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Nerve membrane sodium channels as the target site of brevetoxins at neuromuscular junctions

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1 Actions of two structurally related toxins, T-17 and brevetoxin-B, isolated from the red-tide dinoflagellate, *Ptychodiscus brevis*, were studied on the giant axon of the squid and the neuromuscular junctions of the frog and rat.

2 T-17 toxin caused a large increase in the frequency of miniature endplate potentials at nanomolar concentrations. In one typical case with a frog endplate, the frequency increased from 1.9 s^{-1} before application of 3.5 nM T-17 to 69.3 s^{-1} within 5 min after application. In the rat muscle, the mean frequency increased from 1.39 s^{-1} in control to 11.93 s^{-1} after application of 23.2 nM T-17.

3 The increase in miniature endplate potential frequency was reversed by the addition of 1 μM tetrodotoxin, and was not observed in a solution containing elevated Mg^{2+} and reduced Ca^{2+} concentrations.

4 External or internal application of T-17 toxin (2–5 μM) or brevetoxin-B (10–30 μM) to intact or internally perfused squid axons caused a depolarization of the membrane. This depolarization was abolished by the removal of external Na^+ or by addition of tetrodotoxin to the external solution.

5 In voltage clamped squid giant axons, exposure to T-17 toxin or brevetoxin-B increased the non-inactivating component of the tetrodotoxin-sensitive sodium current. The sodium current was activated at potentials 15 to 40 mV more negative than control.

6 It is proposed that these toxins modify a fraction of the sodium channels to a form which opens at potentials more negative than normal and which inactivates to a lesser extent. This mechanism would predict a depolarization of the nerve membrane at the neuromuscular junction, thus explaining the increased discharge of transmitter.

Introduction

Blooms of *Ptychodiscus brevis* (red-tides) are responsible for massive fish kills that occur along the Florida coast. These dinoflagellates contain a variety of extremely potent, lipid-soluble neurotoxins. Crude extracts containing these agents have been reported to cause repetitive discharges of action potentials in neurones (Westerfield *et al.*, 1977; Parmentier *et al.*, 1978); an increase in transmitter release at the neuromuscular junction (Gallagher & Shinnick-Gallagher, 1980); neuromuscular block (Baden *et al.*, 1984); and depolarization of the muscle membrane (Gallagher & Shinnick-Gallagher, 1980).

A variety of toxins from *P. brevis* have been successfully isolated and purified by several groups of investigators (Shimizu *et al.*, 1976; Baden *et al.*, 1979; Padilla *et al.*, 1979; Risk *et al.*, 1979; 1982), and the chemical structures of some of these toxins have been recently identified (Risk *et al.*, 1979; Lin *et al.*, 1981; Golik *et al.*, 1982; Chou & Shimizu, 1982; Baden, 1983). The backbone is composed of 11 contiguous ether rings linked to form a rigid ladder-like structure. Two lipid-soluble toxins have been isolated by Baden and his co-workers from laboratory cultures of *P. brevis* and purified by thin-layer chromatography (Baden *et al.*, 1979; 1981). These toxins are designated T-17 and T-34 based on their migration during thin-layer chromatography. T-34 is identical to brevetoxin-B and GB-2 described by Lin *et al.* (1981) and Chou & Shimizu (1982), respectively. In T-17 the aldehyde function at the terminal carbon-42 in T-34 is reduced

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to the corresponding primary alcohol (Baden, 1983).

This paper describes the actions of T-17 toxin on the frog and rat neuromuscular junction and the actions of T-17 toxin and brevetoxin-B on the squid giant axon, the latter preparation being ideal for voltage clamp of the fast, peak transient sodium current. The results at the neuromuscular junction suggest that the increase in discharge of transmitter quanta caused by T-17 toxin is likely to be a result of depolarization of the presynaptic nerve terminal. The experiments with squid giant axons have indeed demonstrated that T-17 toxin and brevetoxin-B depolarize the nerve membrane. The tetrodotoxin (TTX)-sensitive sodium current is modified so that the channel activation occurs at large negative membrane potentials with partial removal of channel inactivation, thereby causing a membrane depolarization.

