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Kinetics of Interaction of Batrachotoxin and Tetrodotoxin on Rat Diaphragm Muscle

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SUMMARY

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The interaction between batrachotoxin (BTX) and tetrodotoxin (TTX) and local anesthetics (procaine and lidocaine) was examined on rat diaphragm muscle at 37°. At low concentrations TTX shifted the curve for BTX-induced membrane depolarization to the right, and at higher concentrations it depressed the maximal membrane depolarization induced by BTX. A plot of log (dose ratio − 1) vs. log TTX concentration gave a straight line with a slope of 1.31. The estimated dissociation constant K_B of TTX was 30.2 nM. Both procaine and lidocaine inhibited BTX-induced membrane depolarization when applied either before or during exposure to BTX; a form of noncompetitive antagonism was disclosed. Although TTX reversed the depolarization induced by BTX, the persistent and irreversible action of BTX became apparent after TTX had been washed from the bath. When procaine and lidocaine were applied in a similar manner the repolarization of the muscle membrane was incomplete; upon washing in drug-free physiological solution, the membrane potential and frequency of spontaneous miniature end plate potentials returned toward control values. During the protective action of procaine and lidocaine, sodium activation associated with an action potential was only slightly reduced. The results suggest that TTX and BTX react with different sites along the same sodium channel, but that procaine and lidocaine probably interfere with the binding of BTX to its receptor site.

INTRODUCTION

Batrachotoxin, a steroidal alkaloid obtained from the skin secretions of the Colombian arrow poison frog, *Phyllobates aurotaenia*, irreversibly increases the resting sodium permeability in electrogenic membranes of various animal species in which the membrane current is mainly carried by sodium ions (1, 2). This action of BTX[2] is reversibly inhibited by tetrodotoxin, and the depolarizing effect is prevented by lowering the external sodium concentration (3, 4). It is likely that the site of action of BTX is different from that of TTX. In support of this hypothesis, it has been shown that BTX has no effect on the binding of radioactive TTX to rabbit vagus nerves (5),

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[2] The abbreviations used are: BTX, batrachotoxin; TTX, tetrodotoxin; MEPP, miniature end plate potential.

and TTX is unable to exert its antagonistic effect on BTX-induced depolarization in chronically denervated rat skeletal muscle (6). Additionally, the depolarizing action of BTX on lobster giant axon is antagonized by prior treatment with sulfhydryl reducing agents, which do not alter the action of TTX on the action potential-generating mechanism of the preparation (7).

The purpose of the present study was to investigate quantitatively the interaction between TTX and BTX in an innervated skeletal muscle preparation. Since procaine, but not lidocaine, halted or prevented the membrane depolarization produced by BTX in squid giant axon (8), the interaction between these local anesthetics and BTX on the rat diaphragm muscle was also studied.

MATERIALS AND METHODS

All experiments were carried out *in vitro* on isolated left diaphragm muscles from young adult male rats (150–200 g) of the Wistar strain. The techniques for preparation of the muscle and for stimulation and recording are described in detail elsewhere (3, 9, 10).

The effect of BTX alone or in combination with TTX was determined on the isolated rat diaphragm muscles, and a semilogarithmic plot was constructed. Each point on the curve represents the average membrane potential of 40–97 individual fibers from at least three muscles recorded between 50 and 60 min after addition of the toxin. To test the type of antagonism between BTX and TTX, the influence of TTX on the maximum response to BTX was assessed. Assuming that the antagonism was of a noncompetitive nature, it should be possible to depress the maximum response to BTX. The dose ratio test of competitive antagonism as formulated by Arunlakshana and Schild (11) is not discriminatory in this case, since it can be shown that a similar relationship applies to the interaction between an irreversible agonist and a noncompetitive, reversible antagonist (see RESULTS).

An apparent dissociation constant K_B of TTX was estimated using the Schild equation:

$$\log (DR - 1) = \log [TTX] - \log K_B$$

where DR is the ratio of equiactive concentrations of BTX in the presence and absence of TTX. When log (DR − 1) is plotted against log TTX concentration, a straight-line relationship with a slope of 1 is consistent both with competitive antagonism and with the interaction of an irreversible agonist and a noncompetitive, reversible antagonist. An estimate of K_B was obtained from the intercept of the regression line on the concentration axis when log (DR − 1) was zero.

