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CONVULSANT AND POSSIBLE ANTICHOLINERGIC ACTIONS OF DENDROTOXIN IN THE AMPHIBIAN SPINAL CORD

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1 Dendrotoxin (DTOX)6, 6a and 5,6-1, fractions of the venom isolated from the green mamba (*Dendroaspis viridis*) promoted both spontaneous and stimulus-coupled rhythmic activity and antagonized the cholinergically mediated ventral root-dorsal root potential (VR-DRP) of frog spinal cord. The different time course and reversibility of these two effects indicates that the toxin has two entirely separate sites of action on the frog spinal cord.

2 Since DTOX 6 neither blocked nor enhanced responses of ventral and dorsal roots to glutamate, γ-aminobutyric acid (GABA), β-alanine, glycine or aspartate, it is unlikely that its convulsant action resulted from an alteration of the postsynaptic actions of inhibitory or excitatory amino acids.

3 An alteration in the threshold for action potential generation could perhaps contribute to the convulsant action of DTOX 6, although other mechanisms such as blockade of the release of unspecified inhibitory substances cannot be excluded.

4 In addition to the lack of effect on amino acid responses, DTOX failed to block the polysynaptic DR-VRP or DR-DRP pathways, which are mediated at least in part by amino acid neurotransmitters. Although this would be consistent with a specific action of DTOX at the cholinergic synapse of the VR-DRP pathway, this site of action has not yet been demonstrated unequivocally. Other possible mechanisms whereby DTOX could block VR-DRP are discussed.

Introduction

Toxin fractions isolated from the venom of the green mamba, *Dendroaspis viridis* promote convulsant activity and antagonize the ventral root-dorsal root potential (VR-DRP) of the amphibian isolated hemisected spinal cord (Miledi & Szczepaniak, 1975; Smith, Padjen, Quik & Collier, 1980; Quik, Smith, Padjen & Collier, 1980). Since dendrotoxins (DTOX) are known to block neuromuscular transmission (Banks, Miledi & Shipolini, 1974) and the polysynaptic VR-DRP pathway contains a cholinergic synapse (Kiraly & Phillis, 1961; Mitchell & Phillis, 1962; Barker, Nicoll & Padjen, 1975b; Kudo, 1978), the blockade of this response has been attributed to a central antinicotinic action of the toxin (Szczepaniak, 1974; Miledi & Szczepaniak, 1975; see also, Patrick, Stallcup, Zavanelli & Ravdin, 1980). The mechanism whereby the toxin induces convulsant activity is not known. In our previous study (Quik *et al.*, 1980) these two actions were shown to be the effect of a single venom component since purification of venom fractions to a chromatographically homogenous component did not distinguish the two phenomena.

In the present study on the frog spinal cord we have compared the action of DTOX with the action of nicotinic antagonists and with the action of a convulsant (4-aminopyridine, 4-AP). The first objective was to test whether blockade of VR-DRP results in convulsant activity or whether the production of convulsions can result in the blockade of VR-DRP. Both of these possibilities were eliminated, indicating that the single toxin component has two separate sites of action in the frog spinal cord. The second objective was to characterize better the convulsant activity and to test specifically whether it resulted from blockade of the action of inhibitory amino acid neurotransmitters or enhancement of excitatory transmission.

Methods

The VR-DRP (ventral root-dorsal root potential), DR-DRP (dorsal root-dorsal root potential) and DR-VRP (dorsal root-ventral root potential) of the isolated hemisected spinal cord of the frog were examined using sucrose gap recording as described by Barker, Nicoll & Padjen, (1975 a, b); preparations were superfused with oxygenated Ringer solution at 15°–20°C. For examination of field potentials in the dorsal horn, hemisected frog spinal cords were placed in a small plastic chamber and their dorsal and ventral roots led into pools of mineral oil and stimulated with bipolar platinum electrodes. The same electrodes

