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ACTIONS OF THE CRUDE VENOM OF THE SYDNEY FUNNEL-WEB SPIDER, *Atrax robustus* ON AUTONOMIC NEUROMUSCULAR TRANSMISSION

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1 The effects on mammalian autonomic neuromuscular transmission of the crude venom of the female Sydney funnel-web spider *Atrax robustus*, have been investigated.

2 At doses of 10 μg/ml or lower the indirectly elicited twitch-like responses of the rat anococcygeus preparation were inhibited. At doses greater than 10 μg/ml there was an initial reduction in the twitch-like response followed by a sustained contracture of the tissue.

3 The long-lasting contracture caused by the venom was abolished by the application of phentolamine. It was virtually non-existent in muscle preparations isolated from reserpine-treated rats.

4 In the presence of tetrodotoxin the contracture was smaller and less well maintained than in its absence.

5 The venom caused a small reduction in the amplitude of the indirectly elicited twitch-like response of the longitudinal muscle of the guinea-pig ileum, followed by an increase in the tone of the preparation. The increase in tone was maintained for several minutes and was rapidly abolished by the application of atropine. The presence of venom did not affect control responses to either histamine or acetylcholine.

6 Inhibitory transmission in the rat anococcygeus preparation was unaffected by the venom.

7 The neurally-mediated twitch-like responses of both guinea-pig and rat vas deferens were inhibited by the venom at doses below 10 μg/ml. At higher doses the inhibition was accompanied by spontaneous contractions, and at doses in excess of 100 μg/ml the inhibition of twitch-like responses was transient and was followed by a potentiation of the motor response and extensive spontaneous activity. The preparation became quiescent 20 min after the application of venom and the evoked response was abolished after 60 min.

8 The venom had qualitatively similar effects on motor transmission in the human vas deferens as on the rat and guinea-pig preparations. However, the human preparations were 50 to 100 times more sensitive to the effects of the venom.

Introduction

The Sydney funnel-web spider, *Atrax robustus* is a large aggressive spider; it is responsible for several cases of severe poisoning every year in and around the city of Sydney. Symptoms of poisoning may appear within 10 min of envenomation. They include nausea and vomiting, abdominal pain and diarrhoea, profuse sweating, brisk salivation, lachrymation, severe hypertension and dyspnoea (Sutherland, 1978a). During this period, local and generalised muscle fasciculations appear which may be severe, and the patient may lapse into profound coma. Within an hour of envenomation, the severely poisoned patient may become normotensive, and muscle fasciculation and evidence of hyperactivity in the autonomic nervous system may subside. In the occasional, fatally envenomated patient, death may occur after 15 min to 24 h (Sutherland, 1978b), sometimes from asphyxia and sometimes from cardiac arrest. In the latter event, the cardiac arrest may be preceded by a prolonged period of hypotension.

There is no antivenom available and treatment of the patient is symptomatic. It is imperative, therefore, that the mode of action of the venom be fully understood, and in this paper some observations on its

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action on a variety of animal smooth muscle preparations and on the human isolated vas deferens are described. Some of the results were communicated at the 6th International Symposium on Animal, Plant and Microbial toxins held in Uppsala in 1979 and have been published in the form of an abstract (Harris, Sutherland & Zar, 1979).

Methods

Experiments were carried out on isolated preparations obtained from male rats or guinea-pigs and on pieces of vas deferens obtained from two young men undergoing vasectomy and orchidectomy respectively. The isolated preparations were carefully cleaned in a dish containing a bathing fluid of the following composition (mM): NaCl 112.90, KCl 4.69, $CaCl_2$ 2.52, $MgSO_4$, $7H_2O$ 1.5, KH_2PO_4 1.18, $NaHCO_3$ 25.0 and glucose 11.0. They were mounted in a 10 ml organ bath containing the bathing fluid maintained at 37°C and equilibrated with 95% and 5% CO_2. The muscular contractions (and relaxations) were monitored continuously with isometric strain-gauges, and were recorded on a pen-recorder. Electrical stimulation, when required, was delivered through two parallel, vertical built-in platinum electrodes in the organ bath. The electrodes were connected to a stimulator (Bell & Stein, 1971) designed to deliver up to 30 V at 800 mA.

