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SITES OF ACTION OF MOJAVE TOXIN ISOLATED FROM THE VENOM OF THE MOJAVE RATTLESNAKE

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1 Mojave toxin isolated from the venom of the Mojave rattlesnake (*Crotalus scutulatus scutulatus*) produced an irreversible blockade of the contractile response of the mouse hemidiaphragm to stimulation of the phrenic nerve *in vitro*, at concentrations of 0.16 to 20 μg/ml; the response to direct stimulation was not affected over a testing period of several hours.

2 Mojave toxin (1 to 4 μg/g) was injected into the tail vein of mice and the intoxicated hemidiaphragm preparation was removed either for testing the contractile response or for intracellular recording.

3 In fully intoxicated hemidiaphragms the contractile response to indirect stimulation was either small and transient or absent, whilst the response to direct stimulation was well maintained.

4 Intracellular recording showed that resting membrane potentials of the muscle fibres were within the normal range. Endplates were difficult to locate but miniature endplate potentials (m.e.p.ps) were recorded at sites at which neurally evoked responses either could not be detected or did not exceed 2 mV which corresponds to transmitter release of a few quanta only.

5 The mean frequency of m.e.p.ps at fully intoxicated endplates was not significantly different from controls but potassium depolarization produced only a small increase in m.e.p.p. frequency relative to the control response. A 50 Hz tetanus had no effect on m.e.p.p. frequency.

6 When a sub-lethal dose (3 μg) of Mojave toxin was injected into one hindlimb of mice and the tissues examined at 72 h, there was histological evidence of myonecrosis.

7 The isolated perfused heart of the rat was exposed to recycled Mojave toxin (50 and 100 μg/ml) but showed no change in rate or force of ventricular contraction.

8 Post-mortem examination of intoxicated mice showed a frequent incidence of localized areas of interstitial and intra-alveolar haemorrhage in the lungs. Other organs including skin and muscle were not affected.

9 Mojave toxin showed antigenic similarities to crotoxin, the lethal neurotoxin in the venom of the South American rattlesnake, as determined by the ability of antiserum raised against crotoxin to neutralize Mojave toxin.

10 With systemic Mojave intoxication of rapid onset, the cause of death was respiratory paralysis. However, the toxin acts at multiple sites at differing rates of action. With a slower rate of intoxication, impaired respiration may act synergistically with cardiovascular changes to produce circulatory failure. The desirability of using an antivenin with a high titre against Mojave toxin is indicated.

Introduction

A limited number of investigations have been made on purified toxins of the rattlesnake (*Crotalus* spp.) indigenous to North America. Of the eighteen species of rattlesnakes found in the continental United States, the venom of the Mojave rattlesnake (*Crotalus scutulatus scutulatus*) is the most toxic so far identified, having an LD_{50} value of 0.18 μg per g body wt. in mice (Tu, 1977). This is comparable in toxicity to the venom of the South American tropical rattlesnake (*C. durissus terrificus*). The principal lethal toxin, Mojave toxin, was isolated from the venom of the Mojave rattlesnake (Bieber, Tu & Tu, 1975). It has a molecu-

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lar weight of 22,000 with an isoelectric point of 4.7 indicating that the toxin is an acidic protein. The peptide backbone conformation of the Mojave toxin was found to be predominantly α-helix as studied by Raman spectroscopy (Tu, Prescott, Chou & Thomas, 1976). Mojave toxin has a molecular weight of 22,000 as determined by the Sephadex gel method; however, it becomes 12,000 when determined by the gel electrophoresis method using sodium dodecyl sulphate (Bieber *et al.*, 1975). This strongly suggests that Mojave toxin consists of subunits. Recently, it has been shown that indeed this is the case as Mojave toxin can be dissociated into an acidic protein and a basic protein with phospholipase A activity (Cate & Bieber, 1978). When the two subunits are combined, the intact Mojave toxin can be reconstituted.

Poisoning from the bite of North American rattlesnakes can lead to the onset of shock (Russell, 1979) and, in anaesthetized animals, many of these venoms produce circulatory failure associated with vasodilatation and hypovolaemia. In contrast, poisoning by the venom of the South American rattlesnake involves neurotoxic symptoms and respiratory paralysis (see review by Lee & Lee, 1979). When injected into anaesthetized rabbits, Mojave toxin (1 mg/kg) produced an immediate fall in systemic blood pressure with gradual recovery. A later fall in blood pressure was associated with dramatic changes in the ECG and the primary cause of death was considered to be a direct cardiac action (Bieber *et al.*, 1975).

