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Potassium channel blocking actions of β-bungarotoxin and related toxins on mouse and frog motor nerve terminals

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1 β-Bungarotoxin and other snake toxins with phospholipase activity augment acetylcholine release evoked from mouse motor nerve terminals before they produce blockade. This action of the toxins is independent of their phospholipase A_2 activity, but the underlying mechanism for the facilitation of release is unclear. To determine whether the toxins affect ionic currents at motor nerve terminals, extracellular recordings were made from perineural sheaths of motor nerves innervating mouse triangularis sterni muscles.

2 Perineural waveforms had a characteristic shape, with two major negative deflections, the first being associated with nodal Na^+ currents and the second with terminal K^+ currents. Block of the K^+ currents revealed a Ca^{2+}-dependent component.

3 During the facilitatory phase of its action, β-bungarotoxin (150 nM) reduced the second negative component of the perineural waveform by 30–50%.

4 The reduction could be a consequence of a decreased K^+ ion contribution or of an increase in the current carried by Ca^{2+}. As β-bungarotoxin had similar effects in solutions which contained no added Ca^{2+}, it is unlikely to be acting on the Ca^{2+} current. Also, it is unlikely to be blocking the Ca^{2+}-activated K^+ current, which is suppressed in zero Ca^{2+} conditions.

5 Other prejunctionally active snake toxins (taipoxin, notexin and crotoxin) had similar effects to those of β-bungarotoxin, but a similar basic phospholipase of low toxicity from cobra venom had no effect.

6 Thus, β-bungarotoxin and related toxins block a fraction of the K^+ current in the motor nerve terminals of mouse preparations. Such an effect could explain the facilitation of acetylcholine release caused by these toxins before the onset of presynaptic blockade.

7 In frog cutaneous pectoris preparations, β-bungarotoxin reduced endplate potential amplitude but had little effect on perineural waveforms. Therefore, the consequences of toxin binding must be different in frog terminals.

Introduction

Several snake venoms contain toxins that act prejunctionally to inhibit the release of acetylcholine from motor nerve terminals (see Harris, 1984; Chang, 1985). These toxins, which include β-bungarotoxin, notexin, taipoxin and crotoxin, are phospholipases A_2, and it is likely that their presynaptic blocking actions are in some way related to their enzyme activity. Before the onset of the irreversible block of transmitter release, the toxins produce characteristic changes in the pattern of release evoked by nerve stimulation. These effects are independent of phospholipase activity because they can be seen at low temperature and in the absence of Ca^{2+} (which is essential for phospholipase activity), and with chemically inactivated toxins. The early changes in release are different in different species. In isolated nerve-muscle preparations from the rat and mouse, release may be slightly depressed for a few minutes and then augmented for 15–60 min (Lee & Chang, 1966; Chang *et al.*, 1973; Su & Chang, 1984); in frog nerve-muscle preparations, there is a persistent depression of acetylcholine release (Caratsch *et al.*, 1981).

The mechanisms underlying the early changes in transmitter release induced by the prejunctional neurotoxins are unknown. In order to determine

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whether the facilitatory effects seen in mouse preparations could be a consequence of changes in properties of action potentials in motor nerve endings, nerve terminal spikes were recorded using extracellular microelectrodes (Brigant & Mallart, 1982; Gundersen *et al.*, 1982; Mallart, 1984; 1985a; Penner & Dreyer, 1986; Anderson & Harvey, 1987). Similar experiments were performed on frog neuromuscular preparations. A preliminary account of some of these results was presented at the 6th European Society of Neurochemistry meeting, Prague, September 1986 (Anderson *et al.*, 1987).

Methods

Mouse triangularis sterni preparation

Experiments were performed on the left triangularis sterni nerve-muscle preparation (McArdle *et al.*, 1981) isolated from 17–22 g male mice (A strain, Bantin and Kingman; and Balb C strain). The complete dissection of the muscle with its three nerves was performed under continuous perfusion with physiological salt solution (aerated with 95% O_2 plus 5% CO_2) of the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1 and $NaHCO_3$ 25 to buffer at pH 7.3.

The preparation was pinned thoracic side downwards to the base of a 2–3 ml tissue bath and perfused at a rate of 5–10 ml min^{-1} with the physiological solution described above, to which (+)-tubocurarine (14–37 μM) was added to abolish postsynaptic activity. When Ca^{2+} ions were replaced by Ba^{2+} ions, a solution of the following composition (mM) was used: NaCl 154, KCl 5, $BaCl_2$ 2.5, glucose 11 and HEPES 5 to buffer at pH 7.3. In all Ca^{2+} substitution experiments, a 20 min equilibration period was allowed. Experiments were performed at 18–22°C. The intercostal nerves were stimulated via a suction electrode every 2 s with pulses of 50 μs duration and supramaximal voltage. In some experiments, procaine (10–300 μM) was used to suppress repetitive activity.