The basic physiological solution had the following composition: NaCl, 135.0 mM; KCl, 5.0 mM; $MgCl_2$, 1.0 mM; $CaCl_2$, 2.0 mM; $NaHCO_3$, 15.0 mM; Na_2HPO_4, 1.0 mM; glucose, 11.0 mM. A mixture of 95% O_2 and 5% CO_2 was passed through the bathing solutions, and temperature was kept constant at 37° throughout the experiments. The pH of the solutions was 7.1–7.2. BTX was added to the physiological solution from a refrigerated stock solution (0.19 mM) prepared in absolute ethanol. A stock solution of TTX (Sankyo Company, Ltd., Tokyo) (0.31 mM) in deionized, redistilled water was made and diluted as necessary to make the TTX physiological solution. Local anesthetic (procaine, lidocaine) solutions were prepared freshly for each experiment.

RESULTS

Interaction between BTX and TTX on Membrane Potential and Miniature End Plate Potential Frequency

Exposure of the innervated rat diaphragm muscle to various concentrations of BTX resulted in an increase in spontaneous MEPP frequency with a concomitant depolarization of the surface fibers. The typical effects of BTX (10 nM) are shown in Fig. 1A. After a latency of 20 min the toxin elicited an increase in spontaneous MEPP frequency from about 5 sec^{-1} to more than 500 sec^{-1}, followed by periodic oscillations between 200 and 500 sec^{-1}, and then could no longer be observed. The increase in MEPP frequency occurred simultaneoulsy with a gradual decline of the postsynaptic membrane po-

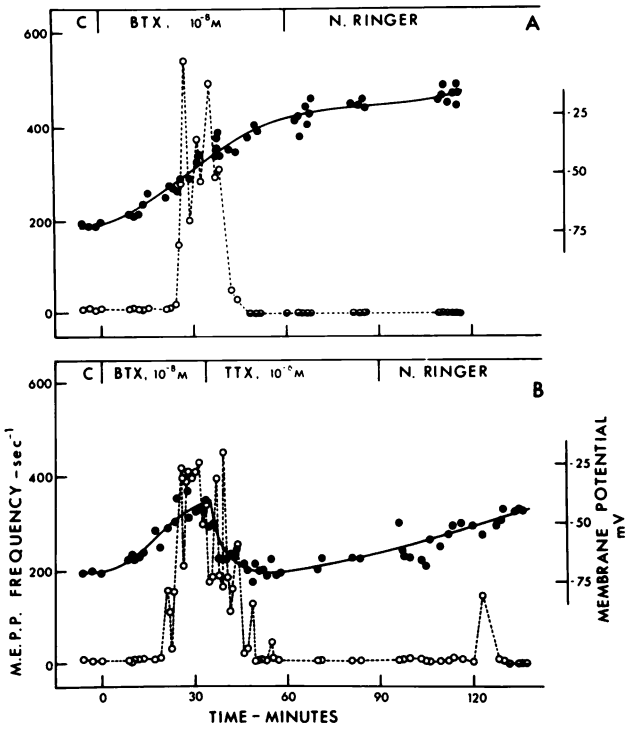

FIG. 1. *Effect of BTX on membrane potential and MEPP frequency in rat diaphragm muscle*

A. The muscle was exposed to BTX for 60 min at 37°, during which time the membrane potential (●——●) and MEPP frequency (O- - -O) were determined. After 60 min the muscle was washed with physiological solution (N. RINGER). ⊖, no detectable spontaneous MEPPs. B. After a 30-min exposure to BTX, the muscle was exposed to TTX for 60 min and then washed with physiological solution.

tential from −75 mV to about −25 mV in 1 hr. The removal of the toxin from the bath by washing with physiological solution did not restore the spontaneous MEPPs, and the membrane potential continued to decrease to −15 mV.

When a muscle was exposed to BTX (10 nM) for 30 min (Fig. 1B) and then washed with physiological solution containing TTX (1.0 μM), the membrane potential and MEPP frequency were restored to their control values within 20 min. The persistent action of BTX was subsequently observed when the preparation was rinsed with toxin-free physiological solution; during this period transmitter release fluctuated and then could no longer be observed, and the muscle membrane potential progressively decreased to −40 mV within 40 min.