Mojave toxin has biochemical similarities to crotoxin, the principal lethal toxin in the venom of the South American rattlesnake, in respect to molecular weight, subunit structure and phospholipase A activity (see review by Habermann & Breithaupt, 1978). It was important to make a detailed examination of the sites of action of Mojave toxin, particularly in relation to the cause of death as (a) crotoxin blocks the release of transmitter from motor nerve terminals (Chang & Lee, 1977; Hawgood & Smith, 1977); (b) some respiratory impairment was present in Mojave intoxication of the anaesthetized animal (Bieber *et al.*, 1975); (c) Mojave rattlesnake venom is poorly neutralized by the commercially available polyvalent antivenin (Wyeth) in comparison with venoms from other North American rattlesnakes (Glenn & Straight, 1977; 1978).

A preliminary account of some of this work has been given (Gopalakrishnakone, Hawgood, Holbrooke, Marsh, Santana de Sa & Tu, 1979).

Methods

Lyophilized Mojave rattlesnake venom was obtained from the Miami Serpentarium. Mojave toxin was isolated by the method of Bieber *et al.* (1975). The venom was fractionated with DEAE-Sephadex in 0.5 M Tris buffer, pH 8.3. The toxic fraction was further purified with DEAE-Sephadex in 0.05 M sodium acetate buffer, pH 5.3. Homogeneity of the toxin was examined by polyacrylamide gel electrophoresis.

Contractile responses

Experiments were carried out on adult male TO mice of 24 to 28 g weight. The phrenic hemidiaphragm preparation (Bülbring, 1946) was isolated and mounted in an organ bath containing 10 ml of physiological saline of the following composition (mM): NaCl 137, $NaHCO_3$ 15.0, NaH_2PO_4 1.0, KCl 2.5, $CaCl_2$ 2.0, $MgCl_2$ 1.0 and glucose 11.0, which was aerated with 95% O_2 and 5% CO_2. Preparations were stimulated indirectly and directly by means of alternate rectangular voltage pulses at a combined frequency of 12/min. The stimulus parameters used in indirect stimulation were 0.05 ms duration and usually 1 V which was supramaximal in strength. To determine the necessary parameters for direct stimulation, indirect stimulation was blocked with (+)-tubocurarine (4 μg/ml) added to the bathing solution during the control period, then supramaximal strength (20 to 25 V) was chosen for pulses of 0.3 to 0.5 ms duration. The preparation was washed three times to allow rapid recovery of indirect response. Isometric tension was recorded with a force displacement transducer and displayed on a Washington 400 pen recorder (G. Washington Ltd., Sheerness, Kent). The resting tension was adjusted to give the maximum contractile response.

Intracellular recordings

The left phrenic nerve-hemidiaphragm preparation was isolated and mounted in a bath containing 25 ml of physiological saline. The entry of gas was so arranged that a bubble lift maintained a flow of solution over the muscle. The nerve was stimulated through a suction electrode. Resting membrane potentials, endplate potentials (e.p.ps) and spontaneous miniature endplate potentials (m.e.p.ps) were recorded with glass microelectrodes filled with 3 M KCl using standard techniques. Recordings were displayed on a Mingograf 34 pen recorder with a frequency response of 700 Hz and time constant of 0.15 s. Mean m.e.p.p. frequency (per s) was determined from the number of m.e.p.ps recorded in a period of 20 to 30 s, and mean amplitude from 40 to 110 m.e.p.ps. To make allowance for the effect of different values of the resting membrane potential (E_m) at endplates, a potential of 70 mV was chosen arbitrarily and the mean amplitude adjusted by multiplying by the factor $70 - 15/E_m - 15$, where 15 mV represents the equilibrium endplate potential (Katz & Thesleff, 1957).

Perfused rat heart

Mojave toxin was tested on the Langendorff preparation of the perfused rat heart using a recycling technique which delivered Krebs bicarbonate saline at a perfusion pressure of 60 to 100 mmHg and flow rate of 4 to 5 ml min^{-1} g^{-1}. After allowing the preparation to stabilize for 10 min on free-flow perfusion (i.e. effluent running to waste) a control recycling of 10 ml Krebs saline was performed for 10 min by collecting the effluent, filtering, gassing with 95% O_2 and 5% CO_2 and returning to the heart by a peristaltic pump. The heart was then returned to free-flow for 10 to 20 min. The recycling procedure was then repeated with 10 ml Krebs bicarbonate saline containing Mojave toxin at a concentration of either 50 or 100 μg/ml. Following this recycling, the hearts were perfused on free-flow for 15 to 60 min. During the entire experiment myocardial contractility was monitored by means of an isometric transducer connected to the tip of the ventricle with output to a Washington MD2 oscillograph.