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were routinely used to record VR-DRP, DR-DRP and DR-VRP but only propagated potentials are observed, unlike the d.c. potentials detectable by means of the sucrose gap technique. Glass microelectrodes were filled with 3 M NaCl and their tips broken to give resistances of 5–10 megohms. Electrodes were slowly advanced into the dorsal horn of the isolated spinal cord until a characteristic field potential could be observed for optimal recording of both the presynaptic volley and the postsynaptic response following DR stimulation. All potentials were monitored with a conventional electrophysiological recording system. The high impedence amplifier was connected to the bath by means of Ringer and/or agar bridges fitted to calomel electrodes. The traces illustrated were filmed from an oscilloscope or recorded on a rectilinear pen recorder (30 Hz bandwidth, Brush 2400). The Ringer inlet tube was fitted with a tap system which allowed the superfusion of the chamber containing the preparations with various drug solutions. For testing samples of toxins, the flow of the Ringer was stopped and a sample of the toxin dissolved in Ringer applied from a micropippette as previously described (Quik *et al.*, 1980). The composition of the Ringer solutions used in all experiments was (mM): NaCl 115, $CaCl_2$ 2, KCl 2, HEPES buffer (adjusted to pH 7.3 with NaOH)10 and D-glucose 8. *Dendroaspis* venom was purchased from Miami Serpentarium, Miami, Florida or Sigma, St. Louis, Mo. Purification of the toxin from either source by the method of Quik *et al.* (1980) yielded fractions 6, 6a and 5, 6–1 (DTOX 6, DTOX 6a, DTOX 5, 6–1, respectively). Fraction 6 and 6a correspond to 4–11 of Shipolini, Bailey, Edwardson & Banks (1973) and contain 5, 6–1 and possibly other toxins; fraction 5, 6–1 contains a single active component equivalent to 4–11–3 of Shipolini *et al.* (1973). Venom of *Bungarus multicinctis* was purchased from Sigma, St. Louis, Mo and α-bungarotoxin was purified according to the method of Chang & Lee (1963) as modified by Berg, Kelly, Sargent, Williamson & Hall (1972). Dihydro-β-erythroidine was a gift

