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THE EFFECTS OF A TOXIN ISOLATED FROM AUSTRALIAN TIGER SNAKE *(Notechis scutatus scutatus)* VENOM ON AUTONOMIC NEUROMUSCULAR TRANSMISSION

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1 The effects on mammalian autonomic neuromuscular transmission of a purified toxin from the crude venom of the Australian tiger snake, *Notechis scutatus scutatus*, have been investigated.

2 The toxin had no measurable effect on transmission in either the rat anococcygeus, the rat vas deferens, or the longitudinal muscle of the guinea-pig ileum.

3 The toxin induced a contraction of longitudinal smooth muscle of the ileum. The tissue relaxed in spite of the continued presence of the toxin, and remained insensitive to further doses. The contractile response was not mediated by either cholinergic or histaminergic mechanisms.

4 The toxin caused an inhibition of the response to field stimulation of the guinea-pig vas deferens and the guinea-pig seminal vesicle. The inhibition was spontaneously reversible, and the preparations remained insensitive to further doses of the toxin. The effects of the toxin were not mediated by either prostaglandins or by noradrenaline.

5 The inhibitory effects of a variety of compounds known to act at presynaptic sites were also blocked by exposure to the toxin; inhibition caused by postsynaptic activity was unaffected by the toxin.

6 It is suggested that the toxin inhibits transmission in the guinea-pig vas deferens and seminal vesicle by a presynaptic mechanism.

7 The possibility that the mechanism involves phospholipase A_2 activity is discussed.

Introduction

Notexin, a toxin isolated from the crude venom of the Australian tiger snake, is one of the most completely characterized snake toxins so far studied. It is a basic protein, of 119 amino acids in a single chain, cross-linked by 7 disulphide bridges. The amino acid sequence is known, and some of its biochemical properties have been documented (Karlsson, Eaker & Ryden, 1972; Halpert & Eaker, 1975; Halpert, Eaker & Karlsson, 1976).

The toxin was originally classified as a presynaptically active neurotoxin on account of its activity at the mammalian skeletal neuromuscular junction (Harris, Karlsson & Thesleff, 1973; Cull-Candy, Fohlman, Gustavsson, Lüllman-Rauch & Thesleff, 1976), although it is now clear that it is also one of the most potent myotoxins known (Harris, Johnson & Karlsson, 1975; Pluskal, Harris, Pennington & Eaker, 1978).

In this paper we describe our observations on the effects of the toxin on autonomic neuromuscular transmission.

Methods

All experiments were performed on isolated preparations obtained from male guinea-pigs and rats. The animals were killed by concussion and exsanguination. 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 22.2. The cleaned preparations were mounted in a 10 ml organ bath containing the bathing fluid maintained at 37°C and equilibrated with 95% O_2 and 5% CO_2. The preparations were allowed to equilibrate in the bathing fluid for approximately 95 min before being exposed to any drug. The muscular contractions (and relaxations) were monitored continuously with isometric strain-gauges, and were recorded on a pen-recorder (Bryan Southern Instruments). 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) which is designed to deliver up to 30 V at 800 mA.

Preparations

The following preparations were used:

Guinea-pig vas deferens. The method of isolating, desheathing and mounting the guinea-pig isolated vas deferens preparation was as described by Ambache & Zar (1971). A resting tension of approximately 0.5 g was maintained throughout the experiment. The parameters of electrical field stimulation used were: trains of 5 pulses at 10 Hz repeated every 60 seconds. Pulse duration was routinely 1 ms delivered at supramaximal voltage.

Plexus-containing longitudinal muscle of guinea-pig ileum. Large guinea-pigs weighing over 400 g were used. The preparations were made from any part of the ileum excluding the terminal 20 cm portion, in the manner described by Paton & Zar (1968). A resting tension of approximately 0.3 g was applied to the preparation. The parameters of electrical field stimulation used were: single pulses of 0.2 ms duration delivered at a supramaximal voltage every 20 seconds.

Guinea-pig bladder. An isolated preparation of the guinea-pig detrusor muscle was prepared and set up as described by Ambache & Zar (1970b). The preparation was maintained at a resting tension of 0.5 gram. The parameters of electrical field stimulation used were: trains of 5 pulses at 10 Hz repeated every 60 seconds. Pulse duration was 0.2 ms at supramaximal voltage.

Guinea-pig seminal vesicle. Segments of seminal vesicle 5 to 6 cm in length were prepared and set up at a resting tension of approximately 0.5 gram. The parameters of electrical field stimulation used were as described for the guinea-pig vas deferens preparation.

Rat vas deferens. The removal, preparation, mounting and stimulation of the preparation was as described for the guinea-pig vas deferens preparation.

Rat anococcygeus. The rat anococcygeus preparation was made as described by Gillespie (1972). The parameters of electrical stimulation used were as described for the guinea-pig vas deferens. The preparation is normally in a fully relaxed state in the organ bath, and each train of electrical pulses evokes a neurogenic motor response. A neurogenic inhibitory response can be obtained provided that (a) motor transmission is paralysed and (b) the tone of smooth muscle is raised. In our investigation both of these conditions were fulfilled by immersing the preparation in clonidine (3×10^{-8} M). Clonidine initiates a rise in tension by stimulating postsynaptic adrenoceptors, and blocks motor transmission by inhibiting the release of noradrenaline (Idowu & Zar, 1976).