To study the protective action of TTX against BTX-induced membrane depolarization, muscles were first incubated with different concentrations of TTX (0.1–3.0 μM) for 30 min, and then various concentrations of BTX were applied in combination with the same concentrations of TTX for 60 min. The membrane potentials of the muscles were continually recorded from individual surface fibers, and the mean membrane potential at the end of BTX plus TTX treatment was used to construct a semilogarithmic plot for BTX-induced depolarization in the absence and presence of increasing concentrations of TTX. Figure 2 shows that a progressive increase in the concentration of TTX caused a parallel shift of the curve for BTX-induced depolarization to the right. There is also some indication that the maximum response to BTX is depressed at higher concentrations of TTX. The form of these experimental curves is consistent with the theoretical curves shown later (see Fig. 5) and favors interpretation in terms of a noncompetitive type of antagonism (see *Theoretical Considerations* below).

The apparent dissociation constant K_B of

TTX acting on rat diaphragm muscle at 37° was estimated (Fig. 3) from the same results and is 30.2 nM.

Theoretical Considerations: Quantitative Description of Dose-Response Curve of BTX (Irreversible Agonist) and Influence of TTX (Noncompetitive Antagonist)

Definition of symbols and assumptions. E is the steady-state membrane potential in the presence of concentration A of agonist (BTX); E_0, the resting membrane potential in the absence of agonist; E_{Na}, the equilibrium potential for sodium ions; E_m, the steady-state membrane potential at maximum depolarization by saturating concentrations of agonist; R, the resting membrane resistance; g, the mean conductance of a single sodium channel opened by agonist; N, the number of sodium channels opened in the presence of agonist; N_m, the maximum number of sodium channels opened by saturating concentrations of agonist; N_B, the number of sodium channels blocked in the presence of antagonist; and K_B, the dissociation constant of antagonist.

The following assumptions are made. (a) The rate of binding of the irreversible agonist is directly proportional to its concentration A [i.e., $N = N_m(1 - e^{-kAt})$, where k is the rate constant of association, and t is a fixed incubation time]. (b) The equivalent electrical circuit shown in Fig. 4A is an adequate description of the steady-state properties of the membrane, and the parameters R and E_{Na} are not materially altered as a consequence of the action of BTX. In reality it is clear that any substantial increase in sodium permeability will result in a net inward movement of sodium ions and a consequent displacement of E_{Na}. However, resting sodium permeability is extremely low, and large depolarizations may therefore result from small conductance increases (13). It is also known that the resting membrane potential of BTX-treated muscle recovers fully on substitution of a sodium-free bathing solution (3). These observations lend support to the assumption that E_{Na} remains essentially constant, and consequently that there is no secondary redistribution of other ions. (c) TTX binds reversibly to block the sodium

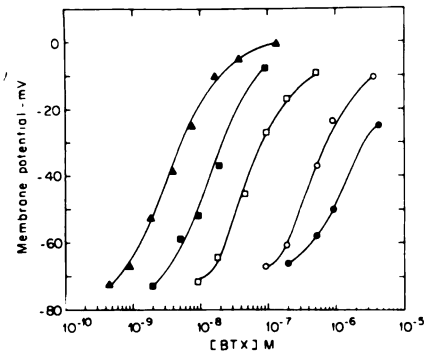

FIG. 2. *Effect of TTX on membrane depolarization induced by BTX in surface fibers of rat diaphragm muscles*

▲, TTX-free; ■, TTX, 0.1 μM; □, TTX, 0.3 μM; ○, TTX, 1.0 μM; ●, TTX, 3.0 μM. The standard errors of the mean are too small to be shown. The muscles were exposed to different concentrations of TTX for 30 min, and then varying concentrations of BTX were applied in combination with the same concentrations of TTX. The membrane potentials recorded at 55—60 min of exposure to BTX plus TTX were used to construct the curves.

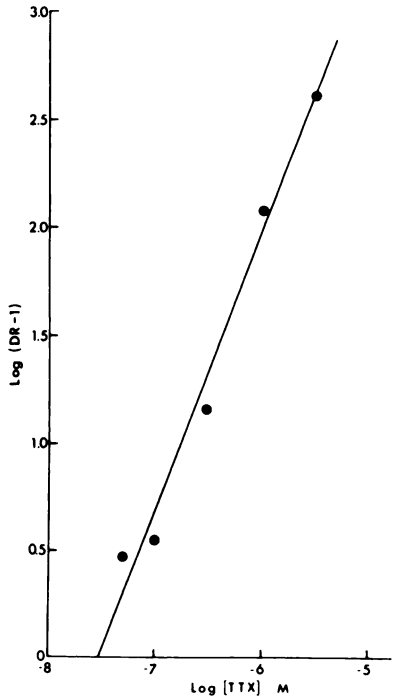

FIG. 3. *Determination of dissociation constant K_B of TTX, using BTX as agonist, from plot of log (DR-1) against log [TTX]*

The data for this figure were taken from Fig. 2. Slope = 1.3 ± 0.06 (mean ± SE).