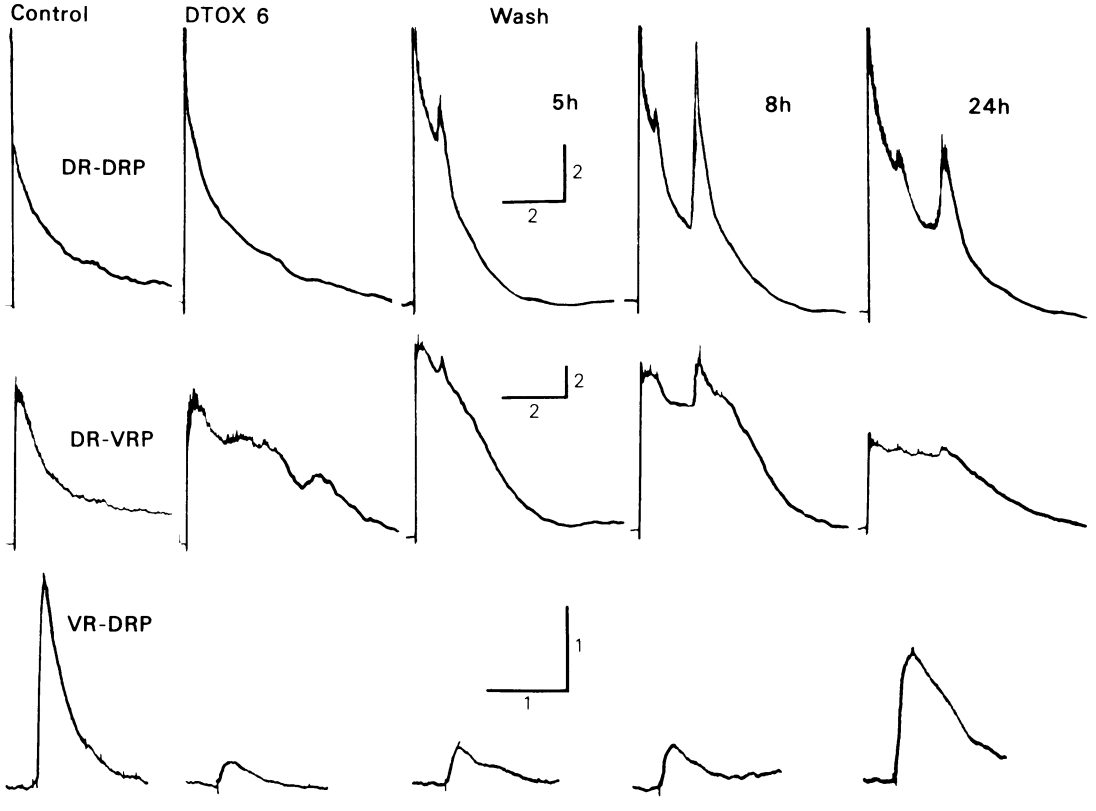

Figure 1 Effect of dendrotoxin (DTOX) 6 on synaptic potentials in the frog spinal cord examined by means of the sucrose gap technique. Control: DR-DRP, DR-VRP and VR-DRP responses. DTOX 6: same responses recorded after 60 min incubation in DTOX 6 (40 μg/ml). Wash: recovery of responses following 5, 8 and 24 h of wash with normal Ringer. Note that top of DR-DRP response is limited by excursion of pen. Also note multiple discharges persisting for 24 h following toxin treatment but progressive although incomplete recovery of VR-DRP. Calibration bars 1 or 2 s, 1 or 2 mV as indicated.

from Merck-Frosst, Pointe Claire. All other drugs were obtained from Sigma.

Results

The experiments described in the following sections (a–c) were designed to investigate whether a single purified DTOX fraction acts at a single site to promote both convulsant activity and VR-DRP blockade or whether the toxin has two independent sites of action. Section (d, e) describes experiments that attempt to characterize the effects of the toxin and provide information about possible mechanisms of action.

(a) Does blockade of VR-DRP result in convulsant activity?

VR-DRP was totally blocked or drastically reduced after 60 min incubation in the presence of DTOX 6 (40 μg/ml; 4 preparations), DTOX 6a (10–80 μg/ml; 11 preparations) or DTOX 5,6–1 (15 μg/ml; 1 preparation). The toxins also promoted spontaneous convulsion-like discharges and increased both the amplitude and duration of DR-DRP and DR-VRP; DTOX 6A (10 μg/ml) enhanced DR-DRP to 142 ± 9% and DR-VRP to 144 ± 32% of control amplitude ($n = 4$). Higher doses of this toxin (40–80 μg/ml) had similar effects; the DR-DRP was enhanced to 182 ± 18% and DR-VRP to 154 ± 10% of control amplitude ($n = 7$). Similar enhancements were observed with DTOX 6 and DTOX 5,6–1. Some recovery of VR-DRP was observed after prolonged washing but the convulsant activity persisted for 24 h in 12 preparations where recovery from DTOX 6, 6a or DTOX 5,6–1 was examined. A typical experiment is illustrated in Figure 1.

Since Miledi & Szczepaniak (1975) have suggested that the convulsant effects of DTOX are not inconsistent with its anticholinergic effects, we examined the actions of various centrally acting anticholinergic drugs to investigate whether they produced DTOX-like convulsant activity. Dihydro-β-erythroidine (2×10^{-6} M) (DHβE) specifically reduced VR-DRP to less than 36% of control after 35 min incubation. Total blockade of the responses was observed with higher doses (10^{-4} M) or longer periods of incubation. Complete recovery of VR-DRP took at best 3 h and with higher doses recovery was often incomplete. Unlike DTOX, DHβE never produced rhythmic discharges nor any marked enhancement of DR-VRP or DR-DRP in any of the eight preparations tested.

Mecamylamine (5×10^{-4} M) was not as specific an antagonist as DHβE since it depressed DR-DRP and DR-VRP as well as VR-DRP; VR-DRP was not blocked by α-bungarotoxin (25 μg/ml) or atropine (1.4 μM). Neither mecamylamine, α-bungarotoxin nor atropine showed any tendency to induce DTOX-like convulsant activity. Although (+)-tubocurarine is known to promote convulsant activity in the isolated spinal cord of the frog, this is probably due to an anti-GABAergic rather than anticholinergic action of the drug (Nicoll, 1975).

In addition, pretreatment with DHβE failed to protect preparations from the convulsant effect of DTOX 6. Thus, all experiments in this section suggest that DTOX-induced convulsions do not result from blockade of a cholinergic synapse.

