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Effects of brevetoxin-B on motor nerve terminals of mouse skeletal muscle

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1 The effects of brevetoxin-B, a red tide toxin, on motor nerve terminal activity were assessed on mouse triangularis sterni nerve-muscle preparations. The perineural waveforms were recorded with extracellular electrodes placed in the perineural sheaths of motor nerves.

2 At 0.11 μM, brevetoxin-B increased the components of waveforms associated with sodium and potassium currents while it decreased the calcium activated potassium current and the slow calcium current of the nerve terminal. The fast calcium current and slow potassium current were not affected.

3 At 1.11 μM, brevetoxin-B decreased all of the components of waveforms associated with sodium, potassium and calcium currents.

4 It is concluded that brevetoxin-B affects sodium, potassium as well as calcium currents in the nerve terminal. The effects may contribute to its pharmacological actions on synaptic transmission.

Keywords: Brevetoxin; nerve terminal current; red tide toxin; neuromuscular transmission

Introduction

The dinoflagellate *Ptychodiscus brevis* (formerly *Gymnodinium breve*) is responsible for numerous fish kills during its blooms ('red tides') along the coast of the Gulf of Mexico and for human toxicity due to ingestion of contaminated shellfish (Steidinger, 1979). In nerve and muscle preparations, Shinnick-Gallagher (1980) reported that the crude fraction of *Gymnodinium breve* toxin depolarized the resting membrane potential of rat diaphragm. Tetrodotoxin antagonized the toxin depolarized membrane. Wu *et al.* (1985) reported that T-17, a fraction of purified toxin isolated from *Ptychodiscus brevis* caused an increase in the frequency of miniature endplate potentials in rat and frog neuromuscular junctions. Similar results were found in rat hemidiaphragm (Gallagher & Shinnick-Gallagher, 1985). T-17 also depolarized the squid giant axons causing the sodium channels to open at the normal resting potential (Wu *et al.*, 1985). Based on the electrophysiological studies on vertebrate synaptic transmission and on squid giant axon, it was suggested that the brevetoxin-enhanced transmitter release was caused by the effect of brevetoxins on the sodium channel in the motor nerve terminal. However, there is no direct evidence proving brevetoxin acts on the sodium channel in the motor nerve terminal.

Brevetoxin-B can be isolated in crystallized form (Lin *et al.*, 1981; Baden *et al.*, 1981; Chou & Shimizu, 1982) and the method of perineural waveform recordings allowed the characterization of channels in the nerve terminals (McArdle *et al.*, 1981; Penner & Dreyer, 1986). The effects of various toxins on motor nerve terminal currents have been well studied (Anderson *et al.*, 1988; Anderson & Harvey, 1988a, b). The aim of this study was to elucidate the possible modes of action of brevetoxin-B on the nerve terminal currents.

Methods

Experiments were carried out *in vitro* on the isolated triangularis sterni nerve-muscle preparation (McArdle *et al.*, 1981) of adult I.C.R. mice (*Mus musculus* from Institute of Cancer Research, U.S.A.) of either sex weighing between 17–25 g. Tissues were immersed in a physiological solution containing (mM): NaCl 115, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.0, $NaHCO_3$ 25, Na_2HPO_4 1.0 and glucose 11. The bath was maintained at room temperature (22–25°C) and continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2, maintaining the physiological solution at pH 7.3. (Penner & Dreyer, 1986). For recording the perineural waveform, preparations were visualized at a $\times$400 magnification by a Zeiss microscope equipped with Normarksi interference contrast optics (Dreyer *et al.*, 1979). The preparation was continuously perfused (3–6 ml min^{-1}) with modified Krebs solutions of the compositions described above.

Signals following nerve stimulation through a suction electrode were recorded inside the endothelial tube of nerve bundles (containing 2–4 nerve fibres) with glass microelectrodes filled with 0.5 M NaCl (resistance 4–10 megaohm). The reference electrode was a silver/silver chloride wire in the recording chamber. The potential difference between the recording electrode and the reference electrode in the bath was measured by a high impedance unity gain amplifier (Axoclamp-2), displayed on a dual beam storage oscilloscope and simultaneously stored on FM tape. Wave-forms were evoked by stimulating the motor nerve via a suction electrode every 2–30 s with supramaximal pulses of 0.05 ms duration. To avoid the contribution of postsynaptic responses, the preparation was treated with (+)-tubocurarine (30 μM). Investigations on the 'Ca currents' were made in the presence of tetraethylammonium chloride (TEA, 1 mM) and 3,4-diaminopyridine (3,4-DAP, 100–500 μM) as indicated in the results.

