electricus* electroplax. It has been found, however, that *cis*- and *trans*-Bis-Q undergo a slow interconversion at room temperature (Delcour et al., 1982; Huebner & Varnadove, 1982). This raises the possibility that the receptor becomes both activated and partially desensitized prior to the light-induced cis–trans isomerization. Techniques developed to separate and to characterize the two pure isomers (Delcour et al., 1982; Nerbonne et al., 1983) made it possible to investigate the effects of *cis*- and *trans*-Bis-Q on receptor-controlled transmembrane processes in well-characterized receptor-containing membrane vesicles (Sachs et al., 1982).

This paper provides quantitative information on the response of the acetylcholine receptor from *E. electricus* and from *Torpedo californica* to the cis and trans isomers of Bis-Q, obtained by studying the kinetics of the receptor-mediated cation flux in native vesicles with a quench–flow technique [for a review see Hess et al. (1983)]. A wide range of Bis-Q concentrations was used, and it was possible to determine (i) the dissociation constant of the receptor–ligand complexes prior to receptor desensitization, (ii) the channel-opening equilibrium constant, (iii) the rate coefficient for receptor inactivation (desensitization), and (iv) the dissociation constant for receptor inhibition. In *E. electricus*, the effects of *trans*-Bis-Q on receptor-controlled flux could be accounted for in terms of the model that accounts for the action of suberyldicholine and acetylcholine (Pasquale et al., 1983; Takeyasu et al., 1983; Shiono et al., 1984). The constants determined for the receptor-controlled ion translocation process initiated by *trans*-Bis-Q, which are reported here, indicate that elementary steps in the channel-opening process of the receptor of *E. electricus* and *T. californica* electroplax cannot be determined by using Bis-Q. In *E. electricus*, *cis*-Bis-Q is not an agonist, but at concentrations greater than 1 μM, it binds to the re-

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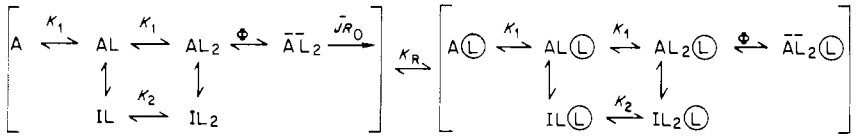
Table I: Minimum Mechanism and Pertinent Equations[a]

	eq no.	ref
		
$M_t/M_\infty = 1 - \exp[-[J_A[[1 - \exp(-\alpha t)]]/\alpha] + J_I t]]$	1	Cash & Hess, 1980
$\ln [(M_\infty - M_t)\ (M_\infty)^{-1}]_T / \ln [(M_\infty - M_t)\ (M_\infty)^{-1}]_{T=0} = -\alpha T$	2	Aoshima et al., 1981
$J_A = \bar{J}R_0L^2/[L^2\ (1 + \Phi) + K_1^2\Phi + 2K_1\Phi L][1 + L/K_R]$	3a	Cash & Hess, 1980; Hess et al., 1981
$(\bar{J}R_0/J_A - 1)^{1/2} = \Phi^{1/2} + \Phi^{1/2}K_1/L$	3b	Hess et al., 1981
$1/J_A = 1/\bar{J}_A + (L/K_R)\ (1/\bar{J}_A)$	3c	Pasquale et al., 1983

[a] K_1 and K_2 are the intrinsic dissociation constants of the complexes involving the active (A) or inactive (I) forms of the receptor, respectively. Φ is the channel-closing equilibrium constant ($\Phi = [AL_2]/[\bar{A}\bar{L}_2]$). $\bar{J}$ is the specific reaction rate for the cation translocation process (Hess et al., 1981) and is directly related to the conductance of the channel (Hess et al., 1984). R_0 indicates the moles of receptor per liter of internal volume of the vesicles, and K_R is the dissociation constant for the regulatory site of the receptor. Equation 1 describes the flux curves in terms of three rate constants: J_A, the rate coefficient for ion flux mediated by the active receptor before the onset of desensitization, α, the rate coefficient for inactivation (desensitization), and J_I, the rate coefficient for the ion flux mediated by an equilibrium mixture of active and inactive (desensitized) receptor forms; J_A and J_I are directly related to $\bar{J}R_0$ (see eq 3a, for instance). The ligand binding to the regulatory (inhibitory) site is identified by a circled "L". The rate of desensitization, α, can be calculated directly according to eq 2, where M_∞ is the final $^{86}Rb^+$ content of the vesicles, M_t is the radioactivity inside the vesicles at the end of the time for which ion flux is allowed to proceed, and T refers to the time of preincubation of the vesicles with ligand before $^{86}Rb^+$ flux was induced (Aoshima et al., 1981). The concentration dependence of J_A is given by eq 3a. Equation 3b represents the linearized form of eq 3a at concentrations where no inhibition by the agonist binding to the regulatory site is observed. Equation 3c corresponds to the concentration dependence of the agonist inhibition at concentrations where the activating sites are saturated. $\bar{J}_A$ is the calculated valuc of J_A that would be obtained in the absence of receptor inhibition by the ligand (see the text).