(b) *Does the production of convulsions result in the elimination of VR-DRP?*

On the basis of the above experiments it is tempting to speculate that the convulsant and VR-DRP blocking actions of the toxin reflect actions at two distinct sites. On the the other hand, one must examine the opposite possibility: the mere productions of convulsions could impair transmission in the VR-DRP pathway. This control is especially relevant since testing of DTOX during the interruption of superfusion (Quik *et al.*, 1980) could cause anoxia, a condition to which the VR-DRP is particularly sensitive (unpublished observations). Therefore, we examined the effect of 4-AP (Saade, Chanelet & Lonchampt, 1971a, b) on the VR-DRP. This substance was chosen since it produces convulsions which superficially resemble those produced by DTOX. In an attempt to mimic any possible deleterious effects of convulsions in the absence of superfusion, experiments were done as follows. The preparation was superfused with Ringer solution containing 4-AP (10 μM), which produced depolarization and activity on both roots; DR-DRP and DR-VRP responses were enlarged and associated with multiple discharges (Figure 2). The superfusion was then interrupted and responses recorded after 1 h in the 'stationary bath' containing 4-AP (10 μM). Although this procedure often caused a slight reduction in the amplitude of VR-DRP (Figure 2, centre column), this effect was never accompanied by any significant blockade of VR-DRP comparable to the effects of DTOX even when convulsant activity was more intense than that induced by DTOX. In some preparations, stimulation of the VR-DRP pathway in the presence of 4-AP produced large convulsant-like discharges.

(c) *Differential time course of the convulsant and VR-DRP blocking actions of dendrotoxin*

The preceding experiments indicate that DTOX has two separate sites of action: (1) to produce convulsions; (2) to block VR-DRP. The most convincing evidence in support of this idea is the different rate of onset and reversibility of the two effects. As illustrated in Figure 3, convulsant activity in frog spinal cord was observed after about 7 min exposure to

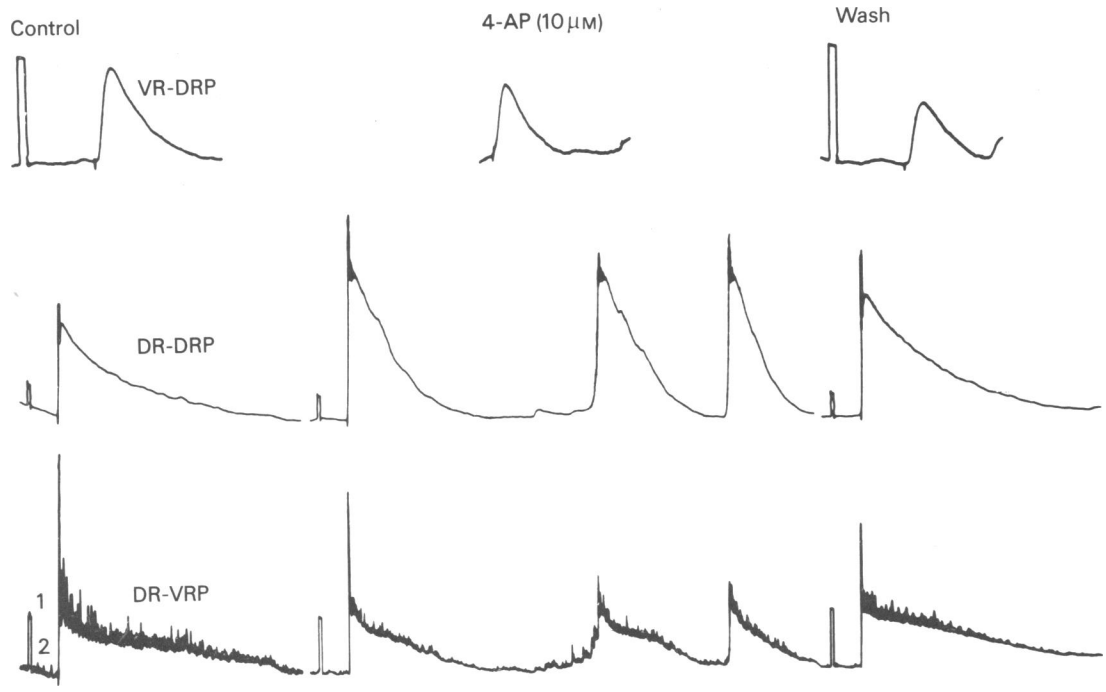

Figure 2 Effect of 4 aminopyridine (4-AP) on synaptic responses in frog spinal cord. Left hand traces: control VR-DRP, DR-DRP and DR-VRP responses. Centre traces: responses recorded after 76 min in the presence of 4-AP (10 μM). The superfusion of the preparation with 4-AP was stopped 60 min before these records were taken (in an attempt to mimic any deleterious effects of convulsions recorded in the absence of superfusion). Note multiple spike discharges following DR stimulation and slight reduction of VR-DRP amplitude. Right hand traces: responses recorded 2 h 50 min after the re-establishment of superfusion and washing with normal Ringer. Note that the DR-DRP is still slightly enlarged compared with control and that VR-DRP was slightly reduced. Traces from rectilinear pen recorder. Calibration pulse = 100 ms/2 mV.