As the shape of the waveform recorded was very dependent on the electrode position, waveforms were monitored continuously from the same site before and throughout application of drugs.

Materials

Tetraethylammonium, 3,4-diaminopyridine, (+)-tubocurarine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Brevetoxin-B was purified by Hong-Nong Chou according to the method of Chou & Shimizu (1982) and it was dissolved in absolute ethanol as a stock solution.

Results

Effect on the perineural waveforms

The effect of brevetoxin-B on the nerve terminal currents was tested in nerve-muscle preparations immobilized by pretreatment with (+)-tubocurarine (30 μM). Figure 1a shows a nerve

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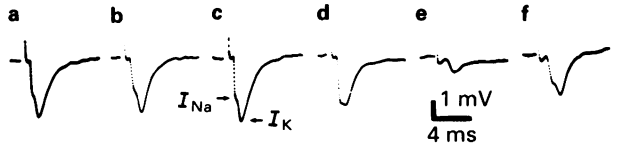

Figure 1 The effects of brevetoxin-B on nerve terminal current of mouse motor nerve terminal. The preparation was incubated in normal physiological solution containing 2.5 mM $CaCl_2$ and (+)-tubocurarine (30 μM). The nerve was stimulated at 0.5 Hz. (a) An example of a perineural waveform; (b), (c), (d) and (e) were perineural waveforms 10 min after brevetoxin-B (0.055, 0.11, 0.55, 1.11 μM) application, respectively. (f) Perineural waveform 30 min after (e) and washing. Note that sodium current (I_{Na}) and potassium current (I_K) showed a biphastic perineural waveform.

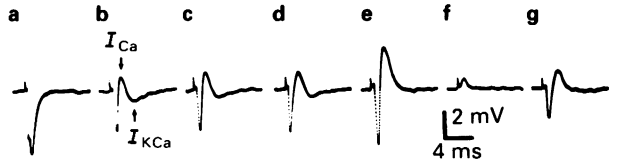

Figure 2 The effects of brevetoxin-B on calcium-dependent potassium current (I_{KCa}) of mouse motor nerve terminal. The perineural waveforms were recorded in the same area of nerve terminal. The nerve was stimulated at 0.03 Hz. At (a) the preparation was incubated in normal physiological solution containing 30 μM (+)-tubocurarine and 2.5 mM calcium; (b) perineural waveform recorded 20 min after addition of 30 μM (+)-tubocurarine ((+)-Tc) and 500 μM 3,4-diaminopyridine (3,4-DAP). At (c), (d), (e) and (f) brevetoxin-B, (0.011, 0.055, 0.11, 1.11 μM) were added respectively for 10 min. At (g) brevetoxin-B was washed with physiological solution containing 500 μM 3,4-DAP and (+)-Tc (30 μM) for 30 min from (f). Note that brevetoxin-B decreased the calcium-dependent potassium current when it increased the sodium and calcium (I_{Ca}) currents in the nerve terminal as shown in (e).

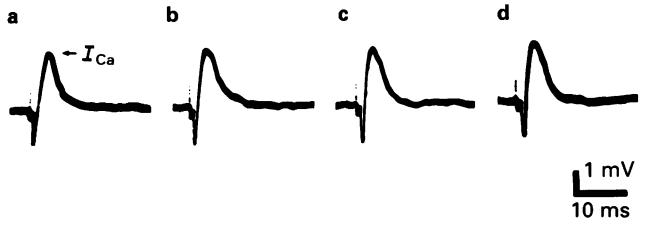

Figure 3 The effects of brevetoxin-B on the slow potassium current of motor nerve terminal. Calcium current (I_{Ca}) was displayed after suppression of the fast potassium current and calcium-dependent potassium current by tetraethylammonium (TEA, 30 mM). The perineural waveforms were recorded in the same area of motor nerve terminal. (+)-Tubocurarine (30 μM) and TEA (30 mM) were present throughout the experiment from (a) to (d). The nerve was stimulated at 0.03 Hz. (a) Control signal; at (b), (c) and (d), brevetoxin-B (0.011, 0.055, 0.11 μM) were added respectively for 10 min. Note that brevetoxin-B did not affect the calcium current, indicating that brevetoxin-B did not alter the slow potassium current of the nerve terminal.