Presynaptic waveforms were recorded by a glass microelectrode (filled with 2 M NaCl, resistance 5–15 MΩ) placed inside the perineural sheath (near endplate areas) of one of the branches of an intercostal nerve (see Mallart, 1985a; Penner & Dreyer, 1986). The potential difference between the silver/silver chloride reference electrode in the bath and the recording electrode was measured by a high impedance unity gain electrometer (W-P Instruments, model M-701), displayed on a dual beam storage oscilloscope and simultaneously recorded on FM tape (Racal 4DS). Usually 20–25 waveforms were recorded at each time period. 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 toxins and drugs. Physiological solution (10–20 ml) containing the toxin at the desired concentration was perfused onto the preparation for 30 min and recycled after aeration. After this exposure period, toxin-free solution was used to perfuse the tissue. Unless stated otherwise, three preparations were used for each experimental condition, and values quoted are means ± s.e. mean.

Frog cutaneous pectoris preparations

Experiments were performed on the cutaneous pectoris preparation of the frog (*Rana pipiens*) (Dreyer & Peper, 1974). Frog Ringer solution contained (mM): NaCl 111, KCl 2, $NaHCO_3$ 2. In order to reduce the calcium-dependent phospholipase activity of the toxin, the Ringer solution contained 6 mM $SrCl_2$ and 1 mM ethylene glycol-*bis*-(β-aminoethylether)-*N*, *N'*-tetraacetic acid (EGTA). The pH of the Ringer solution was adjusted to 7.2, and experiments were performed at room temperature (20–22°C).

In some experiments, endplate potentials (e.p.ps) were recorded by conventional intracellular recording techniques. In other experiments, extracellular recordings of nerve terminal activity were made as described above for mouse preparations. β-Bungarotoxin was added directly to the tissue bath.

Materials

β-Bungarotoxin was a gift from Dr E. Karlsson, Uppsala University, Dr R.C. Hider, Essex University, and Prof. C.C. Chang, Taiwan National University. β-Bungarotoxin was also purchased from Boehringer Mannheim. Notexin, taipoxin, crotoxin, and *Naja naja siamensis* basic phospholipase A_2 were gifts from Dr E. Karlsson, Dr D. Eaker, Uppsala University, Dr C. Bon, Pasteur Institute, Paris, and Dr R.C. Hider, respectively. Other chemicals were obtained from the Sigma Chemical Co., Poole, Dorset.

Results

Effects of β-bungarotoxin on mouse preparations

Pilot experiments using isolated phrenic nerve-hemidiaphragm preparations of the mouse were performed in order to determine an appropriate concentration of β-bungarotoxin for studies on the mechanism of its facilitatory activity. In order to reveal any facilitation of neuromuscular transmission

in twitch tension experiments, the safety factor was reduced by partially paralysing preparations by a reduction in the external Ca^{2+} concentration (see, for example, Su & Chang, 1984). Under these conditions any increase in the release of acetylcholine is revealed by an increase in twitch height. β-Bungarotoxin at 0.15 μM (about 3 μg ml^{-1}) increased twitch height to 266 ± 46% of control (mean ± s.e. mean, $n = 6$). At room temperature, this increase started within 5–10 min of addition of the toxin and was maintained for 60 min. There was no initial depression of twitches in 5 out of 6 experiments.

Perineural recordings in control experiments Control experiments were performed on mouse triangularis sterni preparations to determine the reliability of the extracellular recording technique over the period of the experiments. As previously shown (Mallart, 1985a; Penner & Dreyer, 1986), the waveform recorded from inside the perineural sheath of terminal branches of motor nerves consists of a small positive (upward) deflection, followed by two larger negative deflections (Figure 1A). The positive waveform has been ascribed to longitudinal current inside the sheath; the first negative spike is associated with the inward Na^+ current in the last few nodes of Ranvier; and the second negative deflection has been ascribed to the local circuit currents generated by the outward K^+ current that is localised to the terminals of the nerve (Konishi, 1985; Mallart, 1985a; Penner & Dreyer, 1986). The inward Ca^{2+} current of the nerve terminals is not distinguishable under normal conditions and is only revealed after blocking outward K^+ currents.

In three control experiments in solutions containing 2.5 mM Ca^{2+}, the perineural waveform was stable for over 90 min: the variation was a decrease of 5 ± 3% and 9 ± 3% in the amplitudes of the first and second negative spikes, respectively. The second negative waveform was abolished by the addition of the K^+ channel blocker, 3,4-diaminopyridine (Figure 1A). In preparations exposed to 200 μM 3,4-diaminopyridine, the reduction of the second negative spike often revealed a small positive component. This is thought to be associated with the inward Ca^{2+} current at the nerve terminals (Mallart, 1985a,b; Penner & Dreyer, 1986).