ceptor and induces desensitization. In contrast to the effect of *trans*-Bis-Q on the *E. electricus* receptor, the compound does not induce any detectable ion flux in *T. californica* vesicle preparations.

Experimental Procedures

Receptor-rich vesicles were prepared from the electric organ of *E. electricus* (World Wide Scientific Animals, Apopka, FL) as described (Kasai & Changeux, 1971; Fu et al., 1977) and were equilibrated in eel Ringer's solution (169 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 3 mM $CaCl_2$, 1.5 mM sodium phosphate, pH 7.0). In experiments where a transmembrane voltage was established, the vesicles were prepared in 90 mM NaCl, 1 mM $CaCl_2$, and 1.5 mM sodium phosphate, pH 7.0, and then mixed in the quench–flow machine with an isotonic arginine solution (87 mM arginine hydrochloride, 1 mM $CaCl_2$, 1.5 mM sodium phosphate, pH 7.0). The *T. californica* vesicles were prepared according to published procedures (Sobel et al., 1977; Delgeane & McNamee, 1980) and equilibrated with 90 mM NaCl and 1.5 mM sodium phosphate, pH 7.4. Acetylcholine bromide was obtained from Sigma, *d*-tubocurarine chloride from Calbiochem, and $^{86}RbCl$ from New England Nuclear. All other chemicals were reagent grade. *trans*-Bis-Q was purchased from Molecular Probes (Junction City, OR). Pure *cis*-Bis-Q was obtained by high-performance liquid chromatography on a μBondapak C_{18} column (Waters Associates) from a solution of *trans*-Bis-Q (10 mg/mL) photolyzed by a nitrogen laser (λ = 337 nm; energy = 6.5 mJ per 10-ns pulse). The isomers were separated as described (Delcour et al., 1982) and then stored, in 100 mM NaCl and 10% ethanol, at −20 °C. Before each experiment, solutions of *cis*-Bis-Q were prepared in eel Ringer's solution by evaporation of the ethanol and dilution with the appropriate buffer, and the purity of the cis and trans samples was checked by absorbance spectroscopy (Delcour et al., 1982). The ion-flux measurements were made at 1 °C, where the thermal stability of the isomers was assured for the duration of the experiment, and in the dark with Sylvania red striplight F40R Lifeline as a darkroom safelight.

Acetylcholine receptor mediated $^{86}Rb^+$ translocation, measured before and during inactivation, was followed in the millisecond to second time region by using a quench–flow technique adapted for use with membrane vesicles (Hess et al., 1979; Cash & Hess, 1981). Flux curves were generated in the presence of Bis-Q or of acetylcholine by using the continuous and pulse modes. The rate coefficients for ion flux, J_A, and for receptor inactivation, α, were obtained by fitting the experimental points to eq 1 (Table I) with a nonlinear least-squares (Bevington, 1969) computer program. As described previously (Aoshima et al., 1981), the value of α was also obtained from direct measurement of the flux activity remaining after exposing the receptor to the ligand for various periods of time prior to measuring influx rates. The control experiments and the experimental details of chemical kinetic measurements made in the presence of a transmembrane voltage have been described (Takeyasu et al., 1983). In each experiment, measurements with the same concentration of acetylcholine or of *trans*-Bis-Q were included. This allows one to normalize the J_A values obtained with each preparation of vesicles. Detailed procedures for making quench–flow measurements and for evaluation of the constants have been published (Cash & Hess, 1981; Hess et al., 1983).