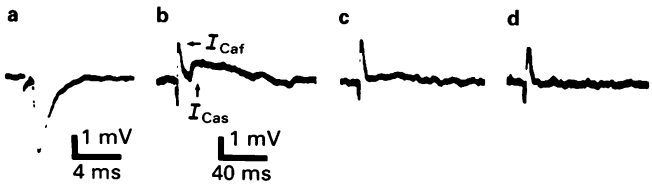

Figure 4 The effects of brevetoxin-B on presynaptic calcium currents in mouse motor nerve terminals. The nerve was stimulated at 0.3 Hz. (a) Control signal; from (b) to (d), the preparation was incubated in a solution containing tetraethylammonium (1 mM) and 3,4-diaminopyridine (100 μM). At (c) and (d) brevetoxin-B (0.11, 0.55 μM), respectively was further added to the preparation for 10 min. Note that brevetoxin-B did not alter the fast calcium current (I_{Caf}) of the motor nerve terminal, while it decreased the slow calcium current (I_{Cas}).

signal following supramaximal nerve stimulation. It consisted of a predominant biphasic negativity which was often preceeded by a small positivity. This waveform closely resembled focally recorded signals obtained at the transition between myelinated axon and non-myelinated terminal where evidence has been presented that the first negativity is due to Na^+ influx (I_{Na}) and the second negative phase corresponds to a passive current generated by the K^+ efflux (I_K) in the nerve terminals (Brigant & Mallart, 1982; Penner & Dryer, 1986; Anderson & Harvey, 1988a, b).

Brevetoxin-B affected the perineural waveform in a concentration-dependent manner. The concentration-effect relationships of brevetoxin-B on the perineural waveforms are shown in Figure 1. At 0.11 μM, brevetoxin-B increased both the sodium and potassium currents in the nerve terminal (Figure 1c). At a higher concentration (1.11 μM), brevetoxin-B decreased both sodium and potassium currents in the nerve terminal (Figure 1e). The effect of brevetoxin-B on the perineural waveform was reversible. After 30 min of washing, the perineural waveform was recovered. Similar results were obtained in five experiments.

Effect of brevetoxin-B on the presynaptic calcium-activated potassium current

For investigations of calcium-activated potassium currents (I_{KCa}) in the nerve terminal, 3,4-DAP (500 μM) was added to the bath solution. This concentration ensured maximal responses and consistently revealed the typical triphasic signals shown in Figure 2b. The signal component of the calcium-activated potassium current was decreased after addition of brevetoxin-B (0.11 μM) as showing in Figure 2e. Note that the sodium and outward current (calcium current, I_{Ca}) in the nerve terminal were increased after brevetoxin-B (0.11 μM)-treatment (Figure 2e). However, the sodium and calcium currents in the nerve terminal were decreased if a higher concentration of brevetoxin-B (1.11 μM) was applied (Figure 2f).

After repetitive washing for 30 min, the sodium and calcium currents in the nerve terminal recovered while the I_{KCa} blocked by brevetoxin-B had not yet recovered (Figure 2g). Similar results were found in 4 experiments.

Effect on slow tetraethylammonium-resistant potassium current

A dose of TEA as high as 30 mM failed to elicit full calcium plateau responses. Prolonged calcium responses could only be obtained by subsequent addition of 3,4-DAP (200 μM). This was taken as evidence for the presence of a TEA-resistant K^+-current in mammalian motor nerve terminals (Penner & Dreyer, 1986). We studied the sensitivity of this current to brevetoxin-B by looking for the ability of the compound to promote full calcium plateau in 30 mM TEA-treated preparations. As shown in Figure 3, the calcium plateau could not be elicited after addition of brevetoxin-B (0.11 μM). If a higher concentration of brevetoxin-B (1.11 μM) was applied, the calcium current as well as the sodium current was decreased. The results indicate that brevetoxin-B has no effect on the slow TEA-resistant potassium current. Similar results were found in 4 experiments.