As it has been demonstrated that the motor nerve terminals of the mouse triangularis sterni muscles have a calcium-dependent K^+ current ($I_{K,Ca}$) that can be blocked by tetraethylammonium (TEA) (Mallart, 1985b), the effects of this drug were investigated in control experiments. Addition of 1 mM TEA to a preparation bathed in a solution containing 2.5 mM Ca^{2+} and 200 μM 3,4-diaminopyridine caused an increase in the delayed positive waveform (Figure 1B). Increasing the concentration of TEA to 10 mM

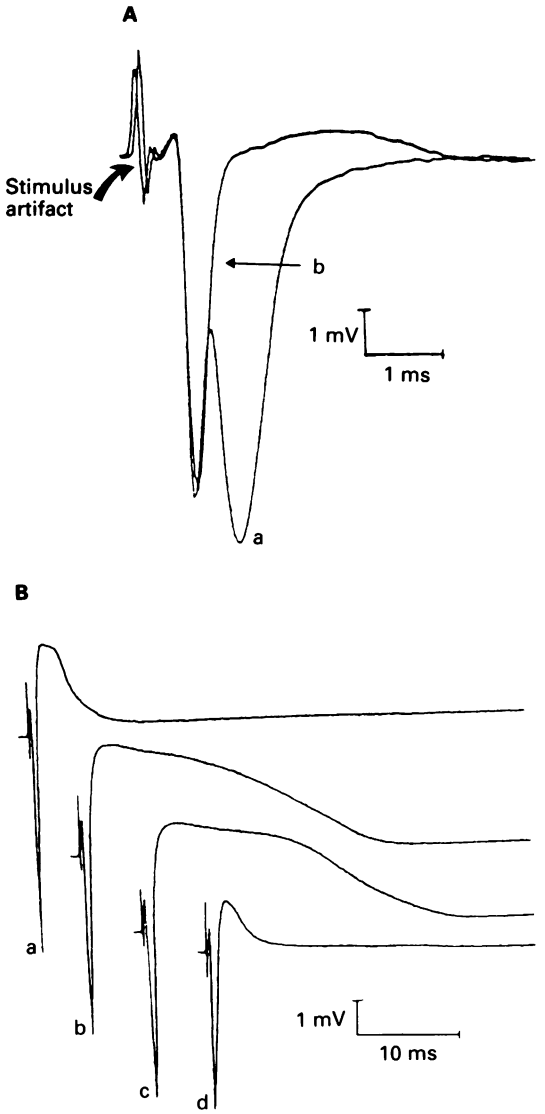

Figure 1 (A) Effect of 3,4-diaminopyridine (0.2 mM) on the extracellularly recorded perineural waveform from a mouse triangularis sterni preparation. (a) Normal waveform. (b) Waveform after addition of 3,4-diaminopyridine. Note that the second negative component of the control waveform (a) has been abolished and that the resulting waveform (b) has a small, slow inward component. In this and subsequent figures, waveforms are averages of 20–25 records. (B) Effect of increasing concentrations of tetraethylammonium (TEA) in the presence of 0.2 mM 3,4-diaminopyridine. (a)–(c) Waveforms in the presence of 1, 10 and 20 mM TEA, respectively; (d) waveform after the addition of 0.2 mM $CdCl_2$. Note the appearance of a positive component of the waveform whose duration is TEA-dependent and which is reduced by cadmium.

led to a further prolongation of the positive waveform (to 350 ± 23% of control), but a further increase of the TEA concentration to 20 mM had little effect on the waveform (mean duration = 317 ± 26%). The amplitude and duration of the positive waveforms were markedly decreased by the addition of 200 μM Cd^{2+} (Figure 1B); the mean duration was reduced to 84 ± 18% of control.

In control experiments in which the solution was changed from one containing 2.5 mM Ca^{2+} to one with no added Ca^{2+}, there was little change in the first negative component (94 ± 9% of control), but the second negative waveform decreased in amplitude to 75 ± 5%, possibly because of a reduction in the contribution from $I_{K,Ca}$ (Figure 2A). Addition of 200 μM 3,4-diaminopyridine abolished the second negative deflection and revealed a positive waveform (Figure 2A). This positive signal is probably due to Na^+ flowing through Ca^{2+} channels following the loss of the Ca^{2+}-dependent selectivity of the Ca^{2+} channels (see Kostyuk *et al.*, 1983; Almers & McCleskey, 1984; Almers *et al.*, 1984; Hess & Tsien, 1984); a similar effect has been demonstrated to occur at nerve terminals of the frog neurohypophysis in low Ca^{2+} solutions (Obaid *et al.*, 1985). Under these conditions, there is apparently little contribution from $I_{K,Ca}$ because addition of TEA did not augment or prolong the positive waveform (Figure 2B).