Methods

All neuromuscular experiments were conducted at room temperature (23°–25°C) with the isolated hemidiaphragm of male Harlan Sprague-Dawley rats (180–250 g) and the cutaneous pectoris muscle from frogs (*Rana pipiens*). Intracellular recordings from muscle fibres were made by conventional techniques with borosilicate glass microelectrodes filled with 3 M KCl solution. Microelectrode resistances were about 5 megohms for frog muscle and 15 megohms for rat muscle. Miniature endplate potentials (m.e.p.ps) were amplified and recorded on magnetic tape. The amplitude and frequency of m.e.p.ps were analysed off-line with a DEC LSI 11-23 computer. In a few instances photographic records of m.e.p.ps were made from the oscilloscope and these records were analysed manually. Contractions of frog muscle were eliminated by the formamide method of excitation-contraction uncoupling, as described by del Castillo & de Motta (1977).

Purified T-17 toxin was a generous gift from Dr D.G. Baden of the University of Miami School of Medicine. The toxin was dissolved in 95% ethanol to prepare a stock solution, which was subsequently diluted with the saline solutions used to superfuse the muscles. The concentration of ethanol after dilution never exceeded 0.12% (v/v). This concentration of ethanol was without effect on m.e.p.p. frequency, as indicated by a control experiment in which the vehicle alone (0.12% ethanol) was applied to the frog muscle.

Brevetoxin-B was kindly provided by Dr K. Nakanishi of Columbia University. It was dissolved in dimethylsulphoxide (DMSO) and applied at concentrations up to 30 μM (0.1% DMSO, v/v).

Rat muscles were superfused with a modified Liley's solution which contained (mM): NaCl 135, KCl 5, $MgCl_2$ 1, $NaHCO_3$ 12, Na_2HPO_4 and glucose 11. Solutions were bubbled with a 95% O_2–5% CO_2 mixture, which maintained the pH between 7.3 and 7.4. Frog muscles were superfused with a Ringer solution of the following composition (mM): CaCl 111, KCl 4.7, $CaCl_2$ 2 and HEPES 2. The pH of the Ringer solution was adjusted to 7.35 by using the appropriate mixture of HEPES base and the Na salt.

Giant axons isolated from the squid, *Loligo pealei*, obtained at the Marine Biological Laboratory, Woods Hole, Mass., were internally perfused by the roller method originally developed by Baker *et al.* (1962) and modified by Narahashi & Anderson (1967). The axon, mounted in a Plexiglass chamber, was perfused internally with standard internal solution and externally with artificial sea water. The axon was voltage clamped by means of the conventional axial wire technique. External current electrodes consisted of three platinum black-plated electrodes, with the middle one measuring membrane current and the two exterior ones electrically guarding against nonradial current flow into the central plate. Leakage currents and capacitive currents were electronically subtracted from the records. Approximately two-thirds of the series resistance were compensated by a feedback circuit. Membrane potential measurements were corrected for junction potentials. In all experiments, the temperature was maintained at 12 ± 0.5°C as measured by a thermocouple mounted about 2 mm from the axon in the central current-measuring region.

Normal artificial sea water had the following composition (mM): NaCl 450, KCl 10, $CaCl_2$ 50 and HEPES 5. The final pH was adjusted with NaOH or HCl to 8.0, and the osmolarity was 980 mosmol. The standard internal solution had the following composition (mM): NaF 50, K-glutamate 320, K_2HPO_4 15 and sucrose, 333. The pH was adjusted to 7.3 with KOH or HCl. The osmolarity was 1020 mosmol. In order to isolate sodium current in voltage clamp experiments, K^+ ions were replaced by Cs^+ ions internally and tetramethylammonium (TMA) externally on an equimolar basis. When external Na concentration was reduced, it was replaced by TMA on an equimolar basis. In most experiments, external Na concentration was reduced to 100 mM to improve the voltage clamp condition. The holding potential was -100 mV unless noted otherwise.