Reserpine treatment of guinea-pigs

The guinea-pigs received two injections of a freshly prepared reserpine phosphate solution. The dose schedule was 10 mg/kg subcutaneously on day 1 and 10 mg/kg intraperitoneally on day 2. The animals were killed on day 3.

Drugs

The following drugs were used: acetylcholine chloride (BDH); (+)-amphetamine (Mawson & Proctor); atropine sulphate (Sigma); clonidine hydrochloride (Boehringer-Ingelheim); histamine acid phosphate (BDH); indomethacin (Sigma); isoprenaline sulphate (Burroughs-Wellcome); mepyramine maleate (May & Baker); (−)-noradrenaline bitartrate (Koch-Light); phentolamine methanesulphonate (Ciba); (±)-propranolol hydrochloride (Sigma); prostaglandin E_2 (Upjohn) and tyramine hydrochloride (Sigma).

Toxins

The toxins were obtained from D. Eaker, Institute of Biochemistry, University of Uppsala. They are defined as follows:

Notexin. A basic protein of 119 amino acids with a formula weight of 13,574.

Notexin/p-bromophenacyl bromide. Histidine at position 48 of notexin is specifically modified by incubation with *p*-bromophenacyl bromide at 30°C in an appropriate medium. The modified compound (PBP-notexin) has been prepared and characterized by Halpert *et al.* (1976).

Naja nigricollis *phospholipase* A_2. A basic protein homologous with notexin. It has higher catalytic activity against ovolecithin than notexin in the absence of deoxycholate. In the presence of deoxycholate, notexin has higher activity. The compound has been characterized by Obidairo, Tampitag & Eaker (Eaker, personal communication).

Other chemicals

All other chemicals used were obtained from the usual commercial sources. They were routinely of the highest grade available.

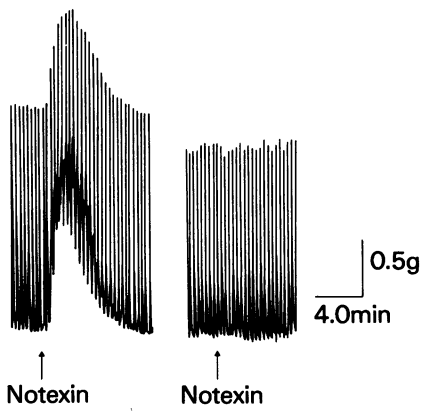

Figure 1 An isolated, innervated longitudinal muscle strip from guinea-pig ileum in which twitch-like responses were generated every 20 s following field stimulation (single pulses, 0.2 ms duration, supramaximal intensity). Notexin, (5.0 μg/ml) was added as indicated. Note the mechanical response induced by the first dose of notexin, the lack of response to the second dose of notexin, and the relatively slight effect on the electrically induced response. The interval between the two panels represents a time period of 120 min during which the preparation was repeatedly washed in fresh Krebs-Henseleit solution.

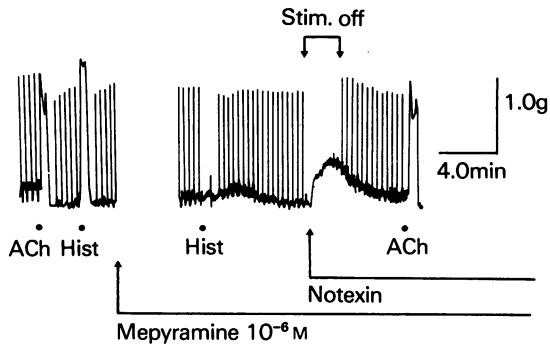

Figure 2 An isolated, innervated longitudinal muscle strip from guinea-pig ileum stimulated as in Figure 1. At ACh, acetylcholine, (10^{-7} M) and at Hist, histamine (3×10^{-7} M) were added. Contact time was held to 1 min, stimulation being suspended during this time. The preparation was then exposed to mepyramine, (10^{-6} M). Thirty min later, the control dose of histamine was added to the bath, followed by notexin, (5.0 μg/ml) and acetylcholine as indicated. Note that the mechanical response to notexin is unaffected by the presence of mepyramine, and that exposure to notexin has no effect on the sensitivity of the preparation to acetylcholine.

Results

Effects on cholinergic transmission

The motor response of the longitudinal muscle of the guinea-pig ileum. Experiments were conducted on five plexus-containing preparations of longitudinal muscle of the guinea-pig ileum. In the longitudinal muscle strips, notexin, (5 μg/ml) caused a contraction of the muscle. The contraction was not maintained, and the muscle relaxed in spite of the continued presence of the toxin. The muscle was insensitive to further doses of toxin, and remained so even after washing the preparation for more than 2 h in normal bathing fluid (Figure 1). This 'desensitization' was apparently specific for the toxin, since exposure to the toxin had no effect on the amplitude of the contractions caused either by histamine (10^{-8} to 10^{-7} M) or by acetylcholine (10^{-8} to 10^{-7} M).

The contraction elicited by notexin was not blocked by atropine (10^{-6} M), thus excluding the possibility that the effect of the toxin is mediated by either a direct or an indirect cholinomimetic mechanism. Mepyramine (10^{-6} M) was similarly ineffective in blocking the contraction (Figure 2). This latter observation is of particular interest since notexin is capable of degranulating mast cells (see Pluskal *et al.*, 1978).