Histological studies

Male TO mice (18 to 28 g) were used in the investigation of structural changes in muscle following a single local injection of a sub-lethal dose of toxin and, in the lungs, following systemic intoxication. Mojave toxin (3 μg in 0.1 ml of 0.9% saline) was injected via a 27 gauge needle into the left hindlimb of 3 mice, in the region of the soleus muscle. Three control animals received similar injections of saline and 2 mice received 16 μg of crotapotin, a non-toxic protein from the venom of *C. durissus terrificus* (gift of Professor E. Habermann, der Justus Liebig Universität, Giessen). The animals were allowed to survive for 72 h then, under ether anaesthesia, fixation was started by the perfusion of formolcalcium through the left ventricle. The whole limb was subsequently decalcified, and the two blocks cut from the calf region were prepared for embedding in wax (Duchen, Excell, Patel & Smith, 1974). Transverse sections were stained with haematoxylin and eosin. At 72 h, necrotic fibres are easily visualized due to extensive cellular infiltration (Duchen *et al.*, 1974). Post-mortem examination was carried out in the majority of mice which were killed at the stage of either partial or full intoxication following intravenous or subcutaneous injection of Mojave toxin. The lungs of 7 mice which received 0.4 to 2 μg/g of toxin were examined histologically. After fixation in 10% formol saline for 3 days, two blocks from each mouse were embedded in wax. Sections from one block were stained with haematoxylin and eosin and sections from the other were stained by the Martius scarlet blue method for fibrin (Lendrum, Fraser, Slidders & Henderson, 1962).

Student's *t* test was used for the statistical analysis.

Antiserum against crotoxin and its neutralization capacity

Antiserum against crotoxin was raised in one New Zealand white rabbit (3.4 kg). The crotoxin complex was reconstituted from its subunits (Hawgood & Smith, 1977) and the dose of 100 μg/kg body wt. was used. The crotoxin solution was emulsified with an equal volume of Freund's complete adjuvant and injected intradermally in about 20 points on either side of the vertebral column. Regular booster injections were given at 3 weekly intervals and the animal was bled through the marginal ear vein after the 4th injection. The presence in the serum of antibodies to crotoxin and to Mojave toxin was tested by the double immunodiffusion method of Ouchterlony (1964).

The LD_{50} of both the sample of Mojave toxin and of the crotoxin complex was determined from dose-response curves. In each case 6 toxin concentrations were used with 6 animals in each group of mice of individual weight 18 to 20 g. The number of animals which survived 24 h was observed. The neutralization capacity of the antiserum to both toxins was determined by an *in vitro* test in which $2LD_{50}$ was selected as the test dose. Six groups of mice with 6 animals in each group were used. The toxin ($2LD_{50}$) was mixed with 0.1 ml of crude antiserum or antiserum of doubling dilution, and incubated at 37°C for 30 min; 0.2 ml of the solution was injected into the tail vein and the number of mice surviving at 24 h was observed.

Results

Effect of Mojave toxin on the development of twitch tension in indirectly and directly stimulated muscle in vitro

Mojave toxin was added to the bathing fluid to give a final concentration of 0.16 to 20 μg/ml or molar concentration of 7×10^{-9} to 9×10^{-7}. After a short latent period, the contractile response was progressively reduced until complete block developed but the response to direct stimulation was unaffected (Figure 1). After 3 h exposure to 20 μg/ml Mojave toxin, the contractile response to direct stimulation was still 75 to 87% control tension (3 muscles). The mean times to 50% block of the contractile response to nervous stimulation are shown in Figure 2. Mojave toxin (10 μg/ml) also blocked the response of the innervated rat diaphragm to indirect stimulation with times to 50% block of 40 and 49 min, in comparison with a mean time of 23.6 min for the mouse hemidiaphragm. Binding of the toxin appeared to be rapid and irreversible as washing at the stage of 80% reduc-

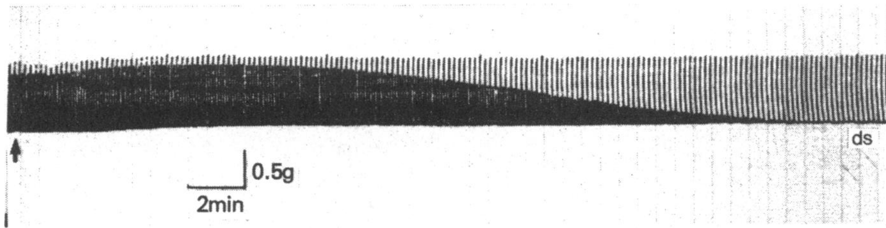

Figure 1 Effect of Mojave toxin (20 μg/ml) on the contractile response of the mouse hemidiaphragm preparation to alternately delivered indirect and direct stimulation. Toxin was added at the arrow. Indirect stimulation rapidly became ineffective but the response to direct stimulation (ds) remained unchanged for more than 2 h. Temperature 37°C.