Results

Experiments with the neuromuscular junction

Effect of T-17 toxin on m.e.p.p. frequency. Application of T-17 toxin at a concentration of 11.6 nM caused a reversible increase in m.e.p.p. frequency at the frog neuromuscular junction. An example of such an experiment with a single endplate is illustrated in

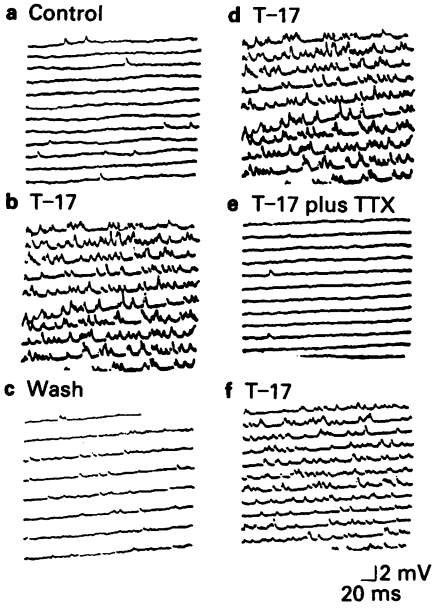

Figure 1 (a–c) Influence of T-17 toxin on the spontaneous miniature endplate potentials (m.e.p.ps). Records (a–c) taken from a single endplate of a frog muscle fibre. (a) Control record taken prior to treatment with T-17 toxin. (b) Record taken after 2 min in T-17 toxin (11.6 nM), showing massive increase in the frequency of m.e.p.ps. (c) Record taken 10 min following washout of T-17 toxin, showing recovery. (d–f) Reversal by tetrodotoxin of T-17 action on m.e.p.ps. (d) M.e.p.ps recorded in the presence of T-17 toxin (11.6 nM). (e) Addition of tetrodotoxin (TTX, 1 μM) rapidly (within 1 min) abolished the toxin-induced m.e.p.p. frequency increase. (f) Washout of TTX, while maintaining T-17 exposure, restored a high m.e.p.p. frequency. Records (d–f) taken from a single endplate of a frog muscle fibre.

Table 1 Effects of T-17 toxin on miniature endplate potential (m.e.p.p.) frequency in the frog

*Fibre**	*m.e.p.p. frequency* (s^{-1})	*Time after T-17 or wash* (min)
Control		
a	0.8	
b	0.7	
c	4.3	
d	1.1	
e	1.9	
T-17 (3.5 nM)		
e	69.3	0–5
f	20.5	5–8
g	12.6	10–18
h	7.8	30–36
i	63.3	36–38
T-17 (11.6 nM)		
j	45.4	3–5
k	71.0	6–9
l	35.8	10–13
m	31.1	14–17
Wash		
i	1.5	6–9
m	4.0	15–16

*All fibres, as indicated by lower case letters (left column), were from the same muscle preparation. In each fibre, m.e.p.p. frequency during 1–4 min periods was determined at various times after T-17, as indicated in the right-hand column.

Figure 1. The m.e.p.p. frequency increased (Figure 1a, b) with a latency of about 15–30 s, and the effect could be reversed (Figure 1c) within 5–15 min during a washout period.

Data from a second preparation in which several fibres were impaled are summarized in Table 1. M.e.p.p. frequencies in five control fibres (a–e in Table 1) varied between 0.8 and 4.3 s^{-1}, but increased markedly in the presence of toxin. When a single fibre (e in Table 1), for example, was held through a solution change into 3.5 nM T-17 toxin, the m.e.p.p. frequency rapidly increased from 1.9 to 69 s^{-1}. High, but variable frequencies of 7.8 to 63 s^{-1} were also recorded from four additional fibres (f, g, h and i in Table 1) sampled in the same preparation in the continued presence of toxin. Concentrations lower than 3.5 nM of toxin were not tested.