Neurogenic 'twitch-like' responses of the longitudinal muscle, elicited by field stimulation were unaffected by notexin, (5 μg/ml) (Figure 1). The electrically-induced 'twitch-like' responses were inhibited by noradrenaline ($1–3 \times 10^{-6}$ M); the inhibitory effect of noradrenaline was also unaltered by notexin.

Effects on non-cholinergic transmission

Motor transmission in the rat anococcygeus. Field stimulation of the rat isolated anococcygeus muscle with trains of 5 pulses at 10 Hz induced a reproducible 'twitch-like' response of approximately 2 g tension. The response has been shown by Gillespie (1972) to be due to the stimulation of post-ganglionic, adrenergic neurones. Notexin (5 μg/ml) had no effect on the 'twitch-like' response of the muscle. Similarly, contractions elicited by the application of noradrenaline (10^{-7} to 10^{-6} M) were unaltered in the presence of notexin.

The motor response of the guinea-pig vas deferens. Field stimulation (5 pulses at 10 Hz repeated every 60 s) was used to generate 'twitch-like' responses in the longitudinal muscle of the guinea-pig vas deferens. There is evidence to believe that these responses are due to the stimulation of post-ganglionic non-

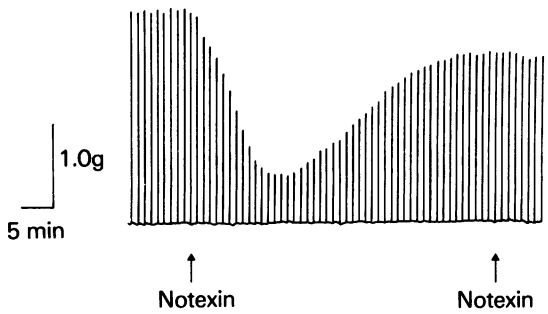

Figure 3 A typical response of a guinea-pig vas deferens preparation to notexin. The twitch-like responses were generated by field stimulation with trains of 5 pulses (pulse duration 1.0 ms, frequency 10 Hz, supramaximal intensity). Notexin, (5.0 μg/ml) was added as indicated. Note the rapid onset of action, reversibility of the effect in spite of the continued presence of the toxin, and the apparent desensitization of the preparation towards notexin.

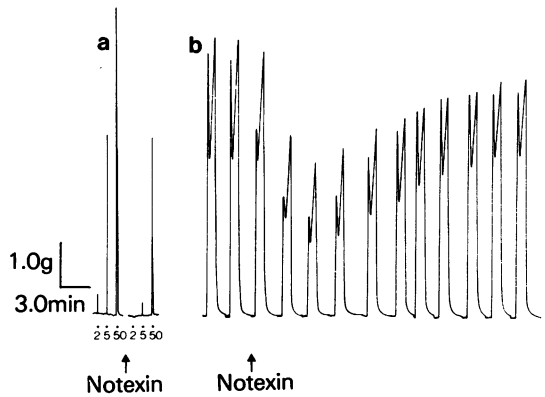

Figure 4 The effect of pulse train length upon the inhibitory effect of notexin in the guinea-pig isolated vas deferens. The twitch-like responses were generated by field stimulation with pulses of 1 ms duration, and supramaximal amplitude delivered at 10 Hz. Two different experiments are illustrated. In (a) a preparation was stimulated with pulse trains of 2, 5 and 50 pulses as indicated. Notexin, (5.0 μg/ml) was then added to the bath. At the 'height' of the inhibition, the response to 2 pulse stimulation was abolished, but that to 50 pulse stimulation was inhibited by only 40%. In (b) a second preparation was stimulated with trains of 90 pulses. After the addition of notexin (5.0 μg/ml) the early response was inhibited by 70% the later response by only 50%.

adrenergic, non-cholinergic neurones (Ambache & Zar, 1971). The introduction of notexin (5 μg/ml) resulted in a very rapid inhibition of the response. The inhibition was maximal by 5 to 15 min after administration of the toxin. In 9 preparations, previously unexposed to any other drug, the mean maximal inhibition was 84 ± 7% (mean ± s.e.mean), the range being 44% to 100%. A characteristic feature of the response was that in spite of the continued presence of the toxin the inhibition began to decline after about 20 minutes. The restored responses were sometimes larger but more often slightly smaller than the responses preceding exposure to the toxin. A typical response is shown in Figure 3. After recovery, the addition of further doses of the toxin was without effect. Even prolonged washing in normal bathing fluid failed to restore the sensitivity of preparations to notexin.

In two experiments we investigated the relationship between pulse-train length, and the degree of inhibition caused by notexin. Figure 4a illustrates an experiment in which a preparation was stimulated with pulse-trains of varying length at a fixed frequency of 10 Hz. At the 'height' of the inhibition, the response to 2 pulse stimulation was abolished, the response to 5 pulse stimulation was reduced by 95% and the response to 50 pulse stimulation was reduced by 40%.