The inactivation rate coefficient, α, was found to be the same within experimental error with all membrane preparations used previously (Cash & Hess, 1980; Cash et al., 1981; Aoshima et al., 1981; Pasquale et al., 1983; Takeyasu et al., 1983). In the experiments described here, α was less by a factor of 2 than the value observed previously. This was attributed to modifications of the dissection of the electroplax during the preparation of the membrane vesicles.

Results

The rate coefficient for ion flux mediated by the receptor prior to desensitization (J_A) was measured, by determining the $^{86}Rb^+$ content of the vesicles at various times, as a function of the *trans*-Bis-Q concentration. The values of J_A, and the final $^{86}Rb^+$ concentration in the vesicles after a 3-s influx, increase with increasing concentrations of *trans*-Bis-Q up to 50 μM. However, in the presence of concentrations greater than 50 μM, the ion-flux rate coefficient decreases with increasing concentrations of *trans*-Bis-Q. This behavior gives rise to the bell-shaped curve of the concentration dependence of J_A shown in Figure 1a. For purposes of comparison, the concentration dependence of J_A observed in the presence of

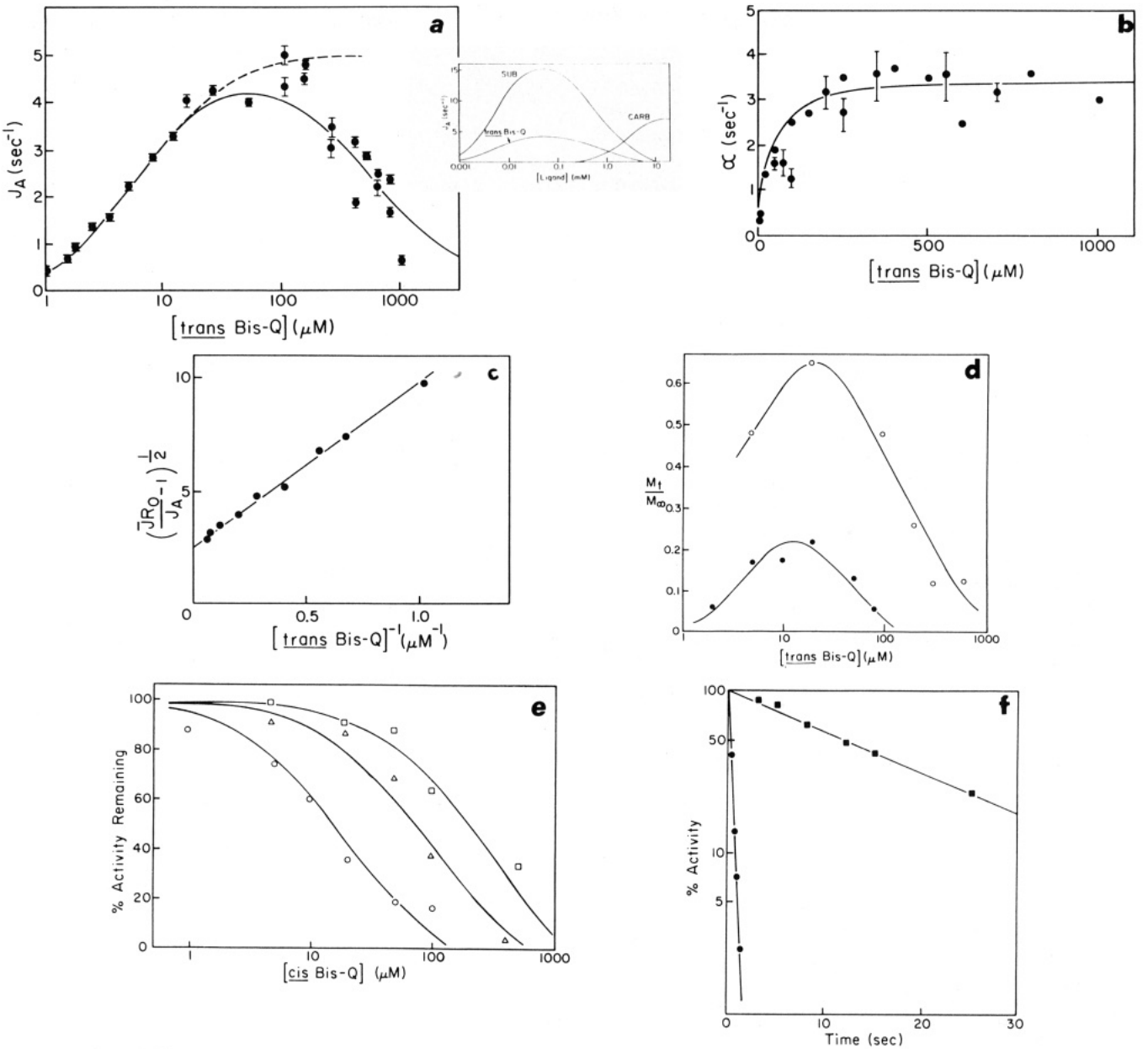


FIGURE 1: Quench–flow measurements of receptor-controlled transmembrane ion flux in the presence of *trans*-Bis-Q or *cis*-Bis-Q in *E. electricus* membrane vesicles, pH 7.0, 1 °C. (a) Dependence of J_A, the rate coefficient for ion flux prior to receptor desensitization, on *trans*-Bis-Q concentrations. The J_A values were determined from influx measurements by using eq 1 (Table I). The solid line was computed by using eq 3a with the following values of constants: $K_I = 3$ μM, $\Phi = 6.3$, and $K_R = 530$ μM. (a, inset) For purposes of comparison the concentration dependence of J_A in the presence of suberyldicholine (Pasquale et al., 1983) and that in the presence of carbamoylcholine (Cash & Hess, 1980) are also shown. (b) Concentration dependence of α, the rate coefficient for inactivation (desensitization). The points with error bars were obtained from direct measurements, as described under Experimental Procedures. The other points were obtained from fitting the experimental influx curves to eq 1. A solid curve is drawn through the experimental points to illustrate that the values of α increase with increasing *trans*-Bis-Q