DTOX 6 or 6a; this effect was essentially irreversible even after about 40 h washing with normal Ringer (Figure 1). On the other hand, blockade of VR-DRP was only observed following 20–50 min exposure to the toxin and, as illustrated in Figure 1, some recovery was observed after 6 to 20 h washing with normal Ringer (see also Quik *et al.*, 1980). Stimulation of ventral roots during recovery from DTOX often produced a response consisting of an initial 'shoulder' representing VR-DRP and a larger peak representing a convulsion-like synchronous discharge.

(d) *Further characterization of the convulsant effects of dendrotoxin*

A number of convulsants, including bicuculline and strychnine, exert at least part of their effect by blocking inhibitory amino acid receptors (Tebecis & Phillis, 1969; Curtis, Duggan & Johnston, 1971; Curtis, Duggan, Felix & Johnston, 1971; Barker *et al.*, 1975a, b). In principle, it is also possible that convulsant activity results from an enhancement of the action of excitatory amino acids. It was therefore of interest to test whether DTOX could modify the postsynaptic actions of putative amino acid neurotransmitters.

The effect of DTOX 6 was examined on responses of frog spinal cord preparations to glutamate, GABA, glycine, aspartate and β-alanine. Experiments were done on seven different preparations in a Ringer solution containing 2×10^{-7}M tetrodotoxin or 2 mM Mn^{2+}/0.4 mM Ca^{2+} to prevent indirect responses to the amino acids (Barker *et al.*, 1975a). DTOX 6 (40–100 μg/ml) failed to affect in any way the responses of both roots to GABA ($5\times10^{-4}-10^{-3}$M; 5 experiments on DR; 8 experiments of VR), glutamate ($2.5\times10^{-4}-10^{-3}$M, 6 experiments on VR; 8 experiments on DR), aspartate ($5\times10^{-4}-10^{-3}$M; 2 experiments on DR; 6 experiments on VR) or glycine (5×10^{-4}M-10^{-2}M) and β-alanine ($5\times10^{-4}-1.5\times10^{-3}$M; 6 experiments on both VR and DR for each drug). DTOX 6 also never altered the response to 8 mM potassium (4 experiments on both roots); this result may be taken to indicate that the toxin had no effect on passive membrane properties. It is also of interest to note that the