When stimulated at 10 Hz with very long pulse-trains (i.e. 90 to 100 pulses) the guinea-pig vas deferens exhibits a biphasic response (Swedin, 1971). Figure 4b illustrates an experiment in which a preparation was stimulated with trains of 90 pulses at 10 Hz repeated every 3 minutes. The initial, rapid first phase of the response was inhibited by 70% by notexin; the slower second phase was inhibited by only 50%. The inhibition of the electrically induced motor response was not due to a non-specific inhibition of muscle excitability since contractions produced by noradrenaline (3×10^{-5} M) before and at various stages during the action of notexin were unaltered (Figure 5).

It is well known that prostaglandins, and in particular prostaglandins E_1 and E_2 inhibit motor transmission in the guinea-pig vas deferens (Euler & Hedqvist, 1969; Ambache & Zar, 1970a). We therefore considered the possibility that the phospholipase A_2 activity of notexin (q.v.) could elevate the level of precursor fatty acids, which by acting as substrates for prostaglandin synthetase could conceivably stimulate the synthesis and thus release of endogenous prostaglandins. However, such a mechanism is probably excluded by the observation that preparations pre-incubated for up to 3 h in indomethacin (5.6×10^{-6} M) responded in a typical fashion to exposure to notexin (Figure 6; cf. Figure 3).

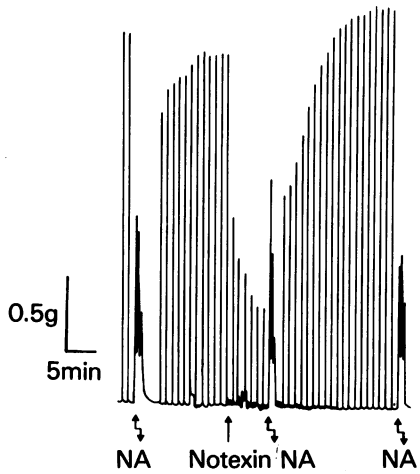

Figure 5 A guinea-pig vas deferens stimulated as in Figure 3. Noradrenaline (NA) (3×10^{-6} M) was added (contact time 1 min) where indicated. Stimulation was stopped during exposure to noradrenaline. The motor response to noradrenaline is unaltered by notexin.

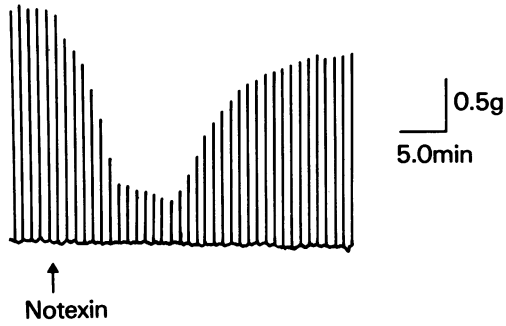

Figure 6 A guinea-pig vas deferens stimulated as in Figure 3. The preparation was exposed to indomethacin, (5.6×10^{-6} M) for 3 h before the addition of notexin, (5.0 μg/ml).

We next considered the likelihood that the effects of notexin were mediated by either the release of endogenous noradrenaline, or by the direct stimulation of inhibitory adrenoceptors.

The first possibility was excluded in experiments in which preparations obtained from 3 guinea-pigs treated with reserpine were used (see Methods for details). The effect of tyramine on these preparations was abolished: tyramine, 10^{-5} M normally causes a sustained inhibition of the motor response of about 75% and in the reserpine-treated preparations the inhibition was $< 5\%$, indicating that the available stores of noradrenaline had been depleted. The response to notexin of preparations from reserpine-treated animals was unaltered.

The second possibility, that notexin exerted its effect by direct stimulation of inhibitory adrenoceptors, was excluded by the observation that the presence of propranolol, (3×10^{-6} M) and phentolamine (10^{-5} M) together, a combination that blocked the inhibitory effect of both noradrenaline (2×10^{-6} M) and tyramine (10^{-5} M), had no effect on the inhibition of the motor response caused by notexin (Figure 7).

In the course of the experiments on the sympathomimetic drugs, we noted that although notexin did not appear to exert its primary action either by releasing noradrenaline or by stimulating inhibitory adrenoceptors, the inhibitory effects of dexamphetamine and tyramine (both indirect sympathomimetic drugs), and of low doses of noradrenaline, were usually partly or even completely blocked by exposure to notexin. In the experiment illustrated in Figure 8, tyramine, (10^{-5} M) and noradrenaline, (2×10^{-6} M) were used to cause an inhibition of the motor response by 86% and 58% respectively. Notexin was then added to the preparation, which responded typically. Thirty min after the recovery of the motor response of the preparation, and in the continued presence of the notexin, tyramine caused a transient and smaller inhibition of 35%, and the inhibitory response caused by noradrenaline was blocked, allowing a potentiated response to be seen.

Altogether 6 experiments were carried out with tyramine and 6 with noradrenaline. Before notexin, tyramine (10^{-5} M) caused an inhibition of the motor response by $73 \pm 4\%$ (mean $\pm$ s.e.mean); after notexin the inhibition was only $28 \pm 3\%$. Similarly, noradrenaline before notexin caused an inhibition of $46 \pm 4\%$ compared with $7 \pm 4\%$ after notexin. In each case the change in sensitivity following exposure to notexin was statistically significant ($P < 0.05$, t test). In control experiments there was no significant change in sensitivity to either of these agents over a period in excess of 1.5 hours.