The effects of T-17 toxin were rapidly reversible. For example, after a washout period of 5 min, the m.e.p.p. frequency in fibre i had declined from 63 to 1.5 s^{-1}. The fibres could also respond to a second challenge of toxin. Following the washout period, T-17 toxin, this time added at 11.6 nM, caused high m.e.p.p. frequencies in four additional fibres (j, k, l and m in Table 1). Interestingly, 11.6 nM toxin appears to have had no additional effect on m.e.p.p. frequency beyond that in 3.5 nM toxin. (Although it is possible that a maximal effect of toxin could occur at 3.5 nM, it cannot be ruled out that the response to 11.6 nM had been reduced as a result of the earlier treatment.) The effect of 11.6 nM toxin was also reversible; the m.e.p.p. frequency in fibre m (Table 1) declined from 31 to 4 s^{-1} after a 15 min washout period.

The m.e.p.p. frequency of the rat diaphragm was also increased by T-17 toxin at a concentration of 23.2 nM. The mean values (± s.e.mean) were $1.39 \pm 0.30\ s^{-1}$ in control and $11.93 \pm 6.4\ s^{-1}$ in toxin (5 fibres). After rinsing with control solution, the m.e.p.p. frequency declined to $1.16 \pm 0.07\ s^{-1}$ (3 fibres). These results are similar to those obtained by Gallagher & Shinnick-Gallagher (1980), who tested a crude fraction of *Ptychodiscus brevis* toxin (1–2 $\mu g\ ml^{-1}$) on the rat hemidiaphragm preparation.

Reversal of T-17 action in high Mg^{2+} – low Ca^{2+} solutions. In order to see whether the T-17 toxin-induced transmitter release was calcium-dependent, one experiment was performed in a solution containing elevated Mg^{2+} (8 mM) and low Ca^{2+} (1 mM). In normal solution that contained 2 mM Ca^{2+} and 1 mM Mg^{2+}, 23.2 nM T-17 toxin increased the m.e.p.p. frequency in a rat hemidiaphragm preparation from 2.18 s^{-1} to 36.5 s^{-1}. However, the application of the high Mg^{2+}-low Ca^{2+} solution containing the toxin caused the m.e.p.p. frequency to decline to 1.38 s^{-1}. When the preparation was again exposed to the normal solution containing the toxin, a high m.e.p.p. frequency was restored (21.8 s^{-1}). Thus calcium is necessary in the external solution for T-17 toxin to stimulate spontaneous transmitter release.

Tetrodotoxin reversal of T-17 action. One possible mechanism of the toxin-induced increase in m.e.p.p. frequency is a depolarization of nerve terminals due to selective opening of sodium channels. If this were the case, TTX would be expected to reverse the m.e.p.p. frequency increase through the selective block of the sodium channel. TTX completely abolished the increase in m.e.p.p. frequency in the frog preparation caused by T-17. T-17 toxin alone (11.6 nM) caused the m.e.p.p. frequency in a single fibre to increase from 1.0 s^{-1} to 120 s^{-1} (Figure 1d). Addition of TTX (1 μM) to the toxin-containing solution caused the m.e.p.p. frequency to return to the control level (Figure 1e). Washout of TTX with a solution containing T-17 toxin (11.6 nM) restored a high m.e.p.p. frequency (91 s^{-1}) (Figure 1f). Similar results were obtained with two additional fibres in two different preparations. These results support the proposition that T-17 toxin increases the probability of the sodium channel being open at the nerve terminal, thereby causing an increase in m.e.p.p. frequency.

Effect of T-17 on m.e.p.p. amplitude. The mean value for m.e.p.p. amplitude was determined from 200–400 m.e.p.ps in each of four rat hemidiaphragm fibres and then averaged. The pattern of amplitude distribution remained unchanged. T-17 toxin decreased the m.e.p.p. amplitude slightly from 0.91 ± 0.18 mV (mean ± s.e.mean) to 0.8 ± 0.1 mV, but the difference is not statistically significant.