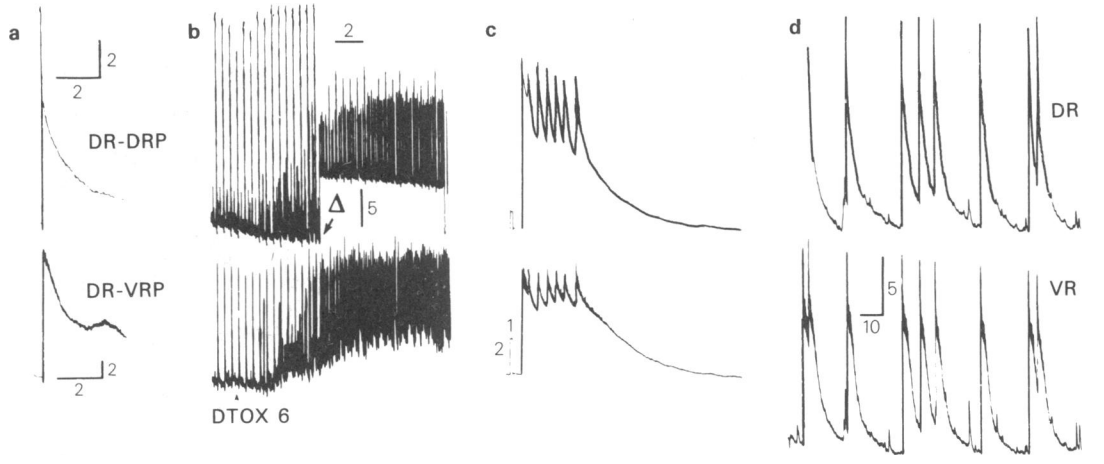

Figure 3 Dendrotoxin (DTOX) 6-induced convulsant activity on ventral and dorsal roots.
(a) Fast chart speed records of DR-DRP and DR-VRP. Calibrations 2 mV/2s.
(b) Slow chart speed records from same preparation following interruption of superfusion. Large deflections indicate DR-DRPs and DR-VRPs elicited once per min. DTOX 6a (final concentration 40 μg/ml) added at arrow. Note depolarization of both roots and increase of stimulus coupled and spontaneous activity. Time scale=2 min, voltage scale as in (a) for both roots. In DR record, gain was changed at point indicated by open triangle, 5 mV calibration then applies. (c) Multiple spike discharges following DR-VRP and DR-DRP in a preparation exposed to DTOX 5, 6–1 (15 μg/ml) calibration 100 ms/2 mV. (d) Intermediate chart speed record to show spontaneous activity recorded on DR (upper trace) and VR (lower trace) following exposure to DTOX 6a (10 μg/ml). Calibration=10 s/5 mV.

convulsant activity produced by DTOX 6 (Figure 1, 3) was not recorded in the presence of manganese Ringer or tetrodotoxin.

Since DTOX clearly enhanced DR-DRP and DR-VRP, it is possible that its convulsant effect could be generated by increase in release of excitatory transmitter. In order to test this possibility we examined the effect of DTOX 6 on both the pre- and postsynaptic components of the field potential recorded in the dorsal horn following stimulation of the dorsal root (DR-DHF) or lateral column (Simpson, 1976). DTOX 6 or 6a (40–100 μg/ml) had little or no effect on the presynaptic spike component or the field potential and slightly enhanced that component presumed to result from the e.p.s.ps in the dorsal horn. Sharp peaks, which are presumed to result from postsynaptic action potentials, sometimes appeared on the postsynaptic field potentials during or following toxin treatment. Such spikes appear in the experiment illustrated in Figure 4, although the postsynaptic field potential presumed to result from the e.p.s.p. was unchanged by DTOX 6. In this and other experiments, the pre- and postsynaptic components of the composite field potential were first identified by examination of the response before, during and after tetanic stimulation at 50 Hz. This high frequency stimulation resulted in the depression of the e.p.s.p. component of the response which progressively recovered when the responses were observed again at a lower frequency of stimulation.

(e) *Effect of dendrotoxin on the response to exogenously applied carbachol*

Superfusion of the frog spinal cord with carbachol (10^{-4}M) produced a biphasic response, depolarization followed by a sm hyperpolarization on both roots. This response was greatly reduced following incubation of the preparation with DTOX 6 (100μg/ml) (Figure 5).

(f) *Protection experiments*

An attempt was made to protect the receptor from the action of DTOX 6 by a nicotinic ligand such as DHβE. However, the doses of DHβE required to block VR-DRP completely produced a slowly reversible antagonism of the response similar in time course to DTOX blockade, so no conclusive demonstration of receptor protection was possible (cf. Chiapinelli & Zigmond, 1978). It is also interesting to note that the action of DTOX 6 (40 μg/ml) on VR-DRP was not blocked by pretreatment of the preparations with α-bungarotoxin (α-BGT) (25 μg/ml). This is not surprising considering that α-BGT does not block nicotinic receptors at neuronal synapses, unlike its action at the neuromuscular junction (Chou & Lee, 1969; Miledi & Potter, 1971; Nurse & O'Lague, 1975; Miledi & Szczepaniak, 1975; Duggan, Hall & Lee, 1976; Brown & Fumagalli, 1977; Hanley, Bennett & Lukasiewicz,