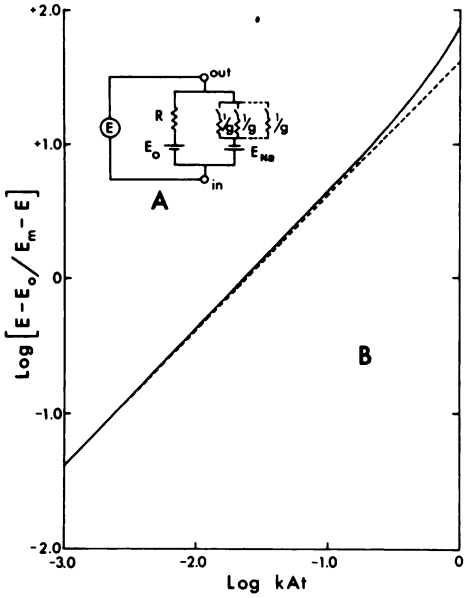

FIG. 4. *Equivalent electrical circuit (A) of muscle membrane and theoretical Hill plot (B)*

The data were obtained from Eq. 3. E is the steady-state membrane potential in the presence of concentration A of the agonist BTX; E_0, resting membrane potential in the absence of agonist; E_{Na}, equilibrium potential for Na^+; E_m, steady-state membrane potential at maximum depolarization by saturating concentrations of agonist; R, resting membrane resistance; g, mean conductance of a single sodium channel opened in the presence of agonist. The value 40 is assigned to $N_m gR$ and is justified by the finding that resting resistance is 40 times greater than active resistance for the membrane of squid giant axon (12). ---, extrapolation of the linear portion of the theoretical curve (slope = 1.0). Further description of the equations and assumptions is given in the text.

channel at a site other than the BTX binding site, and there is no interaction between these sites.

Concentration dependence of membrane depolarization produced by BTX. On the basis of the equivalent electrical circuit shown in Fig. 4A, the following relationship (14) can be applied when N sodium channels are opened by BTX:

$$\frac{E_0 - E}{E_0 - E_{Na}} = \frac{NgR}{1 + NgR} \quad (1)$$

Substituting for N,

$$\frac{E_0 - E}{E_0 - E_{Na}} = \frac{N_m gR(1 - e^{-kAt})}{1 + N_m gR(1 - e^{-kAt})} \quad (2)$$

It is clear from Eq. 1 that the change in membrane potential is 75% of maximal when the activated sodium conductance reaches only 3 times resting membrane conductance. This is a small fraction of the maximal sodium activation (12), and thus fractional saturation of BTX sites is low throughout the dose-response curve when potential change is the measured response.

If $E = E_m$ when $N = N_m$, it follows from Eqs. 1 and 2 that

$$\frac{E - E_0}{E_m - E} = (e^{kAt} - 1)(1 + N_m gR \quad (3)$$

According to Eq. 2, depolarization $(E_0 - E)$ is a hyperbolic function of BTX concentration for small values of the concentration term kAt. Equation 3 shows that the experimental plot of log $\{(E - E_0)/(E_m - E)\}$ vs. log BTX concentration, i.e., a Hill plot, can approximate a straight line of unity slope over a substantial range (Fig. 4B). These relationships may therefore appear to be similar to Michaelis-Menten kinetics although their derivation is quite different.

Influence of TTX on dose-response relationship of BTX. If the number of sodium channels blocked by a given concentration of TTX is N_B, and if TTX acts at a different site from BTX, it follows that in the presence of TTX Eq. 2 is modified to

$$\frac{E_0 - E}{E_0 - E_{Na}} = \frac{N_m gR(1 - e^{-kAt})}{(1 - N_B/N_m)^{-1} + N_m gR(1 - e^{-kAt})} \quad (4)$$

Using Eq. 4, theoretical log dose-response curves were plotted for various values of N_B/N_m (Fig. 5). In this particular instance, when $N_m gR$ is made equal to 40, the maximum response is depressed by only 20% after blockade of 90% of the sodium channels.