The inhibitory effects of noradrenaline are almost certainly due to the stimulation of presynaptically located inhibitory α-receptors. Isoprenaline also inhibits the motor response of the guinea-pig vas deferens preparation, but in this case, the inhibition is mediated by postsynaptically located inhibitory β-adrenoceptors (Ambache & Zar, 1971). The inhibitory effects of isoprenaline (2×10^{-6} M) were unaffected by exposure to notexin.

Although the effect of notexin on the response to the sympathomimetic drugs was clear-cut and unmistakeable, no such unequivocal result was obtained

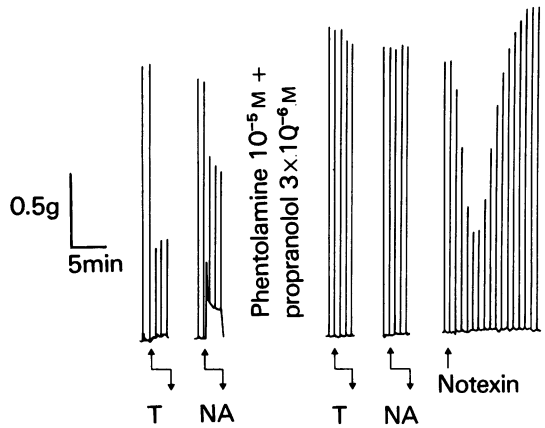

Figure 7 A guinea-pig vas deferens stimulated as in Figure 3. At T, tyramine (10^{-5} M) and at NA, noradrenaline (2×10^{-6} M) were added. The drugs remained in contact with the tissue for 3 minutes. After obtaining control response (first two panels) phentolamine, (10^{-5} M) and propranolol, (3×10^{-6} M) were added to the reservoir of bathing fluid and remained in contact with the preparation for the rest of the experiment; 22 min after the introduction of phentolamine and propranolol, the control experiment was repeated. Tyramine and noradrenaline were both without effect. The response to notexin, (5.0 μg/ml) was normal.

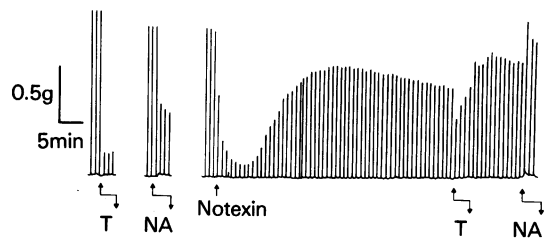

Figure 8 A guinea-pig vas deferens stimulated as in Figure 3. At T, tyramine (10^{-5} M) and at NA, noradrenaline (2×10^{-6} M) were added. The drugs remained in contact with the tissue for 3 minutes. After obtaining control response (first two panels) notexin, (5.0 μg/ml) was added, and remained present for the rest of the experiment. Thirty min after the recovery of the electrically evoked response, the control experiments were repeated.

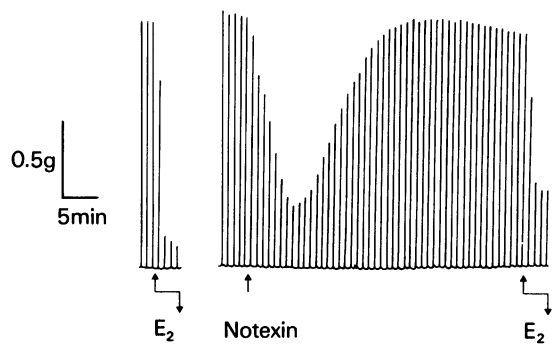

Figure 9 A guinea-pig vas deferens stimulated as in Figure 3. At E_2, prostaglandin E_2, (2 ng/ml) was introduced. The prostaglandin was left in contact for 4 min before the preparation was washed for 40 minutes. Notexin, (5.0 μg/ml) was added as indicated; 20 min after the recovery of the responses, and in the continued presence of notexin, prostaglandin E_2 was introduced again.

when prostaglandin E_2 was used to inhibit the motor transmission. An experiment with prostaglandin E_2 is shown in Figure 9. Before exposure to notexin, prostaglandin E_2, (2 ng/ml) inhibited the motor response by 90% but after exposure to notexin, this value was reduced to 67%. A similar result was obtained in only 4 out of 7 experiments; in the remaining 3 experiments the effects of prostaglandin were unchanged. In no experiment did we observe an increase in the inhibitory effects of prostaglandin after notexin administration. Even so, the mean inhibition caused by prostaglandin in these 7 experiments was $62 \pm 12\%$ before notexin and $37 \pm 6\%$ after notexin. The difference was statistically significant ($P < 0.05$, t test). In control experiments, there was no significant change in sensitivity to prostaglandins over a period of 3 hours.

The motor response of the guinea-pig seminal vesicle. Isolated seminal vesicle preparations were obtained from 2 guinea-pigs. The preparations responded with a 'twitch-like' contraction of approximately 2.5 g when stimulated with trains of 5 pulses at 10 Hz repeated every minute. Notexin, (5 μg/ml) depressed the twitch-like response by about 50%. The inhibition was spontaneously reversed in spite of the continued presence of the toxin, and the preparation remained insensitive to further doses of the toxin.

The motor response of the guinea-pig bladder. A single preparation of a guinea-pig bladder was stimulated with trains of 5 pulses every minute which produced 'twitch-like' responses of the muscle. Notexin (5 μg/ml) induced an inhibition of this response of about 12%. The inhibition was spontaneously reversed in spite of the continued presence of the toxin.