Preparations

The following preparations were used:

Rat anococcygeus In the rat anococcygeus preparation (Gillespie, 1972) trains of 5 pulses at 10 Hz repeated every 60 s were used. The pulse duration was routinely 1 ms delivered at supramaximal voltage.

Rat and guinea-pig vas deferens The isolated tissues were cleaned and desheathed. Portions of the bladder end of the tissues, 1 to 1.5 cm long, were mounted for examination. The preparations were stimulated as for the rat anococcygeus.

Plexus-containing longitudinal muscle of guinea-pig ileum Preparations were set up as described by Paton & Zar (1968) from ileum of large (≃400 g) guinea-pigs. They were stimulated with single pulses repeated every 20 s. The pulse duration was 0.2 ms delivered at supramaximal voltage.

Human vas deferens The tissues were cleaned and mounted as described above. They were stimulated with trains of 5 pulses of 0.2 ms pulse duration at 10 Hz delivered every 3 min.

Reserpine treatment of rats Two rats each received two injections of reserpine. The drug solution was freshly made daily and the dose schedule adopted was 10 mg/kg (s.c.) on day 1 followed by 10 mg/kg (i.p.) on day 2. The animals were used on day 3.

Drugs and chemicals used

The following drugs were used: acetylcholine chloride (BDH); atropine sulphate (Sigma); carbamylcholine chloride (Sigma); histamine acid phosphate (BDH); (−)-noradrenaline bitartrate (Koch-Light); phentolamine methanesulphonate (Ciba); reserpine phosphate (Sigma); tetrodotoxin (Sankyo).

Spider venom

The venom, supplied as a freeze-dried powder, was collected by aspiration from the fangs of female spiders maintained at the Commonwealth Serum Laboratories, Parkville, Victoria. The venom, batch number CSL 3376, was collected during April, 1976, and used during the winter of 1978.

Design of experiments

Little venom was available since most material, particularly the more potent venom of the male spider, is used for immunological studies. Thus each result described or illustrated refers to a single experiment. However, all experiments were done twice and most were repeated 3 times. The majority of experiments were on the rat anococcygeus muscle since its motor response is known to be mediated by the release of noradrenaline from the motor nerve terminals. The rat and guinea-pig vas deferens preparations were used for comparison with the human preparations. The longitudinal muscle of guinea-pig ileum was used as an example of a neuromuscular system in which acetylcholine is the motor transmitter.

Results

Effects on motor transmission in the rat anococcygeus

In a dose of 2 μg/ml the venom caused a reduction of about 25% in the twitch-like response of the indirectly stimulated preparation; in a dose of 20 μg/ml, the response of the tissue was reduced by about 50%. However, the response to higher doses of venom was an initial reduction in twitch-like response followed by a massive and sustained contracture of the tissue. A typical experiment illustrating these effects of the toxin is illustrated in Figure 1.

The inhibition of the motor response caused by the venom was not due to a postsynaptic effect on the

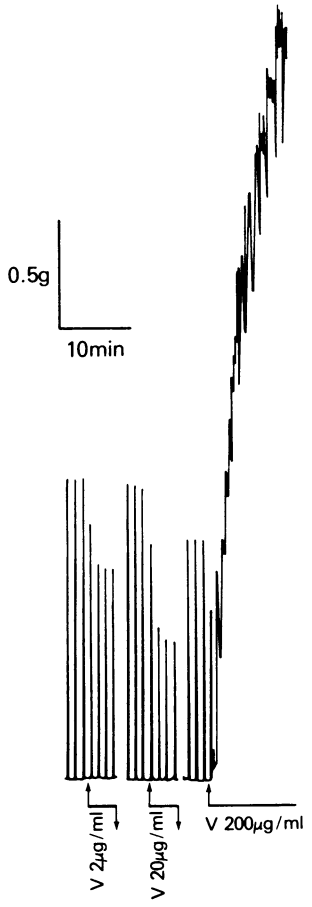

Figure 1 The effects of increasing concentrations of funnel-web spider venom (V) on the responses of the rat isolated anococcygeus preparation to electrical field stimulation. At the low doses, the venom reduced the height of the evoked contraction; at the high dose it caused a prolonged contracture of the muscle.

muscle resulting in a reduction in sensitivity to the motor transmitter (noradrenaline) because contractions elicited by exogenous noradrenaline (2.5×10^{-6} M) were unaffected by the presence of venom (Figure 2).

