Br. J. Pharmac. (1984), **81**, 573–574

Effects of sea-anemone toxin (ATX-II) on the frequency of miniature endplate potentials at rat neuromuscular junctions

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Soleus and extensor digitorum longus muscles were isolated from rats. The muscles were exposed to ATX-II, a toxin isolated from extracts of the sea-anemone *Anemonia sulcata*. The toxin caused a dose-dependent increase in the frequency of miniature endplate potentials in both types of muscle. The increase in frequency could be reversed by the application of tetrodotoxin (TTX), and could be prevented by prior exposure of the muscles to TTX. It is concluded that ATX-II causes a sodium-dependent depolarization of the nerve-terminal membrane.

Introduction The sea-anemone (*Anemonia sulcata*) toxin, ATX-II, is a stable, basic polypeptide of 47 amino acid residues with a formula weight of 4770. It is a single chain polypeptide, cross-linked in three places by disulphide bridges (Béress *et al.*, 1975; Wunderer *et al.*, 1976).

The toxin delays the inactivation of the fast sodium channel in a variety of neuronal membranes, leading to a prolongation of the sodium-dependent action potential (Romey *et al.*, 1976; Bergmann *et al.*, 1976; Rathmayer & Béress, 1976), and the same mechanism is probably responsible for the prolongation of the action potential of vertebrate skeletal muscle fibres (Alsen *et al.*, 1981). The toxin also causes a sodium-dependent depolarization of skeletal muscle fibres.

It is generally held that the toxin does not depolarize motor nerve terminals and Alsen *et al.* (1981) reported that ATX-II (10^{-7} M) failed to cause any increase in the frequency of miniature endplate potentials (m.e.p.ps), even though the appropriate muscle fibres were depolarized by more than 30 mV. It has, however, been reported that ATX-II provokes transmitter release from rat brain synaptosomes in a similar fashion to veratridine, and it was suggested that ATX-II causes a depolarization of the presynaptic membrane (Abita *et al.*, 1977). In this communication we show that m.e.p.p. frequency is increased at rat neuromuscular junctions by exposure to large concentrations of ATX-II, and we suggest that the effect is due to a sodium-dependent depolarization of the nerve terminal.

Methods The experiments were carried out on soleus (SOL) or extensor digitorum longus (EDL) muscles isolated from female Wistar rats weighing 120–160 g. The isolated muscles were maintained in a bathing solution with the composition (mM): K^+ 5.0, Na^+ 150, Ca^{2+} 2.0, Mg^{2+} 1.0, Cl^- 148, $H_2PO_4^-$ 1.0, HCO_3^- 12.0 and D-glucose 11.0. The bathing fluid was aerated with 95% O_2/5% CO_2 and maintained at room temperature.

M.e.p.ps were recorded from putative endplates using standard intracellular techniques.

All results are presented as arithmetic mean ± standard error of the mean. Figures in parentheses represent numbers of observations.

ATX-II was a gift from Dr L. Béress, University of Kiel.

Results Mean m.e.p.p. frequency in control EDL muscle fibres (3.2 m.e.p.ps s^{-1}) was rather greater than that of comparable SOL fibres (1.8 m.e.p.ps s^{-1}). In both types of muscle, ATX-II caused a dose-dependent increase in m.e.p.p. frequency (Table 1).

The increase in frequency in muscle fibres of both EDL and SOL could be reversed by the introduction

Table 1 M.e.p.p. frequency (s^{-1}) in rat extensor digitorum longus (EDL) and soleus (SOL) muscle fibres in the presence of ATX-II.

	Control	*ATX-II* (10^{-7} M)	*ATX-II* (10^{-6} M)
EDL	3.2 ± 0.2 (105)	7.8 ± 2.0 (15)	10.3 ± 1.8 (41)
SOL	1.8 ± 0.2 (109)	7.7 ± 3.6 (20)	15.4 ± 3.7 (34)

Figures are presented as arithmetic mean ± s.e.

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of tetrodotoxin (TTX), 10^{-6} M into the muscle bath. For example, in two experiments on SOL muscles, m.e.p.p. frequency increased from control levels of 1.7 ± 0.21 ($n = 8$) and 1.1 ± 0.16 ($n = 6$) m.e.p.p.s s^{-1} to 43.1 ± 13.4 ($n = 5$) and 28.7 ± 6.6 ($n = 5$) m.e.p.p.s s^{-1} following exposure to ATX-II (10^{-6} M). The introduction of TTX (10^{-6} M) to these muscles, resulted in the reduction of m.e.p.p. frequency to 4.8 ± 1.9 ($n = 8$) and 2.0 ± 0.75 ($n = 7$) m.e.p.p.s s^{-1} respectively. Similar results were obtained with two EDL muscles.

Pre-incubation with TTX prevented the increase in m.e.p.p. frequency caused by ATX-II. For example, in one experiment on a SOL muscle, m.e.p.p. frequency was 1.4 ± 0.20 s^{-1} ($n = 6$) in control bathing fluid. The muscle was incubated for 15 min with TTX (10^{-6} M) before the addition of ATX-II (10^{-6} M). M.e.p.p. frequency was unchanged (1.2 ± 0.1 s^{-1}, $n = 9$) after exposure to ATX-II. Similar results were obtained on a single EDL muscle exposed to ATX (10^{-6} M), and on 1 EDL and 2 SOL muscles exposed to ATX-II (10^{-7} M).

The mean m.e.p.p. frequencies recorded in SOL and EDL muscles exposed to ATX-II, 10^{-6} M (Table 1) were similar to those recorded in muscles exposed to $[K^+]_o = 25$ mM (SOL: 14.5 ± 2.0 s^{-1}, $n = 12$; EDL: 18.8 ± 3.2 s^{-1}, $n = 12$).

Discussion The sea-anemone toxin, ATX-II was shown to cause a dose-dependent increase in m.e.p.p. frequency in muscle fibres of both EDL and SOL muscles of the rat. The effect on m.e.p.p. frequency was marginal at 10^{-7} M and marked at 10^{-6} M. It should be noted that the experiments of Alsen *et al.* (1981), who claimed that ATX-II had no effect on m.e.p.p. frequency, were limited in extent and were made using ATX-II at a concentration of 10^{-7} M. This concentration of ATX-II has marked effects on the evoked endplate potential, and on the muscle fibre membrane of rat SOL muscles.

The increase in m.e.p.p. frequency probably results from a sodium-dependent depolarization of the motor nerve terminal because the effects of ATX-II could be blocked by TTX. Moreover, the observation that the effects of ATX-II could be reversed by TTX indicates that, at least in the short-term, the increase in m.e.p.p. frequency is not associated with physical damage to the nerve terminal.

Increasing $[K^+]_o$ to 25 mM resulted in an increase in m.e.p.p. frequency to values comparable to those caused by exposure to ATX-II (10^{-6} M). It is not possible to measure directly the resting potential of the motor-nerve terminal. If it is assumed that $[K^+]_i$ of the motor-nerve terminal is similar to that of the muscle fibre, the effects of changes in $[K^+]_o$ should be similar in the two tissues. In SOL fibres, the mean muscle fibre membrane was depolarized by 21.8 mV (Em in $[K^+]_o = 5$ mM: -72.5 ± 0.3 mV).

In conclusion, it is suggested that ATX-II causes a sodium-dependent, reversible, depolarization of the motor-nerve terminals of rat EDL and SOL muscle fibres. The depolarization results in an increase in m.e.p.p. frequency. The effects of ATX-II on the motor nerve terminal are similar to those of batrachotoxin (Alburquerque *et al.*, 1971).

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(*Received December 8, 1983.*)