Effect of brevetoxin-B on the presynaptic calcium current

TEA and 3,4-DAP are potassium channel blockers. A combination of both TEA and 3,4-DAP gave rise to a large positive deflection of the presynaptic current, which was blocked by Cd^{2+}, indicating its underlying cause to be a calcium current (Penner & Dreyer, 1986). There were two different presynaptic calcium currents in mouse motor nerve terminal. The fast positive signal component (fast calcium current, I_{Caf}) was attributed to the voltage-dependent calcium channel, responsible for the initiation of transmitter release. The slow

positive signal component (slow calcium current, I_{Cas}) also depended on extracellular concentration although its physiological role remained unknown (Penner & Dreyer, 1986).

The effects of brevetoxin-B on the nerve terminal calcium currents are shown in Figure 4. Brevetoxin-B, at 0.11–0.55 μM, decreased the slow component of the calcium channel in motor nerve terminal. The fast component of the calcium channel was not affected at this concentration. However, at 1.11 μM, brevetoxin-B decreased both fast and slow components of calcium currents in the nerve terminal. The sodium current in the nerve terminal was also decreased. Similar results were found in 5 experiments.

Discussion

In the present experiments, we found that brevetoxin-B (0.11 μM), not only increased the sodium and potassium currents of motor nerve terminal, it also decreased the I_{KCa} of the nerve terminal. At this concentration, brevetoxin-B had no effect on the potassium current of the terminal currents.

The I_{KCa} of the nerve terminal current may be important in regulating nerve terminal excitability (either by altering the frequency of depolarizations, or shortening the duration of depolarization), preventing excessive accumulation of internal Ca^{2+} and thereby inhibiting transmitter release. Therefore, the decreasing of I_{KCa} may contribute in some part to the effect of brevetoxin-B on the transmitter releasing process. It is interesting to note that charybdotoxin, a blocker of calcium activated potassium channel, also produced a moderate increase in the evoked release of acetylcholine after 3,4-DAP pretreatment (Anderson *et al.*, 1988).

Brevetoxin-B (0.11 μM) did not alter the fast calcium current of the nerve terminal (Figure 4), while it increased the outward current in a 3,4-DAP (500 μM)-pretreated preparation (Figure 2e). The reason for the different effects of brevetoxin-B on those currents remained unknown. However, brevetoxin-B also increased the sodium current and calcium-activated potassium current. Any change in the operation of the neuronal sodium current will alter the driving force for the nerve terminal currents.

In the mouse isolated diaphragm preparation, brevetoxin-B initially induced spontaneous muscle twitching, it also increased the frequency of miniature endplate potentials and depolarized the resting membrane potential of the mouse diaphragm (Tsai *et al.*, 1991). Similar results were also found in rat isolated diaphragm and frog cutaneous pectoris muscle (Wu *et al.*, 1985; Gallagher & Shinnick-Gallagher, 1980; 1985). Brevetoxins caused a concentration-dependent depolarization of the crayfish and squid giant axons membranes. Based on the results from rodent skeletal muscle and squid giant axon, it was suggested that the primary action of the toxin on the membrane is to cause the sodium channels to open at the normal resting potential (Wu *et al.*, 1985). However, from the studies on the nerve terminal currents, it appeared that brevetoxin-B affected not only the sodium current, it also acted on the calcium-activated potassium current and slow calcium current in the nerve terminal. It appeared that some of the effects of brevetoxin-B on the signals recorded may be due to alterations in the magnitude of the nerve terminal depolarization.

At 0.11 μM, brevetoxin-B had no effect on the fast calcium current and slow potassium current of the nerve terminal. However, those currents were decreased after treatment with a higher concentration (1.11 μM) of brevetoxin-B. The effect may be due to brevetoxin-B decreasing the sodium current. The sodium current which provided the driving force for nerve terminal currents was decreased after treatment with a high concentration of brevetoxin-B.

It is concluded that brevetoxin-B acts on the sodium, slow calcium and calcium-activated potassium currents of the motor nerve terminal. The effects may contribute to its pharmacological actions on synaptic transmission.

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