The long-lasting contracture caused by large doses of venom was rapidly and completely abolished by the application of phentolamine (10^{-7} M; Figure 3) and was virtually non-existent in muscle preparations isolated from reserpine-treated rats, suggesting that the venom causes the contracture by initiating or accelerating the release of transmitter from the motor nerve terminals. In the presence of tetrodotoxin in a dose that abolished neurally mediated twitch-like responses (5×10^{-7} M), the contracture elicited by the

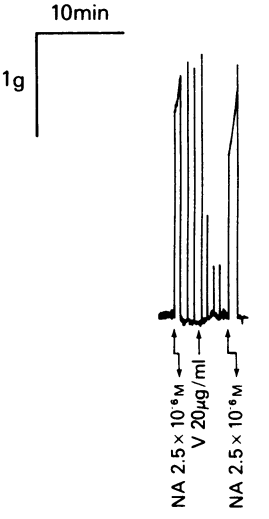

Figure 2 Exposure of the rat isolated anococcygeus preparation to venom (V) resulted in inhibition of the electrically evoked response, but did not affect the sensitivity of the muscle to exogenous noradrenaline (NA).

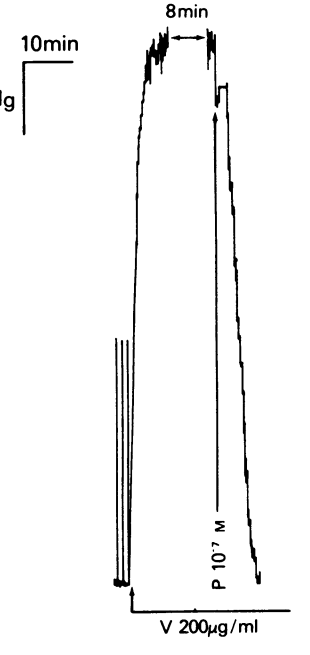

Figure 3 The sustained contracture of the rat isolated anococcygeus preparation caused by large doses of venom (V), is reversed by phentolamine (P) indicating that the venom either stimulates α-adrenoceptors on the muscle, or causes release of transmitter from the motor nerve terminals.

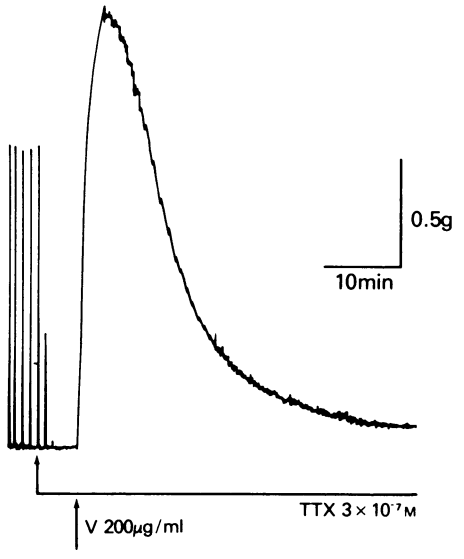

Figure 4 The venom-induced contracture of the rat isolated anococcygeus preparation was partially resistant to the effects of tetrodotoxin (TTX).

spider venom (200 μg/ml) was smaller and less well maintained than that seen in the absence of tetrodotoxin (Figure 4).

Effects on inhibitory transmission in the rat anococcygeus

Inhibitory responses of the preparation were revealed by field stimulation with trains of 5 pulses delivered at 10 Hz every 3 min in the presence of phentolamine (10^{-7} M) and carbachol (10^{-5} M). Funnel-web spider venom (200 μg/ml) had no effect on either the tone of the preparation or on the amplitude or duration of the relaxation induced by the stimulation of the inhibitory nerves.

Effects on motor transmission in the longitudinal muscle of the guinea-pig ileum

A single experiment was carried out on a plexus-containing preparation of longitudinal muscle of the guinea-pig ileum. Spider venom (20 μg/ml) caused a small (<20%) reduction in the amplitude of the evoked twitch-like response of the preparation, followed by an increase in tone. The tension developed was similar to that of the twitch-like response (≃2 g) and was maintained for several minutes; it was rapidly abolished by the application of atropine (10^{-6} M). Control responses to histamine (10^{-6} M) and acetylcholine (10^{-6} M) were unaffected by the venom.