concentrations and then become concentration-independent. (c) Concentration dependence of J_A plotted according to eq 3b, using the values of J_A obtained at concentrations of *trans*-Bis-Q between 1 and 15 μM. The following values were determined: $K_I = 3$ μM and $\Phi = 6$. (d) Voltage dependence of $^{86}Rb^+$ flux induced by *trans*-Bis-Q. The ordinate is the ratio of the $^{86}Rb^+$ content of the vesicles after a 3-s influx (M_t) to the maximum content (M_∞). Symbols: (●) $V_m = -45$ mV; (○) $V_m = 0$ mV. (e) Inhibition of acetylcholine-induced flux by *cis*-Bis-Q. Percent activity remaining is defined as $[\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{+BQ}/\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{-BQ}] \times 100$, where +BQ or −BQ indicates in the presence or absence of *cis*-Bis-Q. M_∞ is the maximal $^{86}Rb^+$ content and M_t the radioactivity in the vesicles during influx. Acetylcholine concentrations are as follows: (○) 0.1 mM; (△) 0.8 mM; (□) 4 mM. The solid lines are drawn to help the reader to differentiate between experiments done at different acetylcholine concentrations. (f) Direct measurement of the rate of inactivation (desensitization). Vesicles were preincubated, for the times shown on the abscissa, with 100 μM *cis*-Bis-Q (■) or 5 mM acetylcholine (●). Then, the solution was mixed with 5 mM acetylcholine, and the influx was allowed to proceed for 700 ms, before quenching with *d*-tubocurarine. The data are plotted according to eq 2 (Table I) with percent activity defined as $[\ln [M_\infty - M_t)(M_\infty)^{-1}]_T/\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{T=0}] \times 100$ (Aoshima et al., 1981).

suberyldicholine or carbamoylcholine is also shown (Figure 1a, inset). This concentration-dependent inhibition is, however, not observed for the rate coefficient for inactivation (desensitization), α (Figure 1b), which continues to increase at concentrations greater than 50 μM to reach a plateau at 200 μM *trans*-Bis-Q. A decrease in the J_A values as the ligand concentration is increased beyond a certain concentration without a concomitant decrease in α was first reported for suberyldicholine (Pasquale et al., 1983) and led to an extension of a previous model (Cash et al., 1981). The extended model (see Table I) includes a regulatory site that is present on all forms of the receptor. Binding to that site leads to a decrease