A similar conclusion applies to the frog neuromuscular junction. The amplitude histograms of m.e.p.p. before (Figure 2a) and during exposure to T-17 (3.5 nM) (Figure 2b), are virtually identical. At this concentration of toxin, the muscle membrane (end-plate region) was depolarized by only 2 mV.

Experiments with the squid giant axon

The experiments with the frog and rat neuromuscular preparations suggest that T-17 toxin increases the probability of the sodium channel being open in the nerve membrane. This would lead to membrane depolarization and a consequent increase in m.e.p.p. frequency. In order to test this hypothesis, experiments were conducted on the squid giant axon.

Depolarization of the membrane. The depolarizing action of T-17 toxin was demonstrated in one intact and two internally perfused axons which were externally perfused with a 100 mM Na^+ sea water. When applied externally, T-17 toxin (1.92 μM) depolarized the membrane by 5–10 mV. Removal of external Na^+ (TMA substitution) or addition of TTX (1 μM) reversibly abolished the depolarization induced by T-17. Similar results were obtained with brevetoxin-B in an additional axon. These findings agree qualitatively

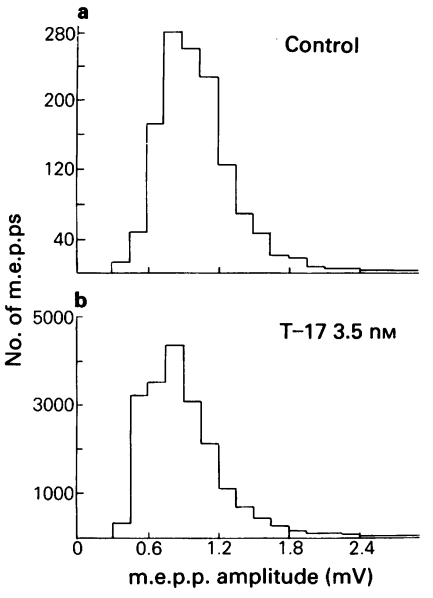

Figure 2 Absence of an effect of T-17 toxin on the distribution of miniature endplate potential (m.e.p.p.) amplitudes in a frog muscle endplate. (a) Histogram of m.e.p.p. amplitudes pior to addition of the toxin. (b) In the presence of 3.5 nM T-17 toxin. Although the frequency of m.e.p.ps was increased by T-17 toxin, the peak value and shape of the distribution were virtually unaffected.

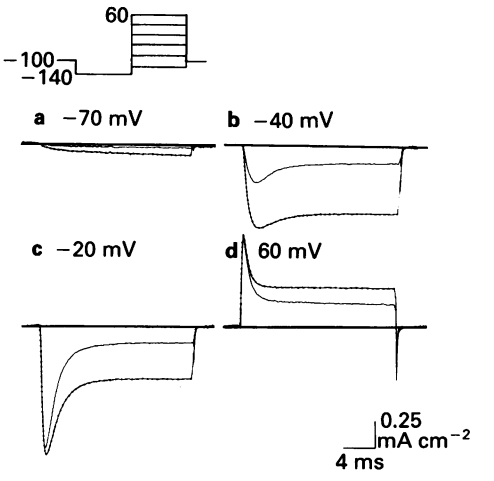

Figure 3 Effects of internally applied 30 μM brevetoxin-B on sodium currents recorded from a squid giant axon. Sodium currents during 20 mV step depolarizations to −70, −40, −20, and +60 mV are shown. A 20 ms prehyperpolarization to −140 mV was applied from the holding potential of −100 mV before the test step depolarizations (inset). Each of panels (a–d) shows superimposed records in control and brevetoxin-B. The toxin caused both peak and steady-state sodium current to increase. Na concentrations in external and internal perfusates were 100 mM and 50 mM, respectively. Temperature 12.2°C.

with those of Huang *et al.* (1984) who have made extensive analyses of the depolarizing action of T-17 toxin on crayfish axons.