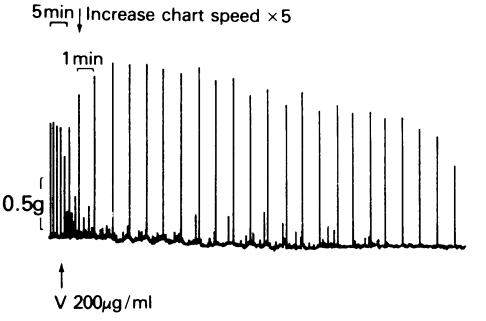

Figure 5 The rat isolated vas deferens preparation responds to venom (V) with a transient inhibition of the neurally-evoked contraction followed by an increase (approximately 50%) in the amplitude of the evoked response. Spontaneous activity is apparent between neurally evoked contractions.

Effects on motor transmission in the guinea-pig and rat vas deferens

The neurally mediated motor response of both the guinea-pig and the rat vas deferens was inhibited by funnel-web spider venom (2 μg/ml) by about 20%. At higher doses (20 μg/ml) the inhibition was accompanied by spontaneous contractions. At a dose of 200 μg/ml, the venom caused a slight (≃20%) and transient inhibition of the motor response, which was followed by a potentiation of the motor response by about 50%. The preparations also became spontaneously active, but this activity declined after about 10 min contact with the venom, and the preparations became quiescent after 20 min (Figure 5). The evoked response of the preparation was abolished after about 60 min.

Effects on motor transmission in the human vas deferens

Qualitatively, the effects of the venom on transmission in the human isolated vas deferens were similar to those seen in the rat and guinea-pig preparations. However, the human preparations were 50 to 100 times more sensitive. Thus, in the human preparation obtained during an orchidectomy, 4 μg/ml of venom caused a 50% reduction followed by a massive potentiation of the neurally mediated twitch-like response and the appearance of spontaneous phasic contractions (Figure 6). The spontaneous contractions were reminiscent of those caused by the application of noradrenaline to normal human isolated vas deferens (Thompson & Zar, unpublished). Increasing the dose of the venom to 12 μg/ml caused a further increase in the twitch-like response of the human preparation (Figure 6) and very large spontaneous phasic contractions appeared. The spontaneous contractions disappeared 3 to 4 min after exposure to the higher dose of

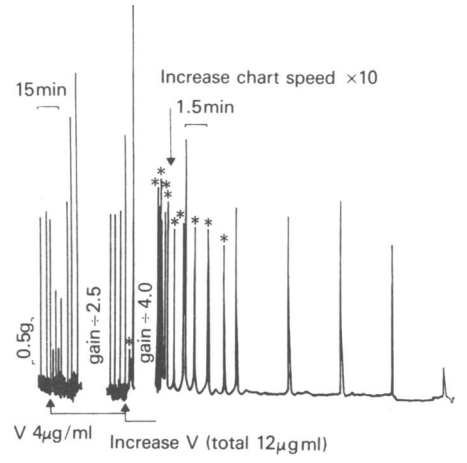

Figure 6 Vas deferens preparations obtained from a 23 year old man undergoing orchidectomy. Pre-surgical medication: minor analgesics (unspecified). The venom (V) caused a transient inhibition followed by a massive potentiation of the evoked contraction. Powerful spontaneous contractions of the muscle appeared (*).

venom, and neuromuscular transmission ceased within 10 min.

Discussion

The experiments described in this paper were designed to provide some information on the effect of funnel-web spider venom on transmission at neuromuscular junctions in the autonomic nervous system. Various isolated preparations were made from rats and guinea-pigs, representing examples where transmission is mediated by noradrenaline (motor response of the rat anococcygeous), acetylcholine (motor response of the longitudinal muscle of guinea-pig ileum) and by an unknown transmitter (motor transmission in the rat and guinea-pig vas deferens; inhibitory response of the rat anococcygeous). The motor transmitter in the human vas deferens is probably noradrenaline (Anton & McGrath, 1977).

With the exception of inhibitory transmission in the rat anococcygeus muscle, transmission in all other animal preparations was impaired by the application of venom in the lowest effective doses. Experiments on both the anococcygeus muscle and the longitudinal muscle of the guinea-pig ileum showed that the presence of venom had no such inhibitory effect on contractions elicited respectively by exogenous noradrenaline or by acetylcholine and histamine, suggesting that the inhibition of the neurally mediated response was prejunctional rather than postjunctional in origin.