in the concentration of the open-channel form, $\bar{A}\bar{L}_2$, at high agonist concentrations but does not affect the rate of desensitization. The expression for the concentration dependence of J_A, according to the scheme shown in Table I, is given by eq 3a (Pasquale et al., 1983). At low *trans*-Bis-Q concentrations, eq 3a reduces to a form that can be rearranged to give eq 3b (Hess et al., 1981). Experimental data obtained with concentrations of 1–15 μM *trans*-Bis-Q are plotted in Figure 1c according to eq 3b (Hess et al., 1981). All the values of J_A were normalized to a vesicle preparation of known R_0 value, R_0 being the moles of receptor sites per liter of internal vesicle volume, and it is assumed that J_A is directly proportional to R_0 (Hess et al., 1981). The specific reaction rate of receptor-controlled ion translocation, $\bar{J}$ (Hess et al., 1981), can be calculated from the conductance of the receptor channel (Hess et al., 1984), which was determined by the single-channel measurement technique (Neher & Sakmann, 1976). The single-channel conductance was found to be ligand-independent in *E. electricus* electroplax cells (Hess et al., 1984), in agreement with single-channel current measurements using rat muscle cells (Gardner et al., 1984). Accordingly, a value of 37 s^{-1} for $\bar{J}R_0$ was used for the evaluation of K_1 and Φ using eq 3b (Hess et al., 1981). The value of the channel-opening equilibrium constant Φ^{-1} of 0.15, obtained from the plot in Figure 1c, indicates that in the absence of a transmembrane voltage the receptor saturated with *trans*-Bis-Q remains mainly in a form in which the channel is closed. A value for K_1, the dissociation constant of *trans*-Bis-Q and the receptor prior to its desensitization, of 3 μM was obtained. The values of K_R were obtained from the best fit of the data according to a linearized form (eq 3c) of eq 3a that applies at high concentrations of *trans*-Bis-Q (data not shown). A K_R value of 530 ± 55 μM was calculated. The solid line in Figure 1a was computed according to eq 3a by using the above parameters. The dashed line is the theoretical curve of the concentration dependence of J_A that would be obtained if the inhibitory effect did not occur. The effect of a voltage of −45 mV across the membrane of *E. electricus* vesicles was studied with various concentrations of *trans*-Bis-Q. The plot of the flux amplitude vs. the concentration of *trans*-Bis-Q (Figure 1d) demonstrates a shift of the bell-shaped curve to lower *trans*-Bis-Q concentrations when the transmembrane voltage was changed from 0 to −45 mV. It was estimated that the *trans*-Bis-Q concentration at which half the maximum inhibition occurs (right-hand part of the curves) is decreased by a factor of 3 as the transmembrane voltage is changed from 0 to −45 mV.

The response elicited by pure *cis*-Bis-Q at concentrations of 1 μM to 1 mM was studied by 1- and 20-s flux measurements. No significant flux activity was observed. However, Figure 1e illustrates that *cis*-Bis-Q binds to the acetylcholine receptor and exerts an inhibitory effect on the acetylcholine-induced $^{86}Rb^+$ flux. The observation that the inhibition is relieved by increasing concentrations of acetylcholine suggests that *cis*-Bis-Q competes with acetylcholine for receptor sites that control channel opening. *cis*-Bis-Q is also capable of inducing desensitization of the receptor (Figure 1f). Vesicles were preincubated with 100 μM *cis*-Bis-Q for times ranging from 3 to 25 s, and the activity of the receptor was then probed with a 1-s influx in the presence of 5 mM acetylcholine. The ion translocation rate of the receptor after exposure to *cis*-Bis-Q follows an exponential decay, with a half-life of 12 s.

trans-Bis-Q does not induce receptor-controlled ion flux in *T. californica* vesicle preparations at concentrations between 100 nM and 1 mM, as measured by the amplitude of a 30-s flux. To determine if *trans*-Bis-Q binds to the receptor, the