Effects of β-bungarotoxin on perineural waveforms When β-bungarotoxin (0.15 μM) was added to preparations in the presence of 2.5 mM Ca^{2+}, there was a selective reduction in the second negative deflection (Figure 3A). This occurred between 5 and 10 min after exposure to the toxin, and the reduction, which was 27 ± 4%, was stable for at least 60 min. There was little or no change in other components of the waveform; for example, the first negative component was 96 ± 9% of control. Similar effects were found when β-bungarotoxin was added to preparations that were equilibrated in a solution in which $CaCl_2$ had been substituted with $SrCl_2$; the average reduction in the second negative waveform after 45 min was 13%. After exposure to β-bungarotoxin, 3,4-diaminopyridine still abolished the second negative waveform and subsequent addition of TEA produced a large positive component (Figure 3B).

With extracellular recording, the signal is the algebraic sum of inward and outward currents in the vicinity of the electrode tip. Hence, the decrease in amplitude of the second negative deflection seen in the presence of β-bungarotoxin could be caused by a reduction in the outward K^+ current at the nerve terminals or by an increase of the inward Ca^{2+} current. Moreover, effects on K^+ current could be

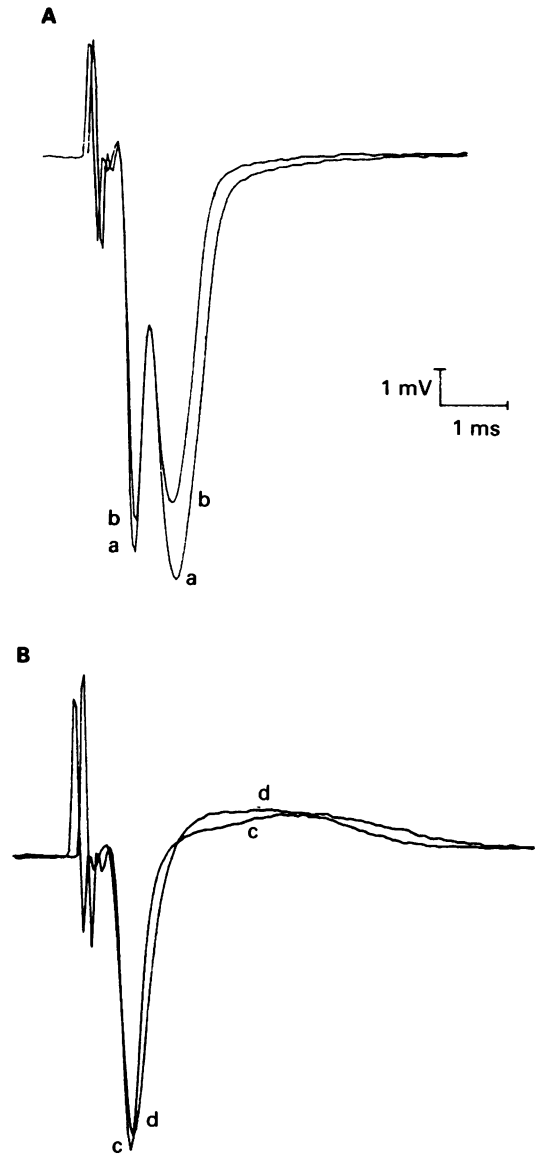

Figure 2 (A) Effect of removal of external Ca^{2+} on normal perineural waveforms of a mouse triangularis sterni preparation. (a) Normal waveform in presence of 2.5 mM Ca^{2+}; (b) waveform in the absence of Ca^{2+}. (B) Effect of 3,4-diaminopyridine and tetraethylammonium (TEA) on the waveform recorded in zero Ca^{2+}. (c) Waveform in the presence of 3,4-diaminopyridine (0.2 mM); (d) waveform in the presence of 0.2 mM 3,4-diaminopyridine and 1 mM TEA. In this and subsequent figures, all records were made from the same site. Note the large reduction (b) of the second negative component of the normal waveform on removal of external Ca^{2+}, and the inability of TEA to induce a large positive component (d).