tion of control tension failed to produce any recovery of response to nervous stimulation and washing 3 times within the latent period, viz 10 to 15 min after the addition of 2.5 μg/ml Mojave toxin gave a mean time to 50% block of 41.3 ± 11.6 min (s.d. of 3 muscles) which was not significantly different from the mean time of 28.7 ± 4.9 min (3 muscles) in the absence of washout.

In vivo *intoxicated muscles*

A bolus injection of Mojave toxin (1 to 4 μg/g) into the tail vein of mice produced early clinical signs of incoordination of movement. The mice were killed at the stage of full intoxication when the limbs appeared paralysed and breathing was laboured, and the innervated hemidiaphragm preparation was removed. The intoxicated hemidiaphragm failed to show a contractile response to nervous stimulation *in vitro* in 3/4 cases, and the small response in the fourth preparation was absent by 30 min. The twitch tension in response to direct stimulation was within the normal range obtained in *in vitro* experiments and was well maintained over the recording period of 90 to 270 min. These results suggested that Mojave toxin had sufficient neurotoxic action *in vivo* to cause death. To confirm this finding and to elucidate the site of action of the toxin, intracellular recording techniques were used to determine any deficit in spontaneous and neurally evoked release of transmitter in *in vivo* intoxicated muscles.

Presynaptic site of action

The resting membrane potentials of single muscle fibres in intoxicated hemidiaphragms were within the range of values recorded from the diaphragm of control mice although the mean value of 66.3 ± 1.2 mV (s.e. mean of 58 fibres, 7 muscles) was somewhat lower than the mean value of 70.8 ± 0.8 mV (s.e. mean of 100 fibres, 5 muscles) recorded from control fibres.

In comparison with control preparations, endplates were difficult to locate in these intoxicated hemidiaphragms but m.e.p.ps could be recorded from sites in which either no neurally evoked response could be detected, or, at 3 endplates, e.p.ps of up to 2 mV in amplitude were recorded which corresponds to a release of transmitter of a few quanta only. The mean frequency of m.e.p.ps was not significantly different from controls recorded at either 23°C or 27°C (Table 1). To test the ability of the nerve terminals to respond to depolarization, the K concentration of the medium was raised from 2.5 to 10 mM. The increase in m.e.p.p. frequency at the severely intoxicated endplates was two fold which was considerably lower than the 12 fold increase observed in controls (Table 1). To test the possibility that action potentials may not be able to invade the nerve terminals, the effect on m.e.p.p. frequency of a tetanus of 50 Hz for 30 s was determined. No significant increase in m.e.p.p. frequency was detected at 11 endplates in 3 muscles when paired data were examined. This is in marked contrast to the tetanic and post-tetanic increases in m.e.p.p. frequency observed at normal mammalian endplates in low Ca, high Mg Ringer (Liley, 1956).

Mean m.e.p.p. amplitudes varied considerably between endplates, probably due to differences in fibre size (Katz & Thesleff, 1957). In intoxicated muscles the overall mean value was 255 ± 97 μV (s.d. of 9 endplates in 4 muscles) in comparison with the overall mean value of 400 ± 168 μV (s.d. of 9 endplates in 4 muscles) in control preparations. As the coefficient of variation was 46.6%, it cannot be ascertained whether intoxication reduced m.e.p.p. amplitudes. The distribution of m.e.p.p. amplitudes at individual endplates was within normal limits.

In another set of experiments, innervated hemidiaphragm preparations were removed from mice at the stage of partial intoxication when limb movement was affected but respiration was still shallow. As phrenic nerve stimulation produced contraction of some muscle fibres, it was necessary to equilibrate the iso-