Another result which follows from Eqs. 2 and 4 concerns the ratio DR of the concentrations A_1 and A_2 of BTX that produce equal depolarization $(E_0 - E)$ in the presence and absence, respectively, of a given concentration of TTX. It can readily be shown that

$$\frac{1 - e^{-kA_1t}}{1 - e^{-kA_2t}} = \left(1 - \frac{N_B}{N_m}\right) - 1 \quad (5)$$

Applying mass action law to TTX binding gives

$$\frac{N_B}{N_m} = \frac{[TTX]}{K_B + [TTX]} \quad (6)$$

From Eqs. 5 and 6,

$$\frac{1 - e^{-kA_1t}}{1 - e^{-kA_2t}} - 1 = \frac{[TTX]}{K_B} \quad (7)$$

For small values of the concentration terms kAt, the left side of Eq. 7 approaches (DR − 1), and the relation is then indistinguishable from that derived by Gaddum *et al.* (15) on the basis of competitive antagonism. An estimate of the dissociation constant K_B for TTX can therefore be obtained by plotting log (DR − 1) against log [TTX] after the manner of Arunlakshana and Schild (11). This plot should begin to deviate from linearity at high concentrations of TTX, but experimental observation of linear relationship with unity slope obviously cannot be taken as evidence of competition in this case.

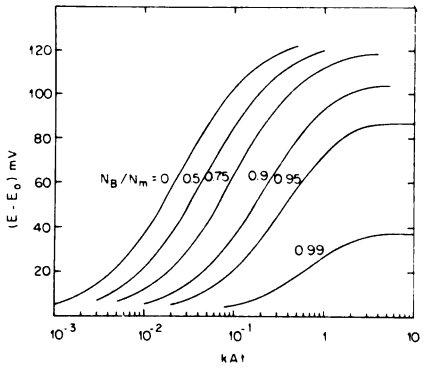

FIG. 5. *Theoretical log dose-response curves obtained from Eq. 4 for different values of N_B/N_m in the range of 0–0.99*

N_m is the maximum number of sodium channels opened by saturating concentrations of agonist; N_B, number of sodium channels blocked in the presence of antagonist. Other abbreviations are the same as for Fig. 4, and a value of 40 is assigned to $N_m gR$. An arbitrary value of 130 mV is given to $(E_{Na} - E)$, based on approximate physiological parameters for mammalian skeletal muscle. See the text for further description of the equations and assumptions.

Effects of Procaine and Lidocaine on Resting Membrane and Action Potentials before and during Exposure to BTX

Neither procaine nor lidocaine had a significant effect on the membrane potential of surface fibers of diaphragm muscles at concentrations in the range 0.1–1.0 mM (Fig. 6). In one series of experiments, muscles were first treated with 1.0 mM procaine for 30 min (Fig. 6C). This procedure blocked neuromuscular transmission within 15 min. After the combination of procaine (1.0 mM) and BTX (10 nM) had been applied to the muscle for 60 min, the membrane depolarized by only 10 mV, and subsequent washing with physiological solution did not appreciably alter the membrane potential. Prior treatment of the diaphragm muscles with smaller concentrations of procaine (0.1 and 0.3 mM) was less effective in protecting the muscle membrane against BTX-induced depolarization. For example (Fig. 6A), 0.1 mM procaine did not affect the increase of spontaneous MEPP frequency induced by BTX. However, a slight delay in the time course of depolarization and a reduction in the amount of membrane depolarization were observed. At 0.3 mM procaine (Fig. 6B), protection was intermediate between that provided by 0.1 and 1.0 mM procaine. Only 15 mV of membrane depolarization were produced after 60 min, and the increase in MEPP frequency was seen only after the preparation had been washed with physiological solution. When the concentration of procaine was kept constant at 0.25 mM and increasing concentrations of BTX were applied in combination with procaine, the amount of membrane depolarization recorded at the end of a 1-hr incubation period was used to construct a double-reciprocal plot (Fig. 7). The divergence of the extrapolated maxima indicates a noncompetitive mechanism for this interaction.