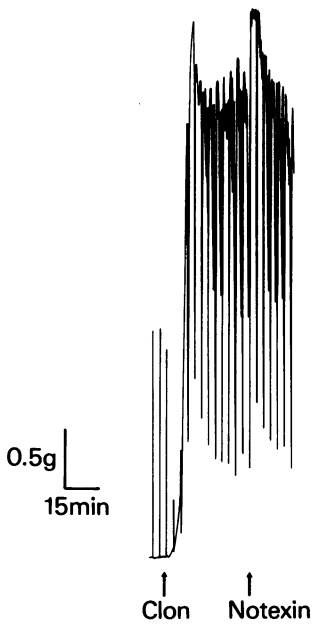

Figure 10 A rat anococcygeous preparation stimulated with trains of 5 pulses at 10 Hz every 3 minutes. Clonidine, (3×10^{-8} M), was used to reveal the inhibitory response of the tissue. Notexin, (20 μg/ml) was added as indicated. Note the relative lack of effect of notexin on the inhibitory transmission.

The motor response of the rat vas deferens. The neurogenic 'twitch-like' response of the rat vas deferens was obtained with field stimulation of 5 pulses at 10 Hz repeated every 60 seconds. Compared with the activity of notexin on the guinea-pig vas deferens, the twitch-like response of the rat vas deferens was relatively unaffected by exposure to notexin, a dose of 20 μg/ml inhibiting the motor response by only 25%. However, the characteristics of the response (rapid onset of action, gradual reversal of the inhibition in spite of the continued presence of the toxin, and refractoriness to further doses of notexin) were as described for the guinea-pig vas deferens. Contractions caused by noradrenaline, (2.5×10^{-5} M) were unaffected by notexin.

Inhibitory transmission in the rat anococcygeous. Inhibitory responses of the rat anococcygeous (Gillespie, 1972) were revealed by field stimulation (5 pulses at 10 Hz every 3 min) in the presence of clonidine, a compound known to act as an adrenergic neurone blocker in this tissue (Idowu & Zar, 1976) at a concentration of 3×10^{-8} M. Notexin, (20 μg/ml) caused a slight, transient rise in the resting tension of the muscle, but had no effect on the absolute amplitude of the inhibitory response (Figure 10).

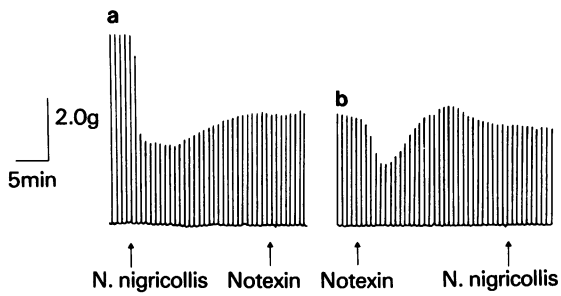

Figure 11 Two different guinea-pig vas deferens preparations stimulated as in Figure 3. In (a) *Naja nigricollis* phospholipase A_2 (5.0 μg/ml) was added as indicated, followed by notexin (5.0 μg/ml) 30 min later. In (b) notexin (5.0 μg/ml) was added as indicated followed by *Naja nigricollis* phospholipase A_2, (5.0 μg/ml) 31 min later.

The effects of compounds related to notexin upon the motor responses of the guinea-pig vas deferens. Many of the biological properties of notexin are virtually abolished if native toxin is reacted with *p*-bromophenacyl bromide (Halpert *et al.*, 1976; Harris & Johnson, 1978). The modified form of the notexin (called PBP-notexin) was inactive (at 5.0 μg/ml) when introduced to the guinea-pig vas deferens preparations. Moreover, exposure to PBP-notexin did not desensitize the preparation to subsequent doses of native notexin.

The phospholipase A_2 of *Naja nigricollis* venom is a homologue of notexin (Eaker, 1975). At a dose of 5.0 μg/ml the compound inhibited the motor response of the guinea-pig vas deferens with qualitatively similar characteristics to those described for notexin. After the (partial) recovery from the effect of *Naja nigricollis* phospholipase A_2, notexin was inactive. Prior exposure to notexin, prevented a response to *Naja nigricollis* phospholipase A_2 (Figure 11).

Discussion

The experiments described in this paper reveal notexin to be largely inactive at autonomic post-ganglionic synapses. The only positive effects of the toxin appear to be the initiation of a contraction of the longitudinal muscle of the guinea-pig ileum and the inhibition of the motor response of the electrically stimulated guinea-pig vas deferens and seminal vesicle

preparations. The actions of notexin in all three tissues had the common features of spontaneous reversibility and long-lasting desensitization.

The contraction of the longitudinal muscle of the guinea-pig ileum elicited by the toxin involved neither a cholinergic nor a histaminergic mechanism, the response being unaffected by the presence of either atropine or mepyramine. The possibility that prostaglandins, bradykinin, substance P or other related autacoids might possibly be the mediator of the contractile response, has not been excluded.