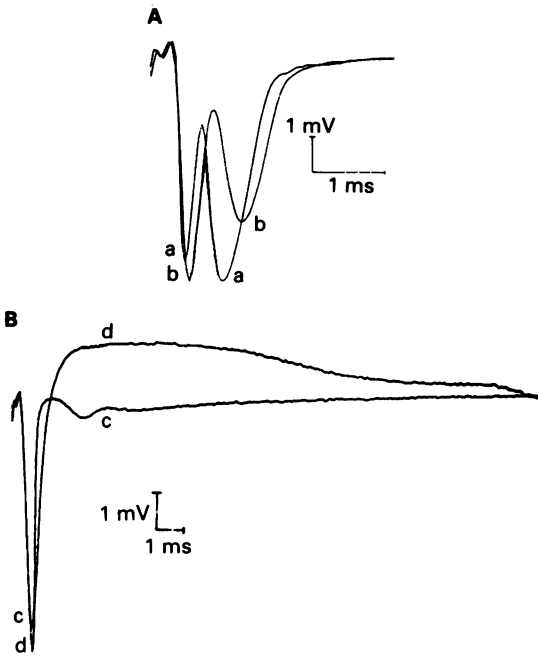

Figure 3 (A) Effect of β-bungarotoxin on the normal perineural waveform of a mouse triangularis sterni preparation. (a) Control waveform; (b) waveform 60 min after exposure to β-bungarotoxin (0.15 μM). Note the large reduction in the second negative component of the waveform. (B) Effect of 3,4-diaminopyridine and tetraethylammonium (TEA) after exposure to β-bungarotoxin. (c) Waveform in the presence of 0.2 mM 3,4-diaminopyridine; (d) waveform after addition of 1 mM TEA in the continued presence of the aminopyridine.

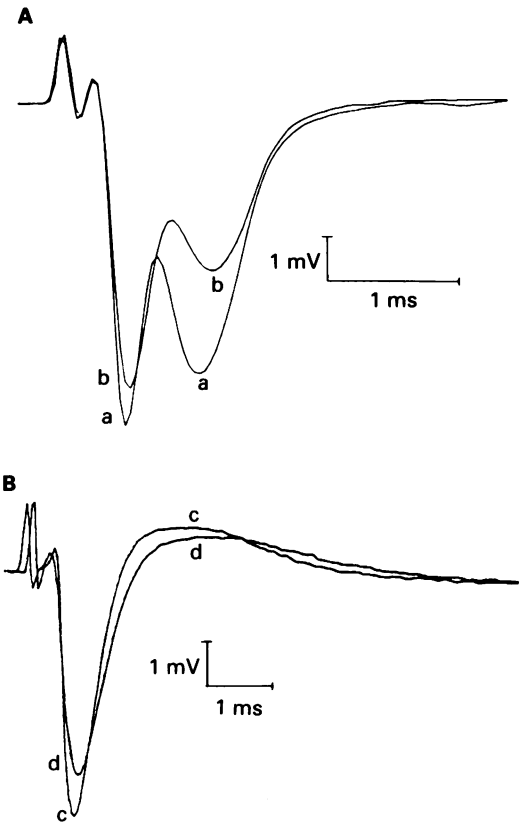

Figure 4 (A) Effect of β-bungarotoxin on the perineural waveform of a mouse triangularis sterni preparation in the absence of Ca^{2+}. (a) Control waveform in the absence of Ca^{2+}; (b) waveform 45 min after addition of β-bungarotoxin (0.15 μM). Note the large reduction in the second negative component of the waveform. (B) Effect of 3,4-diaminopyridine and tetraethylammonium (TEA) after exposure to β-bungarotoxin. (c) After 0.2 mM 3,4-diaminopyridine; (d) after 1 mM TEA in the continued presence of the aminopyridine.

associated with blockade of voltage-dependent K^+ channels or of calcium-activated K^+ channels. Further experiments were performed to distinguish between these possibilities.

Effects in low calcium solutions In the absence of Ca^{2+}, addition of 0.15 μM β-bungarotoxin reduced the second negative component by 36 ± 2%, with only a decrease of 11 ± 1% in the first negative spike (Figure 4A). The effect of β-bungarotoxin was apparent within 5–10 min, and was maintained constant for at least 45 min. Addition of 200 μM 3,4-diaminopyridine abolished the remainder of the second negative wave and revealed a small positive deflection (Figure 4B). As in control experiments in zero Ca^{2+}, subsequent addition of TEA had little effect.

Effects in the presence of potassium channel blockers It is not possible with the available recording methods to measure the Ca^{2+} current of motor nerve terminals under normal conditions. However, a Ca^{2+}-dependent waveform was revealed in the presence of K^+ channel blockers (see Figure 1). In an attempt to look directly at the effects of β-bungarotoxin on the waveform associated with the Ca^{2+} current, experiments were performed on preparations exposed to 600 μM 3,4-diaminopyridine and 10 mM TEA. These concentrations had previously been shown to produce the maximal effects of the compounds. Under these conditions, addition of 0.15 μM β-bungarotoxin had little effect on the positive waveform.