In diaphragm muscles first treated with lidocaine (1.0 mM) and BTX (10 nM) there was a depolarization of the muscle fibers from control values to a mean membrane potential of −58.6 ± 0.8 mV (mean ±SE) after 50–60 min. Washing with physiological solution restored the membrane potential toward normal values (−69.9 ± 0.8

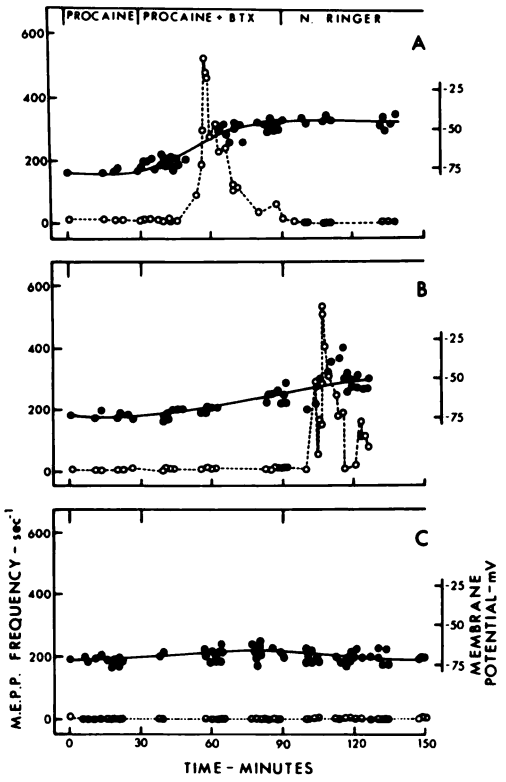

FIG. 6. *Effect of procaine on time course of BTX-induced depolarization of nerve and muscle membranes*

The muscles were treated for 30 min with 0.1 mM (A), 0.3 mM (B), or 1.0 mM (C) procaine, followed by procaine plus BTX (10 mM) for 60 min, and then washed with physiological solution. ●——●, membrane potential; O- - -O, MEPP frequency; ⊖, no detectable spontaneous MEPPs.

mV), and MEPPs reappeared at the control frequency.

When applied during the process of membrane depolarization induced by BTX, both procaine and lidocaine partially restored the membrane potential in rat diaphragm muscles (Fig. 8). The mean membrane potential 30 min after exposure to BTX was −55.0 ± 1.3 mV, and upon application of either procaine (1.0 mM) or lidocaine (1.0 mM) depolarization was halted and a gradual restoration of membrane potential to near control values was observed. After 30 min of procaine application the mean membrane potential was −65.5 ± 0.6 mV and for lidocaine it was −72.0 ± 1.6 mV. A further restoration of the membrane potential and spontaneous transmitter release toward control levels was observed when the muscles were washed with physiological solution alone.

The extent to which the action potential-generating mechanism was affected by the local anesthetics during protection against the membrane depolarization induced by BTX was examined. After a control period the muscles were exposed to the local anesthetics alone for 30 min, then for 60 min in combination with BTX, followed by washing with physiological solution. At the concentrations employed neither procaine nor lidocaine blocked directly elicited action potentials within 30 min of application. However, a significant increase in the threshold of excitation and a 25% decrease in the amplitude of the action potential were observed (Table 1). The shape of the action potentials elicited in the presence of the local anesthetics plus BTX was not significantly different from those obtained in the presence of local anesthetics alone. Upon washing with physiological solution, action potentials recovered to control values.

DISCUSSION

The experimental results show that the receptive sites for BTX and TTX are different, for the following reasons: (a) the depo-

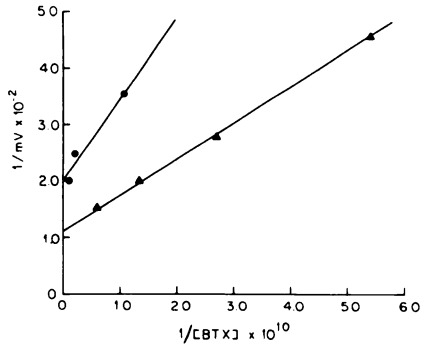

FIG. 7. *Double-reciprocal plot of BTX-induced membrane depolarization in the absence and presence of procaine*

▲, BTX alone for 60 min; ●, BTX plus procaine (0.25 mM). The data for BTX alone were taken from Fig. 2 and represent those values obtained after 55–60 min of exposure to the toxin. The values for BTX plus procaine were obtained from muscles exposed to procaine for 30 min and then to procaine plus BTX for 60 min; recordings were made between 55 and 60 min.