The most pronounced effect of notexin was on the motor response of the electrically stimulated guinea-pig vas deferens. Under the conditions of the experiment, the motor response is elicited by the excitation of the motor nerves supplying the longitudinal muscle. Notexin caused an inhibition of the motor response, the inhibition being both rapid in onset and spontaneously reversible in spite of the continued presence of the toxin. Any given preparation responded only to the first exposure to the toxin. Thereafter, even washing for long periods in fresh bathing fluid failed to restore the sensitivity of the preparation to the toxin. There would appear to be several possible mechanisms by which the inhibition of the motor response is effected, and we consider each possibility in turn.

The toxin could act by inducing a generalized but transient reduction in either the excitability or the contractility of the smooth muscle. If this were the case one should expect a corresponding reduction in the response of the tissue to other spasmogens such as noradrenaline. In our experiments, such a generalized reduction in sensitivity was not seen, and so we reject this possibility.

Next, we consider the possibility that the toxin specifically blocks the postsynaptic receptor activated by the motor transmitter. On the evidence available, it seems inherently unlikely that this is the mechanism of action. Thus, we are unaware of any other situation where a specific antagonist can act as a transient inhibitor to an agonist, the spontaneous reversal of the antagonism being associated with the development of tachyphylaxis specific to the antagonist itself. However, since there is no general agreement on the identity of the motor transmitter (Ambache & Zar, 1971; Euler & Hedqvist, 1975) and since it is possible to obtain only one response to notexin per preparation, it is difficult to design experiments that would provide a definitive answer to this possibility.

If we accept that the mechanism of action of notexin cannot be adequately explained on the basis of postsynaptic activity of any kind, it naturally follows that the inhibition is effected by a decrease in the output of motor transmitter in response to nerve stimulation. In this context, three possibilities come to mind. Firstly, the toxin might activate an inhibitory adrenergic mechanism by initiating the release of endogenous noradrenaline, or by acting directly on inhibitory α-adrenoceptors situated on the motor nerve terminal (evidence for the existence of such receptors is well documented by Ambache & Zar, 1971, and by Euler & Hedqvist, 1975, for example). Our experimental evidence is completely against either of these mechanisms. Thus, in one series of experiments we demonstrated that preparations removed from reserpine-treated animals (in which the depletion of noradrenaline was confirmed by the use of tyramine) remained normally sensitive to notexin, clearly excluding the 'indirect adrenergic' mechanism of action. In another series of experiments, we showed that even in the presence of doses of α- and β-adrenoceptor blocking agents sufficient to abolish the inhibitory response of the preparation to noradrenaline, the potency of notexin was unimpaired. Another possibility is that the presynaptic activity of notexin might be mediated by prostaglandins. Notexin is a phospholipase A_2 (Halpert *et al.*, 1976), and it seems reasonable to suggest that such enzyme activity might lead to the synthesis and release of prostaglandins (see Flower & Blackwell, 1976, for a discussion of the role of phospholipase activity in the normal synthesis of prostaglandins). There is evidence for the presence of prostaglandins in the guinea-pig vas deferens and the inhibitory effects of prostaglandins in this tissue have been demonstrated by Hedqvist & Euler (1972). Moreover, the rat vas deferens preparation which is relatively insensitive to notexin is also relatively insensitive to the inhibitory effects of prostaglandins. However, in view of the fact that pre-incubation of the guinea-pig vas deferens for 2–3 h with a dose of indomethacin known to block the synthesis of prostaglandins (Gryglewski & Vane, 1971) does not lead to a diminution in the potency of notexin, it seems unlikely that the effects of the toxin are mediated by prostaglandins.

Since the actions of notexin cannot be explained by invoking the mediation of either catecholamines or prostaglandins, it seems probable that it acts as a direct presynaptic inhibitor of motor transmission in the guinea-pig vas deferens and probably in the guinea-pig seminal vesicle. If this is the case, then the characteristics of the action of notexin can be explained. Thus the rapid onset of inhibition, and the early onset of tachyphylaxis suggest that notexin behaves as a partial agonist, first stimulating its inhibitory 'receptor' and then either occupying its binding site in an irreversible manner or causing a true desensitization.

A curious feature of the desensitization was that the inhibitory effects of noradrenaline, the indirectly acting sympathomimetic drugs and, to a lesser extent the prostaglandins were also blocked. However, it is of interest to note that the inhibitory response to iso-

prenaline, which is known to suppress motor activity by stimulating postsynaptic β-receptors remained unaffected by exposure to notexin. These observations would support our contention that the site of action of notexin, involving both the inhibition of the motor response, as well as the subsequent desensitization, are presynaptic.

It is tempting to speculate that although the various classes of inhibitory drugs acting at presynaptic sites (namely noradrenaline, the indirectly acting sympathomimetics and the prostaglandins) initially activate their own specific receptors on the nerve terminal, they eventually share a common pathway that leads to inhibition, and that notexin ultimately blocks this final pathway.

It is pertinent to ask whether or not the phospholipase A_2 activity of notexin is involved in its inhibitory action on the guinea-pig vas deferens. Superficially there is evidence to the affirmative. Thus PBP-notexin, which is almost devoid of catalytic activity (Halpert *et al.*, 1976) is inactive, causing neither inhibition in its own right nor modifying the response of the tissue to a subsequent dose of notexin. Further, *Naja nigricollis* phospholipase A_2 (Eaker, 1975) causes a spontaneously reversible inhibition of the motor response, blocks the effects of a subsequent dose of notexin, and is itself blocked by notexin. However, in many situations there would appear to be little direct relationship between catalytic activity and other forms of biological activity in a large number of structurally related toxins (Eaker, 1975; Pluskal *et al.*, 1978; Harris & Johnson, 1978). The relationships between catalytic activity and other forms of biological activity are also difficult to study since even though the relevant toxins are all classified as phospholipase A_2 enzymes, they seem to exhibit considerable differences in their individually preferred substrates.