Modification of sodium current by T-17 and brevetoxin-B. Both T-17 toxin and brevetoxin-B markedly modified the sodium current. Partial reversibility was observed with T-17 after a 30 min wash; in comparison brevetoxin-B was much less reversible. Experiments were made with the axons perfused externally and internally with K^+-free media to eliminate the potassium current. The residual current measured after external application of TTX (1 μM) was subtracted from all records to obtain the TTX-sensitive sodium current.

An example of a series of sodium current records before and during application of 30 μM brevetoxin-B is illustrated in Figure 3. A 20 ms prepulse which hyperpolarized the membrane to −140 mV was followed by 20 ms test pulses to various membrane potentials. Sodium current appeared at membrane potentials more negative than control after application of brevetoxin-B. This is illustrated in Figure 3a for a depolarization to −70 mV. The amplitude of the non-inactivating (steady-state) current, which followed the transient sodium current at potentials less negative than −50 mV, was increased markedly (Figure 3b, c and d) and its magnitude was maintained during depolarizing pulses up to 40 ms in duration (not illustrated). The peak amplitude of the sodium current was also increased, the degree of change depending on the membrane potential (Figure 3b, c and d). However, the kinetics of sodium current activation were largely unaltered. The time for the sodium current to reach its peak was slightly prolonged and the rate of decay was slightly slower than control, and these effects were more noticeable in the negative membrane potential region.

The kinetics of the tail current associated with a step repolarization as applied during the sodium current represent the rate at which the sodium channel is closed. The membrane was repolarized following 0.5 ms and 10 ms depolarizing pulses to +20 mV to examine the rate of sodium channel closing during the peak and steady-state currents, respectively. The kinetics of the sodium tail current remained unchanged after application of brevetoxin-B.

Results qualitatively similar to those with brevetoxin-B were obtained with T-17 toxin.

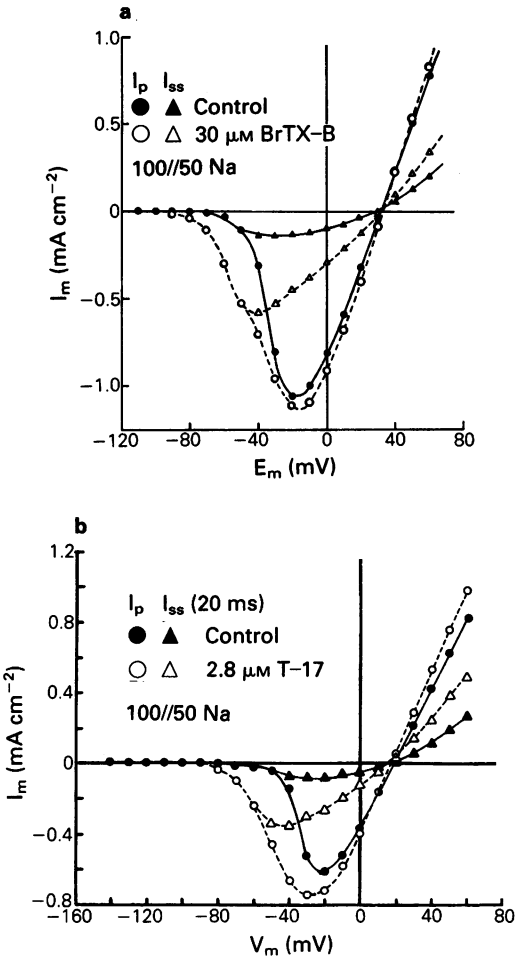

Figure 4 Current-voltage ($I_m - E_m$) relationships for the peak (I_p) and steady-state (I_{ss}) currents in the absence (filled symbols) and presence (open symbols) of 30 μM brevetoxin-B (BrTX-B) (a) or 2.8 μM T-17 toxin (b). Data obtained using the protocol shown in Figure 3. External and internal Na concentrations were 100 mM and 50 mM, respectively.