It is known that Ba^{2+} can block K^+ conductances in nerve membranes (Århem, 1980; Armstrong *et al.*, 1982; Hirst, *et al.*, 1985; Mallart, 1985b), so the effect

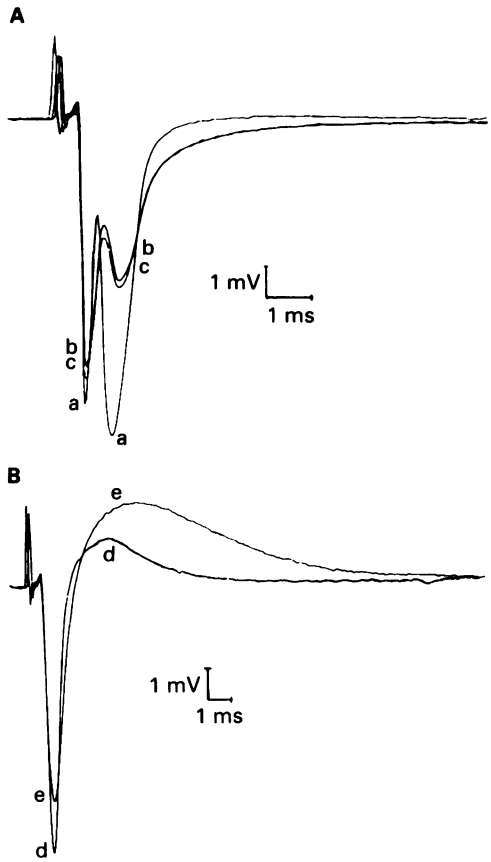

Figure 5 (A) Effect of substituting 2.5 mM Ca^{2+} with 2.5 mM Ba^{2+} on the perineural waveform of a mouse triangularis sterni preparation. (a) Control waveform in the presence of 2.5 mM Ca^{2+}; (b) waveform after substituting Ca^{2+} with 2.5 mM Ba^{2+}; (c) waveform after 60 min in the presence of 2.5 mM Ba^{2+}. Note the large reduction in the second negative component of the waveform after changing to Ba^{2+}. (B) Effect of 3,4-diaminopyridine and tetraethylammonium (TEA) in Ba^{2+}-containing solution. (d) After 0.2 mM 3,4-diaminopyridine; (e) after 1 mM TEA in the continued presence of the aminopyridine.

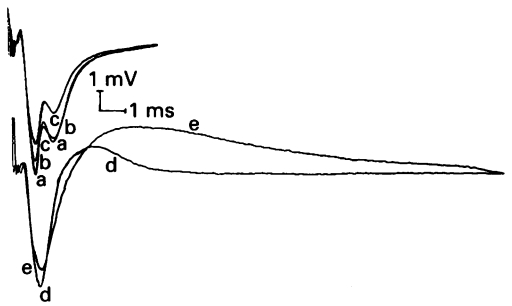

Figure 6 Effect of β-bungarotoxin on the perineural waveform of a mouse triangularis sterni preparation in the presence of Ba^{2+}. (a) Control waveform in solution containing 2.5 mM Ba^{2+}; (b) after 5 min exposure to β-bungarotoxin (0.15 μM); (c) after 60 min; (d) waveform after addition of 0.2 mM 3,4-diaminopyridine; (e) after addition of 1 mM tetraethylammonium in the continued presence of the aminopyridine. Note the decrease in both negative components of the waveform (a–c).

of substitution of Ca^{2+} by 2.5 mM Ba^{2+} was tested. Replacement of Ca^{2+} by Ba^{2+} resulted in extensive repetitive firing of the nerve terminal after single shock stimulation. This was suppressed by the addition of procaine at a concentration that did not reduce the size of the first negative deflection. In control experiments, on substitution of Ca^{2+} by Ba^{2+} there was an initial reduction of 13 ± 1% in the amplitude of the first negative component of the perineural waveform and a more marked decrease (49 ± 5%) in the second negative component (Figure 5A; see also Mallart, 1985b). Recordings were then reasonably stable over 60 min (Figure 5A). 3,4-Diaminopyridine and TEA were still effective in the presence of Ba^{2+} (Figure 5B).

In Ba^{2+}-containing solution, β-bungarotoxin (0.15 μM) no longer acted selectively, but reduced both components of the negative waveform (Figure 6). The decrease was apparent after 5 min, and became larger with time (Figure 6). After 60 min exposure to β-bungarotoxin, both negative deflections were reduced to 82 ± 9%. At this point, 200 μM 3,4-diaminopyridine blocked the second phase of the negative waveform and induced a positive component, which was augmented by the addition of 1 mM TEA (Figure 6).

In an experiment in which the preparation was equilibrated in solution containing 2 mM Ba^{2+} and 200 μM 3,4-diaminopyridine, there was a relatively large inward deflection. β-Bungarotoxin (0.15 μM) had little effect on this component of the waveform, although it did produce a small and progressive decrease in the first negative deflection.

Effects of β-bungarotoxin on frog preparations

As shown previously for sartorius muscle preparations (Caratsch *et al.*, 1981), β-bungarotoxin (0.1 μM) reduced the amplitude of e.p.ps recorded from cutaneous pectoris muscles in strontium-containing Ringer solution (Figure 7). Over a 30–45 min period, e.p.ps evoked at 0.5 or 0.05 Hz were reduced to 10–20% of control amplitude. There was less than a 10 mV fall in resting membrane potential from its control value of −80 mV, and there was little change in the time course of the e.p.ps.