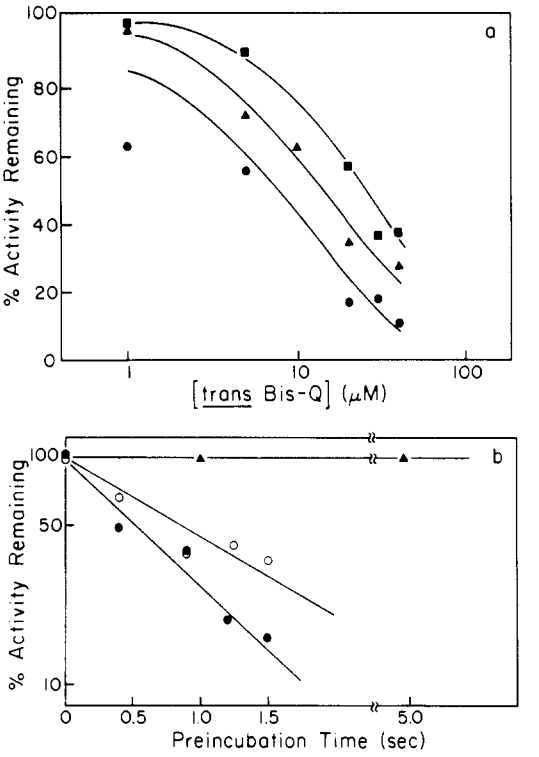


FIGURE 2: Quench–flow measurements of receptor-controlled transmembrane ion flux in *T. californica* vesicles, pH 7.4, 1 °C. (a) Inhibition of acetylcholine-induced flux by *trans*-Bis-Q. Percent activity remaining is defined as $[\ln [M_\infty - M_t)(M_\infty)^{-1}]_{+BQ}/\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{-BQ}] \times 100$, where +BQ or −BQ indicates the presence or absence of *trans*-Bis-Q, M_∞ is the $^{86}Rb^+$ content in the vesicles after 25 ms in the presence of 50 (●, ■) or 80 μM (▲) acetylcholine, and M_t is the $^{86}Rb^+$ content after 13 ms in the presence of both *trans*-Bis-Q and 50 (●), 80 (▲), or 100 μM (■) acetylcholine. The solid lines are drawn to help the reader to differentiate between experiments done at different acetylcholine concentrations. (b) Direct measurement of the rate of desensitization. Percent activity remaining is defined as $[\ln [(M_\infty - M_t)(M_\infty)^{-1}]_T/\ln [(M_\infty - M_t)(M_\infty)^{-1}]_{T=0}] \times 100$, where T is the preincubation time, M_∞ is the $^{86}Rb^+$ content in the vesicles after 25 ms in the presence of 80 μM acetylcholine, and M_t is the $^{86}Rb^+$ content after 13 ms. The concentrations are as follows: (▲) 5 μM *trans*-Bis-Q; (●) 80 μM acetylcholine (α = 1.4 s^{-1}); (○) 80 μM acetylcholine and 5 μM *trans*-Bis-Q (α = 0.8 s^{-1}).

$^{86}Rb^+$ content of the vesicles was measured after exposure for 13 ms to three concentrations of acetylcholine and various concentrations of *trans*-Bis-Q. The results (Figure 2a) show that *trans*-Bis-Q inhibits acetylcholine-induced ion flux in *T. californica* membrane vesicles and that the inhibition caused by *trans*-Bis-Q in the micromolar range can be overcome by increasing concentrations of acetylcholine. This suggests that *trans*-Bis-Q competes with acetylcholine for the receptor sites that control channel opening without causing a measurable ion translocation. The effect of *trans*-Bis-Q on desensitization was also measured directly (Figure 2b). A 5 μM concentration of *trans*-Bis-Q does not desensitize the receptor over a 5-s period, but *trans*-Bis-Q reduces the rate for desensitization induced by 80 μM acetylcholine by about half.

Discussion

The chemical kinetic approach to measurements of transmembrane processes using fast-reaction techniques [for a review see Hess et al. (1983)] has allowed one to determine, in separate measurements, the ion translocation rates of the receptor, both prior to desensitization and after the desensitization process has gone to completion, and the desensitization rates (Cash & Hess, 1980; Aoshima et al., 1981). Consequently, it becomes possible to determine whether receptor

inhibition is due to effects on J_A (Figure 1a) or α (Figure 1b) or both (Pasquale et al., 1983; Takeyasu et al., 1983). The data in Figure 1a,d demonstrate that inhibition involves a voltage-dependent noncompetitive site, which is likely to be the specific regulatory site for suberyldicholine and acetylcholine (Pasquale et al., 1983; Takeyasu et al., 1983). Inhibition by agonists has also been observed in electrophysiological experiments with suberyldicholine (Neher & Steinbach, 1978), decamethonium (Adams & Sakmann, 1978), and both isomers of Bis-Q (Nerbonne et al., 1983; Chabala et al., 1984).