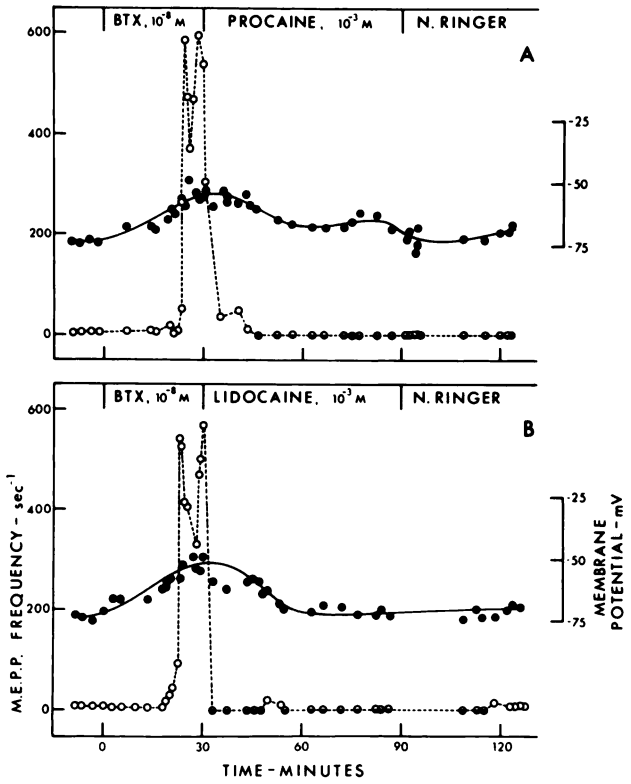

FIG. 8. *Reversal of BTX-induced depolarization by procaine and lidocaine*

○- - -○, MEPP frequency; ●——●, membrane potential; ⊖- - -⊖, no detectable spontaneous MEPPs. The muscles were first exposed to BTX for 30 min, then to procaine (A) or lidocaine (B), followed by washing in physiological solution (N. RINGER).

TABLE 1

Effect of procaine and lidocaine on action potential-generating mechanism before and during exposure to batrachotoxin

The numbers in parentheses refer to the number of fibers examined in at least three muscles. Values are means ± standard errors. The values obtained during the application of local anesthetic alone were recorded between 25 and 30 min after application of the drug. The values during application of local anesthetic and BTX were obtained after 55–60 min of simultaneous exposure, and those during the wash were recorded 30–45 min after the start of the wash.

Treatment	Threshold	Overshoot	Amplitude	Rate of rise
	mV	*mV*	*mV*	*V/sec*
Control	39.4 ± 0.6 (37)	29.1 ± 1.2	79.2 ± 1.2	565 ± 15
Procaine (1 mM)	41.3 ± 1.0[a] (21)	16.1 ± 2.4[a]	64.5 ± 2.3[a]	426 ± 27[a]
Procaine (1 mM) + BTX (10 nM)	44.7 ± 0.8[b] (20)	13.3 ± 2.2[c]	59.2 ± 2.6[c]	404 ± 31[c]
Wash	40.5 ± 1.3[d] (17)	20.6 ± 2.6[a]	72.3 ± 2.5[a]	502 ± 38[d]
Lidocaine (1 mM)	43.8 ± 0.6[a] (27)	18.0 ± 1.4[a]	64.3 ± 1.3[a]	431 ± 9[a]
Lidocaine (1 mM) + BTX (10 mM)	42.0 ± 0.5[b] (50)	16.1 ± 0.9[a]	64.7 ± 0.8[a]	443 ± 8[c]
Wash	41.5 ± 4.3[a] (28)	21.6 ± 1.1[d]	71.6 ± 1.2[d]	522 ± 17[d]

[a] $p < 0.05$ with respect to control.

[b] $p > 0.05$ with respect to procaine (1 mM) or lidocaine (1 mM).

[c] $p < 0.05$ with respect to procaine (1 mM) or lidocaine (1 mM).

[d] $p > 0.05$ with respect to control.

larizing action of BTX on lobster giant axons was antagonized by prior treatment with sulfhydryl reducing agents, which were ineffective in antagonizing the action of TTX on the action potential-generating mechanism in this nerve (7); (b) BTX had no effect on the binding of [^{3}H]TTX to rabbit vagus nerves (16); (c) TTX did not exert its antagonistic effect on BTX-induced membrane depolarization in chronically denervated rat skeletal muscle (6); and (d) TTX blocked the pre- and postsynaptic effects of BTX, but only when TTX was in the bathing medium; otherwise the persistent actions of BTX were present (3, 17). Since the present analysis of BTX action did not take into account the nonlinearities which result from rectification in ionic channels, no attempt was made to fit theoretical curves to the experimental data. However, the change in input conductance seen with large membrane depolarizations induced by BTX was generally very small (13), and this observation appears to justify the use of a simple model. The results therefore conform adequately to the requirements of noncompetitive antagonism between TTX and BTX.