Extracellular waveforms recorded by an electrode inserted into the perineural sheath of fine motor

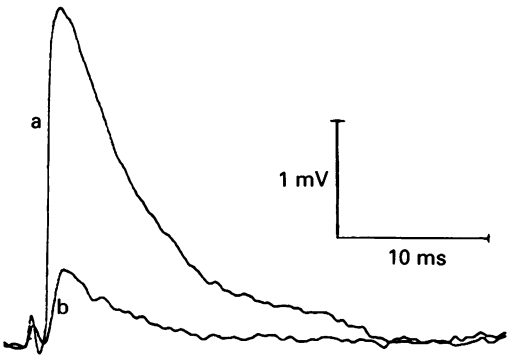

Figure 7 Effect of β-bungarotoxin on nerve-evoked endplate potentials (e.p.ps) recorded from a frog cutaneous pectoris preparation in the presence of 6 mM Sr^{2+} Ringer. (a) Averaged control e.p.p.; (b) averaged e.p.ps from the same neuromuscular junction 30 min after addition of β-bungarotoxin (0.1 μM).

nerve branches were similar in shape to those obtained from mouse triangularis sterni muscles. Exposure to 0.1 μM β-bungarotoxin for 25–40 min had no obvious effect on either of the negative deflections. In one experiment in which the concentration of β-bungarotoxin was increased to 3.1 μM, there followed a progressive fall in the amplitude of both negative deflections. After 90 min, the signal was about half of its original size. Under these conditions the nerve began to fire repetitively in response to single shock stimulation. These may correlate with spontaneous e.p.ps previously noted to be induced at frog neuromuscular junctions by β-bunngarotoxin (Abe *et al.*, 1976).

Effects of taipoxin, notexin, crotoxin, and a basic phospholipase

The three phospholipase toxins were tested on mouse triangularis sterni preparations after establishing effective concentrations on mouse hemidiaphragm preparations. Taipoxin and crotoxin both produced twitch augmentation, but, as previously described (Chang, 1985), notexin produced little augmentation.

Taipoxin (6.5 nM, 0.3 μg ml^{-1}) caused a slow decrease in the amplitude of the second negative spike of perineural waveforms. This effect was apparent after 5–10 min and became more pronounced after 20–60 min. The maximum decrease was about 40% (Table 1). Crotoxin (0.13 μM) also reduced the second negative waveform, although this was accompanied by a slight enhancement of the first negative component (Table 1). Notexin (0.25 μM) rapidly reduced the second negative waveform: after 10 min, it was about 70% of control, with no other change being apparent. With continued exposure to notexin, the second negative component was reduced further, with little change in other parts of the waveform (Table 1). After exposure to taipoxin, crotoxin, or notexin, 200 μM 3,4-diaminopyridine abolished the remaining fraction of the second negative phase of the waveform and induced a positive component, which could be subsequently enhanced by the addition of 1 mM TEA.

Table 1 Effects of taipoxin, crotoxin and notexin on the negative components of the perineural waveform recorded from mouse triangularis sterni preparations

Toxin	*First component* (% of control)	*Second component* (% of control)
Taipoxin	95 ± 10	60 ± 7
Crotoxin	108 ± 4	51 ± 13
Notexin	90 ± 5	41 ± 1

In contrast to the effects of the neurotoxic phospholipases, the basic phospholipase from *Naja naja siamensis* (20 μg ml^{-1}, about 1.5 μM, for 45 min) had no significant effects on the nerve terminal waveform.

Discussion

β-Bungarotoxin acts at the neuromuscular junction to cause an irreversible blockade of acetylcholine release (Chang & Lee, 1963; Chang *et al.*, 1973). Similar effects have been obtained with notexin (Harris *et al.*, 1973; Magazanik & Slavnova, 1978), taipoxin (Kamenskaya & Thesleff, 1974; Su & Chang, 1984), and crotoxin (Chang & Lee, 1977; Hawgood & Smith, 1977). In mammalian nerve-muscle preparations, the toxins generally cause a period when evoked transmitter is increased, before being blocked (Lee & Chang, 1966; Su & Chang, 1984; Chang, 1985). The mechanism underlying this prejunctional facilitatory effect is not known, although it has been postulated that it could be due to a reduction of Ca^{2+} uptake within motor nerve terminals following uncoupling of oxidative phosphorylation in mitochondria (Wernicke *et al.*, 1975), to increased probability of fusion of synaptic vesicles (Strong *et al.*, 1976), to enhanced sensitivity of the release mechanism to Ca^{2+}, or to an increase in Ca^{2+} entry during action potentials (Su & Chang, 1984). Results from the present experiments are consistent with there being an increased Ca^{2+} influx as a consequence of loss of a portion of the outward K^+ current at the nerve terminal. Block of this K^+ current would be predicted to prolong the depolarization of the nerve endings (Brigant & Mallart, 1982), and hence allow the voltage-dependent Ca^{2+} channels to open for longer than normal.