Similarly, in all instances other than that of inhibitory transmission in the rat anococcygeus, there was evidence of the initiation of or an increase in the spontaneous release of the appropriate transmitter. In the anococcygeus and the longitudinal muscle of the ileum, the increase in release of the motor transmitter was indicated by the development of a sustained, large contracture and in the vas deferens preparations by the appearance of spontaneous phasic contractions. In the anococcygeus and the longitudinal muscle of the ileum, the contracture could be reversed rapidly and completely by the application of the relevant neuromuscular blocking drug (phentolamine and atropine respectively). Further evidence that the contracture was caused by the release of the motor transmitter was provided by the observation that the response of the anococcygeus muscle was not present in preparations obtained from reserpine-treated animals.

In skeletal muscle of the mouse, funnel-web spider venom causes fasciculations which are associated with spontaneous electrical activity generated in the motor nerves (Spence, Adams & Gage, 1977; Gage & Spence, 1977); the fasciculations do not appear to have their origin in the muscle fibres themselves (Gage & Spence, 1977). In the anococcygeus muscle, the contracture caused by exposure to higher doses of venom was not prevented by the application of tetrodotoxin, although the duration of the contracture was reduced. It seems probable, therefore, that the increase in the spontaneous release of transmitter in this tissue was only partially due to the initiation of spontaneous electrical activity in either the motor nerve terminals or the axons.

All the animal preparations were rather insensitive to the venom, requiring a dose of about 1 μg/ml for the first signs of activity, and 100 μg/ml for the 'full' expression of activity. This dose range is identical to that established by previous workers for other tissues (Gage & Spence, 1977; Spence *et al.*, 1977; Carroll & Morgans, 1976; 1978; Morgans & Carroll, 1976; 1977), and is consistent with the general view that non primates are resistant to severe systemic poisoning following envenomation by *Atrax robustus* (Sutherland, 1978a). However, it should be noted that almost all work on this venom (with the exception of that of Spence *et al.*, 1977) has used venom from the female spider. Such venom is 6 to 10 times less potent than the venom of the male spider (Sutherland, 1978a); indeed, where the sex of the offending spider has been determined, fatal envenomation of human subjects has always been made by a male spider (Sutherland, 1976).

The relatively low sensitivity of animal preparations to the effects of the venom compared with the sensitivity of the human tissue raises the question as to whether observations made on animal preparations have any relevance to human envenomation.

Experiments on human material are few. However, Carroll & Morgans (1976) found that human isolated intercostal muscle responds to the venom in a qualitatively and quantitatively similar fashion to rodent neuromuscular junctions, an observation supported by a preliminary statement by Gage & Spence (1977) and attributed to Morgans & Spence as an 'unpublished observation'. The venom also has a weak vasoconstrictor action on human isolated temporal artery (Morgans & Carroll, 1977), but venom doses of around 1 mg/ml are required to elicit the vasoconstrictor response. Our own experiments demonstrated that although qualitatively similar, the human vas deferens was 50 to 100 times more sensitive to the effects of the venom than comparable animal preparations (compare Figure 5 with Figure 6).

In summary, Sydney funnel-web spider venom appeared to inhibit the neurally mediated release and to increase the spontaneous release of the transmitter from the majority of neuromuscular synapses in the mammalian autonomic nervous system. This pattern of activity is not unique to the spider venom. The venoms of other arachnids and in particular of the scorpions *Leiurus quinquestriatus*, *Androctonus australis* and *Tityus serrulatus* have been shown to exert similar effects on a variety of autonomic neuromuscular preparations (see for example Diniz, Pimenta, Netto, Pompolo, Gomez, Böhm & Preto, 1974; Tintpulver, Zerachia & Slotkin, 1976; Einhorn & Hamilton, 1977).

It is not known whether the various properties of the venom derive from the activity of a single component (for example, the toxin *Atraxotoxin*; Sutherland, 1973) or whether various components are involved. Nor is the reason for the relatively high sensitivity of human tissues known. These particular problems are to be the subject of further investigation.

Note added in proof

Sutherland (personal communication), has recently isolated a purified IgG (antivenom) from rabbits immunized with male *Atrax robustus* venom. The preparation has been shown to neutralize *in vitro* the venom of both *A. robustus* and *A. formidabilis*.

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