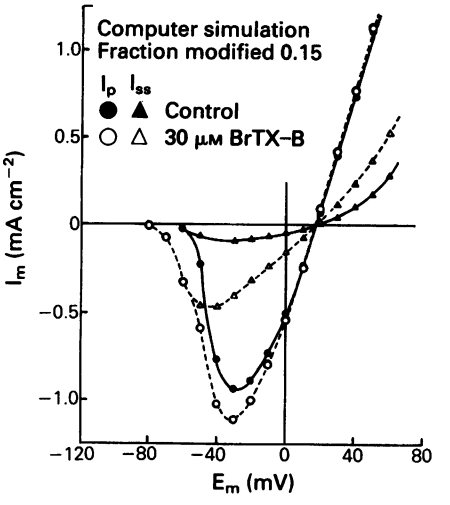

Figure 5 Simulation of current-voltage ($I_m - E_m$) relationships for normal sodium currents and sodium currents modified by 30 μM brevetoxin-B (BrTX-B), as calculated from the Hodgkin-Huxley formalism. I_p, peak current; I_{ss}, steady-state current. Symbols as for Figure 4. See text for further explanation.

However, brevetoxin-B was about one-tenth as potent as T-17; 30 μM brevetoxin-B was required to produce approximately the same effect on the non-inactivating component of the sodium current as 2.8 μM T-17.

The current-voltage relationships before and during exposure to 30 μM brevetoxin-B and 2.8 μM T-17 are shown in Figure 4a and b, respectively, for the peak and steady-state sodium currents. The current-voltage relationship for the peak current was shifted in the direction of hyperpolarization by about 20–40 mV ($n = 4$) by T-17 and 10–25 mV ($n = 4$) by brevetoxin-B. In both toxins, the peak and steady-state sodium currents were increased over the entire potential range tested. In T-17 the potential at which maximum peak current occurred was shifted 10–20 mV ($n = 4$) in the direction of hyperpolarization whereas no shift was observed in brevetoxin-B. Neither of the toxins changed the reversal potentials for the peak and steady-state currents.

The generation of the sodium current at more negative potentials than control and the increase in non-inactivating sodium current in the presence of the toxins can account for the observed depolarization of the resting membrane. The absence of the change in the reversal potential indicates that the selectivity of the sodium channel, at least for Na^+ relative to Cs^+ (which was used as the substitute for internal K^+), was not altered by the toxins.

Simulation by Hodgkin-Huxley formulation. Current-voltage relationships for peak and steady-state sodium currents before and during exposure to brevetoxin-B were calculated using the Hodgkin-Huxley formalism (Hodgkin & Huxley, 1952): $I_{Na} = m^3h(E - E_{Na})$, where I_{Na} refers to sodium current, m and h refer to dimensionless parameters for sodium current activation and inactivation, respectively, and E and E_{Na} refer to the membrane potential and the sodium equilibrium potential, respectively. The activation and inactivation rate constants α_m, β_m, α_h and β_h were calculated for a temperature of 12°C. The currents in

30 μM brevetoxin-B were obtained by assigning 15% of the total current to a modified form in which the activation parameters were shifted by 25 mV in the direction of hyperpolarization and inactivation was removed. The simulated current-voltage relationship is illustrated in Figure 5. The similarity of the calculated and observed curves (Figure 4a) is very striking. However, this simple model was unable to simulate entirely the observed current-voltage relationship for the peak sodium current in T-17 toxin (Figure 4b), in that the maximal peak sodium current was shifted by about 10 mV in the direction of hyperpolarization.