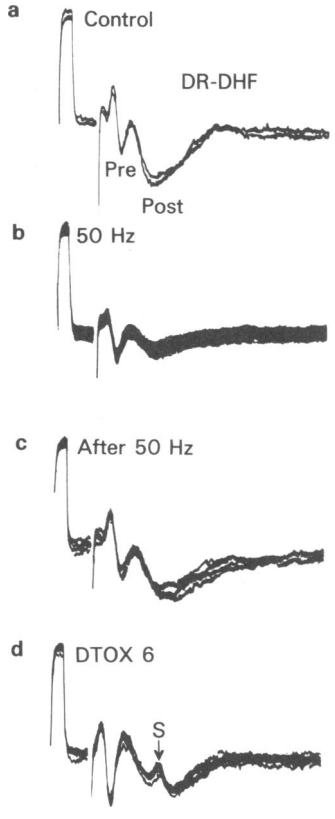

Figure 4 Effect of dendrotoxin (DTOX) 6a on the field potential recorded in the dorsal horn following DR stimulation (DR-DHF). (a) Control response, Pre=presynaptic component; Post=postsynaptic component; (b) response recorded during stimulation at 50 Hz, note depression of postsynaptic component of response; (c) progressive recovery of postsynaptic component of DR-DHF following 50 Hz stimulation; (d) DR-DHF recorded in the presence of DTOX 6a (40 μg/ml). S indicates probable spike on postsynaptic component of DR-DHF although amplitude of e.p.s.p. field potential is unchanged. Traces filmed from oscilloscope. Calibration pulse=2 mV/1 ms.

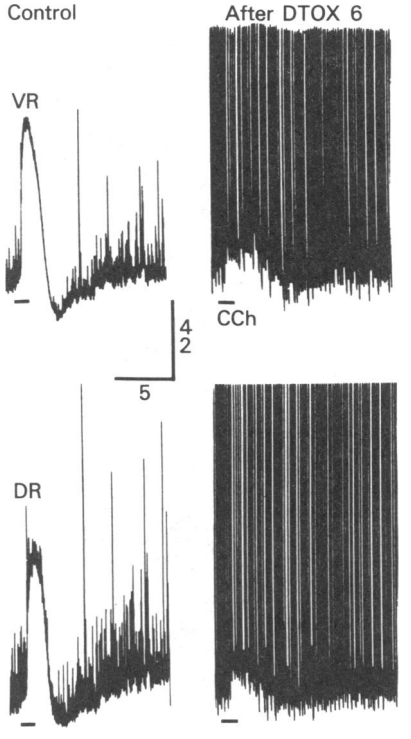

Figure 5 The effect of dendrotoxin (DTOX) 6 on the response of frog spinal cord to carbachol (CCh). Upper traces: sucrose gap recording from Xth ventral root (VR). Lower traces: simultaneous recording from Xth dorsal root (DR). Left hand records: control response of both roots to superfusion with 0.1 mM carbachol (CCh). Right hand records: response to CCh recorded 64 min after incubation of the preparation for 42 min in DTOX 6 (100 μg/ml). Note pronounced increase in spontaneous activity. Spontaneous responses on DR are limited by excursion of pen. CCh response is greatly reduced. Experiment performed in normal Ringer solution without manganese or tetrodotoxin. Black bar indicates duration of exposure to CCh. Calibration=4 mV/5 min for VR, 2 mV/5 min for DR. Traces from rectilinear pen recorder.

1978; Kouvelas, Dichter & Greene, 1978; Morley, Kemp & Salvaterra, 1979; Patrick *et al.*, 1980; but see also Chiappinelli & Zigmond, 1978; Fex & Adams, 1978; Marshall, 1979).

Discussion

Two sites of action for dendrotoxin

As discussed above, the difference in the time course of onset and rate of recovery between the convulsant and VR-DRP blocking actions of DTOX strongly suggests that the toxin has two separate sites of action in the frog spinal cord.

Characterization of convulsant effects of dendrotoxin

The apparently irreversible convulsant action of the toxin may be a consequence of the irreversible binding of the toxin to nerve tissue (Patrick *et al.*, 1980). The present data indicated that DTOX-induced convulsions are not produced as a result of a change in passive membrane properties, antagonism of the action of inhibitory amino acids, potentiation of the actions of excitatory amino acids or, probably, from

an increase in excitatory transmitter release. (The toxin neither changed the presynaptic component nor enhanced the postsynaptic component of the DR-DHF). The possibility remains that the convulsions result from the blockade of release of inhibitory amino acids. A blockade of GABA release is somewhat unlikely since DTOX does not block DR-DRP which is thought to be mediated in part via this transmitter (Barker *et al.*, 1975 a, b). However, it should be noted that impairment of GABAergic transmission with the antagonists, picrotoxin or bicuculline, also does not eliminate this potential. An action of DTOX on release of glycine cannot be excluded. The toxin could also block the release or the postsynaptic action of a range of other putative modulators of synaptic activity in frog spinal cord, for example, 5-hydroxytryptamine (Caulford & Coceani, 1977; Kudo, 1978; Soller & Erulkar, 1979) or enkephalins (Padjen, unpublished observations). Further studies on the pharmacology of the spinal cord may be advanced by testing DTOX as a potential antagonist of the postsynaptic actions or release of these compounds.