The results from external recordings can be ambiguous because of the difficulty in resolving inward and outward currents originating at different distances from the recording site. On the basis of previous work (Brigant & Mallart, 1982; Konishi, 1985; Mallart, 1985a,b; Penner & Dreyer, 1986), the perineural waveform from mouse triangularis sterni preparations has been regarded as being composed of two major components: an inward Na^+-dependent current at the nodes of Ranvier and the first regions of the unmyelinated terminal, and an outward K^+-dependent current which is restricted to the terminal regions. There is also an inward Ca^{2+} current at the terminals, but it is normally much smaller than the K^+ current and consequently it is hidden within the 'K^+ waveform'. At concentrations which increase acetylcholine release, β-bungarotoxin, taipoxin, and crotoxin all produced a selective decrease in the component of the waveform associated with terminal K^+ and Ca^{2+} currents. Notexin has a similar effect, although it is not known whether this toxin can also enhance quantal content in mouse neuromuscular preparations.

The effect of the toxins on the perineural waveform could be due to a decrease in outward K^+ currents or to an increase in the inward Ca^{2+} current at the nerve terminal. Our evidence is consistent with an effect on K^+ currents. Thus, β-bungarotoxin produced similar effects when there was no external Ca^{2+} ions to flow through Ca^{2+} channels, but had no effect on the Ca^{2+}-dependent waveform recorded in the presence of high concentrations of K^+ channel blocking drugs. Furthermore, the actions of β-bungarotoxin were blocked by substitution of Ca^{2+} by Ba^{2+}. As Ba^{2+} can flow through Ca^{2+} channels of motor nerve terminals (Silinsky, 1978), but block some K^+ channels (e.g. Hirst *et al.*, 1985), this result is consistent with β-bungarotoxin acting on K^+ rather than Ca^{2+} channels. Previously, β-bungarotoxin has been shown not to affect the uptake of $^{45}Ca^{2+}$ into synaptosomes (Wernicke *et al.*, 1975).

At the mammalian motor nerve terminal, there are voltage-dependent and Ca^{2+}-activated K^+ conductances (Mallart, 1985a,b). β-Bungarotoxin appears to act on the voltage-dependent K^+ current, because blocking $I_{K,Ca}$ by removal of external Ca^{2+} ions did not affect the action of the toxin. Although Ba^{2+} has some blocking effects on $I_{K,Ca}$ (Mallart, 1985b), it also blocks voltage-dependent K^+ currents; in myenteric neurones of guinea-pig ileum, Ba^{2+} was found to be a selective blocker of a transient I_K (Hirst *et al.*, 1985). During the course of the present experiments, it was found that β-bungarotoxin selectively blocked a fraction of the K^+ current recorded from voltage-clamped neurones in guinea-pig dorsal root ganglia (Petersen *et al.*, 1986). These results are consistent with our findings at the motor nerve terminal. Petersen *et al.* (1986) also demonstrated that dendrotoxin, a facilitatory toxin from green mamba venom (Harvey & Karlsson, 1980), could suppress a further fraction of the ganglionic K^+ current in the presence of β-bungarotoxin. Similarly, at the mouse neuromuscular junction, dendrotoxin inhibits a different component of the terminal K^+ current (Harvey & Anderson, 1985; Anderson & Harvey, 1987).

The early effects of β-bungarotoxin, crotoxin, taipoxin and notexin on mouse neuromuscular junctions are independent of the phospholipase activity of the toxins (see Chang, 1985). Likewise, the effects on the K^+ component of the perineural waveforms are not dependent on the enzymatic actions of the toxins because they were found in conditions that suppress the Ca^{2+}-dependent phospholipase A_2 activity (i.e. low temperature, removal of Ca^{2+}, and substitution of Ca^{2+} by Sr^{2+}). By contrast, the facilitatory phase of β-bungarotoxin's action in frog neuromuscular preparations is associated with phospholipase activity; in the presence of Sr^{2+}, β-bungarotoxin causes a concentration-dependent reduction in acetylcholine release (Caratsch *et al.*, 1981). This was confirmed in the present experiments, and it was found that β-bungarotoxin had no apparent effect on perineural waveforms of frog motor nerves. Previously, β-bungarotoxin, even at $100\,\mu g\,ml^{-1}$ (about $5\,\mu M$), had no gross effect on focally recorded nerve terminal spikes of frog sartorius preparations (Lee & Chang, 1966). Hence, the binding site for the toxin on mouse motor nerve terminals that is somehow associated with K^+ channels appears to be absent from frog motor endings or distinct from K^+ channels.

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