Although the receptive site for BTX appears to involve the participation of sulfhydryl groups, it has been suggested that the receptive site for TTX may involve an ionic group such as a carboxyl moiety (18, 19). Further characterization of the binding site for TTX has been obtained from studies on garfish olfactory nerve suspensions after treatment with various hydrolytic enzymes (20). The latter authors concluded that TTX binds to the nerve membrane with a dissociation constant of 8.3 nM and that the binding component is a protein embedded in phospholipid.

In the present studies the apparent dissociation constant for the binding of TTX to the sodium channels of the diaphragm muscles was found to be 30.2 nM at 37°. This value is compatible with that observed by Benzer and Raftery (20) in garfish olfactory nerve homogenates and that reported for nonmyelinated fibers of rabbit vagus nerve (5). A value of about 6.1 nM at 20° has also been reported for the equilibrium constant of TTX calculated by studying the binding of [^{3}H]TTX on rat diaphragm muscle (16).

The simple relationships derived under RESULTS for the noncompetitive interaction of an irreversible agonist and a reversible antagonist require that the experimental plot of log (DR − 1) vs. log TTX concentration should approximate a straight line of unity slope. The experimental result shown in Fig. 3 is therefore in reasonable agreement with the prediction.

A further requirement (Fig. 4B) is that the experimental plot of log $[(E - E_0)/(E_m - E)]$ vs. log BTX concentration (Hill plot) should also be a straight line with a slope of 1 over a substantial range of concentration. The experimental Hill plot in Fig. 9 is linear (regression coefficient of 0.98) and has a slope of 1.05. This satisfactory agreement suggests that cooperativity is probably not involved in the interaction of BTX with its reactive sites at the sodium channels in the resting state. It is likely that 1 molecule of BTX interacts with 1 receptive site. Furthermore, this analysis indicates that the interaction between BTX molecules and the sodium channel is not altered in the presence of TTX; i.e., there is no allosteric interaction between the binding of one toxin and the other.

Interaction between Local Anesthetics and BTX on Mammalian Skeletal Muscle

The membrane depolarization induced by BTX on the squid giant axon was halted

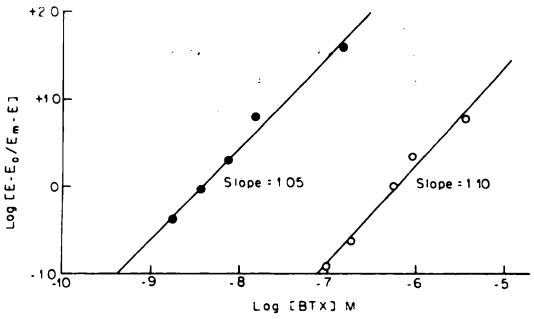

FIG. 9. *Hill plots constructed from depolarization curves of BTX in the presence and absence of TTX*

●, BTX; ○, BTX plus TTX (1.0 μM). The data were taken from Fig. 2. The correlation coefficient (r^2) of both curves is 0.98. E is the mean membrane potential in the presence of toxins at the end of the 60-min incubation period, E_0 is the membrane potential when no drug was present, and E_m is the membrane potential in the presence of saturating concentrations of BTX.

by procaine, but lidocaine was ineffective, and it was therefore suggested that procaine prevents the binding of BTX to the receptor in the axon membrane (8). The present study on rat diaphragm muscle indicates that procaine (Figs. 6 and 7) and lidocaine (Fig. 8) were effective in blocking BTX-induced depolarization. Procaine and lidocaine were also found to restore the membrane potential when applied during the progressive depolarization induced by BTX, but the restoration process was slower than TTX-induced repolarization (Figs. 1 and 8). The decrease of the extrapolated maximum response to BTX by procaine (Fig. 7), taken together with the evidence that procaine can displace BTX from its sites of action, indicates that procaine reduces BTX affinity in a noncompetitive manner. Since the concentrations of procaine and lidocaine used in this study did not block the action potential-generating mechanism, blockade of sodium conductance and blockade of BTX-induced depolarization appear to be related to two separate actions of these local anesthetics (see Table 1).

It therefore appears that BTX increases sodium permeability by an action at a site different from that occupied by TTX. Both procaine and lidocaine interact with the binding site for BTX in a noncompetitive manner and thereby allow sodium permeability in a muscle previously exposed to BTX to return towards the control level.

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