Although DTOX 6 tends to favour the appearance of action potentials on e.p.s.ps and this could imply that the toxin lowers the threshold for action potential generation, this is not necessarily the only mechanism underlying its convulsant effects, even though the time course and irreversibility of both effects are similar. It is interesting to note that similar increases in excitability have been observed with several convulsants, including picrotoxin (Barker & MacDonald, 1980) and tetanus toxin (Dimpfel, 1979). It has been suggested that such effects could underlie the convulsant action of tetanus toxin (Dimpfel, 1980) although an effect on GABA release has been reported (Davies & Tongroach, 1979). Since tetanus toxin also affects ACh release (Bigalke, Dimpfel & Habermann, 1978) with a different time course from its convulsant actions, there seem to be some similarities between its action and those of DTOX which, as noted above, may also reduce GABA (or glycine) release and promote increases in excitability.

Dendrotoxin and blockade of VR-DRP

DTOX 6 greatly attenuated the depolarization recorded on both VR and DR following the administration of carbachol. This is consistent with an action of the toxin on nicotinic receptors; however, it does not prove that this is the site of toxin action because of the indirect nature of these responses (cf. Nicoll, 1975).

DTOX 6 could be antagonizing the carbachol response at any point between the cholinoceptive neurones and the ventral and dorsal roots. Furthermore, it is likely that the response to carbachol is mediated, at least in part, by muscarinic as well as a population of nicotinic receptors (Phillis & Tebecis, 1967; Kudo, Kim & Fukuda, 1978) which may be different from those involved in the VR-DRP pathway. When indirect responses were blocked by using 2 mM Mn^{2+}/0.4 mM Ca^{2+} Ringer (cf. Nicoll, 1975), carbachol or ACh produced only small and variable responses on VR or DR. These responses, presumably resulting from activation of acetylcholine receptors on primary afferents or motoneurones, were not completely antagonized by DHβE. Since this indicates that such responses were not mediated purely by nicotinic receptors, their sensitivity to DTOX 6 was not examined. Also, the direct responses of VR and DR to carbachol or ACh presumably reflect activation of a different population of acetylcholine receptors from those involved in VR-DRP.

In addition to the nicotinic synapse (Kiraly & Phillis, 1961; Mitchell & Phillis, 1962) there is at least one more synapse involved in the VR-DRP pathway where transmission is presumed to be mediated by a neutral amino acid (Barker *et al.*, 1975b). Three lines of evidence indicate that DTOX does not block VR-DRP by interference of transmission mediated by any putative amino acid transmitter. (1) DTOX does not block the responses of VR or DR to GABA, glycine, β-alanine, glutamate or aspartate. (2) DR-VRP and DR-DRP were not blocked by DTOX 6. These responses which are mediated by amino acid neurotransmitters (Barker *et al.*, 1975 b; Padjen & Smith, 1980) were in fact enhanced by DTOX 6. (3) DTOX 6 did not block the field potentials resulting from the e.p.s.ps in dorsal horn interneurones following dorsal root stimulation (Figure 4). Even though DTOX does not block nerve conduction (Miledi & Szczepaniak, 1975), one cannot by a process of elimination of all possible sites of toxin action arrive at the unequivocal conclusion that the site of action is the cholinergic synapse of the VR-DRP pathway. Speculation allows for at least two possible mechanisms whereby the toxin could specifically block VR-DRP: the toxin could antagonize the action or prevent the release of some other hitherto undetected neurotransmitter involved somewhere in the polysynaptic VR-DRP pathway; or the toxin could cause hyperpolarization of interneurones involved in the VR-DRP pathway so that e.p.s.ps generated in them do not exceed the threshold for action potential generation; propagation of the response through the polysynaptic pathway could thereby be prevented. Either of these two possibilities could also account for the observed antagonism of carbachol responses observed with DTOX 6 (Figure 5).

If DTOX does exert its effect by blockade of the cholinergic synapse of the VR-DRP pathway, it is unlikely to have a presynaptic action on ACh release since it does not block nicotinic transmission in sympathetic ganglia (Quik *et al.*, 1980). Both the ACh

releasing pre-ganglionic sympathetic terminal and the ACh releasing recurrent collateral of the VR-DRP pathway are processes of motoneurones. It would therefore seem unlikely that ACh release from one of these terminals is DTOX 6-sensitive while release from the other terminal is not.

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