It was shown that, under conditions where the receptor sites that control the formation of transmembrane channels were saturated with ligand, the maximum flux rate coefficient, $J_{A(max)(obsd)}$, varies with the ligand investigated (Hess et al., 1981; Pasquale et al., 1983). $J_{A(max)(obsd)}$ for suberyldicholine is about twice that for carbamoylcholine (Figure 1a, inset) and about 3 times that for *trans*-Bis-Q. We have suggested (Hess et al., 1981) that the reason for these differences in $J_{A(max)(obsd)}$ values lies in the equilibrium, characterized by the equilibrium constant Φ, between the two receptor–ligand complexes corresponding to the closed-channel (AL_2) and open-channel ($\bar{A}\bar{L}_2$) forms (see Table I). A consequence of the suggested relationship between Φ and $J_{A(max)(obsd)}$ is that once the channel is open, the number of ions that can pass through it per unit time is independent of the ligand used to induce channel opening. This is observed in single-channel current measurements with three different channel-activating ligands tested in *E. electricus* electroplax cells (Hess et al., 1984) and with ten different channel-activating ligands examined in rat muscle cells (Gardner et al., 1984). Differences in the channel-opening equilibrium constant can also account for the observations that *cis*-Bis-Q competes with acetylcholine for the receptor sites that control channel opening but does not induce measurable ion translocation in receptor-containing vesicles. The difference in the effects observed in experiments with *E. electricus* and *T. californica* vesicles using *trans*-Bis-Q may again be ascribed to differences in the value of Φ, because *trans*-Bis-Q binds to the sites that control channel activation in both types of vesicle but does not induce measurable ion translocation in *T. californica* preparations. Differences in the value of Φ can also account for the variable effect of *trans*-Bis-Q on the nicotinic receptors of various other organisms (Lester & Nerbonne, 1982). Differences in the effect of carbamoylcholine on the rate of desensitization of the *E. electricus* and *T. californica* receptors have been reported (Walker et al., 1981; Aoshima, 1984).

Kinetic investigations of elementary steps in the formation of receptor–ligand complexes and of transmembrane channels are likely to require measurements with a microsecond time resolution. Preequilibration of an inactive ligand with the receptor followed by photoconversion in the millisecond time region to an active ligand is a promising approach for obtaining such time resolution. The results presented here demonstrate several drawbacks in the use of Bis-Q in such investigations. (1) The results show that *cis*-Bis-Q is not an inactive precursor of *trans*-Bis-Q, because it competes with acetylcholine for the receptor sites that control the formation of transmembrane channels and it causes the receptor to be desensitized. (2) The rate constants for the opening and closing of transmembrane channels can be obtained, provided a sufficiently wide range of ligand concentrations can be employed in the kinetic measurements. At concentrations of ligand at which the formation of receptor–ligand complexes is fast compared to the formation of the open channels, the determination of the rate constant for channel opening and closing is vastly simplified (Hess et al., 1985). Given the values of the bimolecular rate constants for the formation of small molecule–protein complexes, 10^6–10^9 M^{-1} s^{-1} (Hammes, 1982), and estimates for the rate constants for channel opening (Sakmann et al., 1980; Lester et al., 1980; Land et al., 1984), ligand concentrations of at least 100 μM are likely to be required in order to attain a preequilibrium between the ligand and the closed-channel forms of the receptor. At these concentrations *trans*-Bis-Q becomes an inhibitor of the receptor (Figure 1a). In order to obtain sufficient precision in current measurements with electroplax cells, transmembrane voltages of −80 to −120 mV are required [see, for instance, Hess et al. (1984)]. The experiments on the voltage dependence of receptor inhibition by *trans*-Bis-Q (Figure 1a,d), and previous measurements with suberyldicholine and acetylcholine (Pasquale et al., 1983; Takeyasu et al., 1983, 1986), indicate that *trans*-Bis-Q will become an increasingly better inhibitor as the transmembrane voltage is changed to more negative values.

For the reasons given, we have synthesized other photoactivatable acetylcholine receptor ligands, which may be useful in kinetic investigations of elementary steps in receptor-controlled transmembrane processes (Walker et al., 1986).

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Registry No. *trans*-Bis-Q, 32752-27-5; *cis*-Bis-Q, 69655-54-5; Rb, 7440-17-7; acetylcholine, 51-84-3.

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