Why the motor transmission of the vas deferens and the seminal vesicle of the guinea-pig should be so sensitive to the effects of notexin is not clear. It may reflect either some structural feature of the motor nerve axon or terminal, or it may be related to the specific motor transmitter itself. We have no satisfactory explanation for the relative lack of activity of notexin at other sites.

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References

AMBACHE, N. & ZAR, M.A. (1970a). An inhibitory effect of prostaglandin E_2 on neuromuscular transmission in the guinea-pig vas deferens. *J. Physiol.*, **208**, 30–32 P.

AMBACHE, N. & ZAR, M.A. (1970b). Non-cholinergic transmission by post-ganglionic motor neurones in the mammalian bladder. *J. Physiol.*, **210**, 761–783.

AMBACHE, N. & ZAR, M.A. (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.*, **216**, 359–389.

BELL, P.M.G. & STEIN, R.B. (1971). A digital stimulator built on modular principles using integrated circuits. *J. Physiol.*, **218**, 5P.

CULL-CANDY, S.G., FOHLMAN, J., GUSTAVSSON, D., LÜLLMAN-RAUCH, R. & THESLEFF, S. (1976). The effects of taipoxin and notexin on the function and fine structure of the murine neuromuscular junction. *Neuroscience*, **1**, 175–180.

EAKER, D. (1975). Structure and function of snake venom toxins. In *Peptides: Chemistry, Structure and Biology.* ed. Walter, R. & Meienhofer, J. pp. 17–30. Ann Arbor: Ann Arbor Science.

EULER, U.S. VON & HEDQVIST, P. (1969). Inhibitory action of Prostaglandin E_1 and E_2 on the neuromuscular transmission in the guinea-pig vas deferens. *Acta physiol. scand.*, **77**, 510–512.

EULER, U.S. VON & HEDQVIST, P. (1975). Evidence for an α- and β_2-receptor mediated inhibition of the twitch response in the guinea-pig vas deferens by noradrenaline. *Acta physiol. scand.*, **93**, 572–573.

FLOWER, R.J. & BLACKWELL, G. J. (1976). The importance of phospholipase A_2 in prostaglandin biosynthesis. *Biochem. Pharmac.*, **25**, 285–291.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its responses to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

GRYGLEWSKI, R. & VANE, J.R. (1971). Rabbit-aorta contracting substance (RCS) may be a prostaglandin precursor. *Br. J. Pharmac.*, **43**, 420–421P.

HALPERT, J. & EAKER, D. (1975). Amino acid sequence of a presynaptic neurotoxin from the venom of *Notechis scutatus scutatus* (Australian tiger snake). *J. biol. Chem.*, **250**, 6990–6997.

HALPERT, J., EAKER, D. & KARLSSON, E. (1976). The role of phospholipase activity in the action of a presynaptic neurotoxin from the venom of *Notechis scutatus scutatus* (Australian tiger snake). *Febs Letters*, **61**, 72–76.

HARRIS, J.B. & JOHNSON, M.A. (1978). Further observations on the pathological response of rat skeletal muscle to toxins isolated from the venom of the *Australian tiger snake, Notechis scutatus scutatus. Clin. exp. Pharmac. Physiol.* (in press).

HARRIS, J.B., JOHNSON, M.A. & KARLSSON, E. (1975). Pathological responses of rat skeletal muscle to a single subcutaneous injection of a toxin isolated from the venom of the Australian tiger snake, *Notechis scutatus scutatus. Clin. exp. Pharmac. Physiol.*, **2**, 383–404.

HARRIS, J.B., KARLSSON, E. & THESLEFF, S. (1973). Effects of an isolated toxin from Australian Tiger snake (*Notechis scutatus scutatus*) venom at the mammalian neuromuscular junction. *Br. J. Pharmac.*, **47**, 141–146.

HEDQVIST, P. & VON EULER, U.S. (1972). Prostaglandin-induced neurotransmission failure in the field-stimulated isolated vas deferens. *Neuropharmac.*, **11**, 177–187.

IDOWU, O.A. & ZAR, M.A. (1976). Inhibitory effect of clonidine on a peripheral adrenergic synapse. *Br. J. Pharmac.*, **58**, 278P.

KARLSSON, E., EAKER, D. & RYDEN, L. (1972). Purification of a presynaptic neurotoxin from the venom of the Australian Tiger snake, *Notechis scutatus scutatus*. *Toxicon*, **10**, 405–413.

PATON, W.D.M., & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194**, 13–33.

PLUSKAL, M.G., HARRIS, J.B., PENNINGTON, R.J. & EAKER, D. (1978). Some biochemical responses of rat skeletal muscle to a single subcutaneous injection of a toxin (notexin) isolated from the venom of the Australian tiger snake *Notechis scutatus scutatus*. *Clin. exp. Pharmac. Physiol.* (in press).

SWEDIN, G. (1971). Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. *Acta physiol. scand.*, **83**, supp. 369.

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