Discussion

The present study has clearly demonstrated the actions of T-17 and brevetoxin-B on the nerve membrane sodium channel as the basis for their neuromuscular effects. Recently, T-17 and T-34 (brevetoxin-B) have been shown to cause a TTX-sensitive contracture in the rat neuromuscular preparation which was followed by a block of transmission (Baden *et al.*, 1984). These effects can be explained in terms of the effects on the sodium channel as described below.

T-17 toxin has been found to cause a large increase in the spontaneous release of transmitter at the frog and rat neuromuscular junctions. In a solution containing high Mg^{2+} and low Ca^{2+}, the toxin was no longer able to increase the m.e.p.p. frequency. This suggests that Ca^{2+} influx via voltage-dependent calcium channels in the nerve terminal (Llinás *et al.*, 1981) is required for transmitter release in the presence of toxin. The increase in m.e.p.p. frequency was not accompanied by a change in the distribution of m.e.p.p. amplitudes, indicating that the toxin exerted its effect presynaptically. One mechanism by which m.e.p.p. frequency can increase is by depolarization of the nerve terminal due to a toxin-induced increase in the probability of the sodium channel being in the open state at the resting potential.

Experiments with squid giant axons have directly demonstrated the depolarizing action of the toxins on nerve membranes. Both T-17 toxin and brevetoxin-B (in micromolar concentrations) caused a depolarization that could be reversibly blocked by TTX or by the removal of Na^+ from the external solution. Similar results have been obtained by Huang *et al.* (1984) in their studies of the action of T-17 toxin on crayfish axon membranes. Since, however, nanomolar concentrations of T-17 toxin were effective in depolarizing the crayfish giant axon, this indicates that the toxin is roughly 1000 fold more potent on the membrane of the crayfish axon than on that of the squid axon. It appears that the sodium channels of the presynaptic fibre of the frog neuromuscular junction are also sensitive to nanomolar concentrations of T-17 toxin based on the increase in discharge of transmitter quanta; unfortunately there are no data available at present on the range of effective concentrations for vertebrate axons. The possibility that the toxin acted at a site other than the sodium channel to exert this effect is remote because the high m.e.p.p. frequency caused by the toxin was completely removed by the addition of TTX. How the sodium channels of axonal membranes might have been modified by the toxins to cause a depolarization is discussed below.

The voltage clamp experiments with internally perfused squid giant axons showed a substantial increase in the non-inactivating component of sodium current, and a shift of the membrane potential at which the sodium channels opened by 20 to 40 mV in the direction of hyperpolarization. Similar results were obtained on crayfish axons (Huang *et al.*, 1984). These two effects, which are sensitive to TTX, would predict a substantial increase in sodium permeability of the resting membrane and would thus account for the toxin-induced depolarization. T-17 toxin was virtually without effect on the delayed potassium current of perfused squid axons (Huang *et al.*, 1984); hence the delayed potassium channel should not be involved.

T-17 toxin was approximately 10 times more potent than brevetoxin-B and was more readily removed after washing. The only difference in chemical structures between these two toxins is at the carbon 41 position, T-17 having an alcohol group and brevetoxin-B an aldehyde group.

Effects on sodium currents similar to those reported here have been observed with squid axons and frog nodes of Ranvier exposed to batrachotoxin, grayanotoxin and scorpion venom (Schmitt & Schmidt, 1972; Khodorov, 1978; Seyama & Narahashi, 1981; Tanguy *et al.*, 1984). However, unlike these toxins, T-17 and brevetoxin-B increased the peak sodium current in squid giant axon and had little or no effect on the cation selectivity of the sodium channel. Our findings can be interpreted as indicating that T-17 or brevetoxin-B modified approximately 10–20% of the sodium channel population in an all-or-none manner to a form that activates at a potential 10–40 mV more negative than the control and inactivates more slowly. Alternatively, the findings are consistent with the situation in which the activation and inactivation gating properties of the entire channel population are modified to some extent. Further investigations including single channel studies are required to resolve the mechanisms underlying the effects of T-17 and brevetoxin-B on the sodium channel.

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