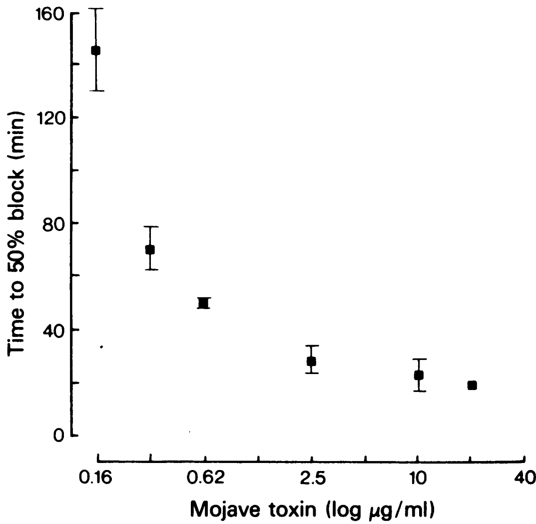

Figure 2 Effect of various concentrations of Mojave toxin on the time to 50% block of the contractile response of the mouse hemidiaphragm evoked by indirect stimulation at 37°C. Each point is the mean result from 3 to 4 muscles and vertical lines show s.d.

lated preparation with (+)-tubocurarine prior to recording. The response of the endplate to a train of stimuli applied to the nerve at 50 Hz for 1 to 2 s was determined. The amplitude of successive e.p.ps in the train was variable and did not show the marked initial decline characteristic of control endplates. At some endplates there was an overall small decline, at others a small degree of facilitation. This was shown clearly in one intoxicated muscle which was equilibrated with 1 µg/ml (+)-tubocurarine when, at 8 endplates, the amplitudes of the 5th and 10th e.p.ps in each train were expressed as a percentage of the amplitude of the 1st e.p.p. The 5th e.p.p. was 117 ± 43.2% (mean and s.d.) and the 10th e.p.p. was 91.5 ± 44.0% of the amplitude of the 1st e.p.p. which is significantly different from the values in a control muscle which was equilibrated with 3 µg/ml (+)-tubocurarine; here the 5th e.p.p. was 46.9 ± 12.6% (mean and s.d. of 7 endplates, $P < 0.005$) and the 10th e.p.p. was 25.9 ± 10.5% ($P < 0.005$) of the amplitude of the 1st e.p.p. An unusual finding was the presence of periodic failure of response within the train at 4 endplates in another intoxicated muscle equilibrated with 3 µg/ml (+)-tubocurarine. There was no decline in amplitude in the 4 to 5 e.p.ps of each group within the train. In all, 29 endplates in 2 intoxicated muscles and 28 endplates in 2 control muscles were examined.

Myonecrosis

In acute *in vitro* experiments, raising the concentration of Mojave toxin to 30 µg/ml produced a variability in the contractile response to direct stimulation which ranged from no change to a reduction to 17% control tension at 2 h. To test if Mojave toxin produces a more slowly developing myotoxicity as has been shown for the crotoxin complex (Gopalakrishnakone & Hawgood, 1979) a sub-lethal dose (3 µg) was injected into the region of the soleus muscle in one hindlimb of 3 mice. At 72 h after the injection of Mojave toxin, soleus muscle fibres were heavily infiltrated with cells indicative of extensive damage (Figure 3a). Leucocytes, other phagocytic cells and cells with prominent nuclei, possibly myogenic cells were seen. Muscle groups surrounding the soleus muscle showed cellular infiltration in a few peripheral fibres. The soleus muscle of mice receiving control injections of either saline or the non-toxic protein, crotapotin, were normal in appearance (Figure 3b). Myonecrosis was not associated with extravasation of erythrocytes.

Table 1 Comparison of miniature endplate potential (m.e.p.p.) frequencies recorded at control and severely intoxicated endplates under various experimental conditions

		M.e.p.p. frequency (per s)	
		Control endplate	*Intoxicated endplate**
Normal medium	(27 ± 1°C)	0.85 ± 0.05 (57,3)**	0.75 ± 0.17 (16,3)
	(23 ± 1°C)	0.57 ± 0.09 (32,2)	0.47 ± 0.06 (38,4)
K 10 mM	(27 ± 1°C)	10.28 ± 0.82 (53,3)	1.57 ± 0.13 (40,3)

Values are mean ± s.e. mean; *1 µg/g Mojave toxin given intravenously; **number of fibres and number of muscles, respectively.

Cardiac contractility

When rat isolated perfused hearts were exposed to a recycling concentration of Mojave toxin of either 50 µg/ml (3) or 100 µg/ml (1), there was no evidence of cardiotoxicity. Toxin recycling caused a slight fall in the strength of contraction but this was no greater than that produced by recycling of normal Krebs bicarbonate saline. In both cases the strength of contraction returned to normal after the resumption of free-flow conditions. Heart rate was unaltered throughout these experiments. The experimental conditions of 10 min contact time with the toxin and subsequent monitoring of cardiac activity for up to 60 min were those in which marked depression of neuromuscular transmission were demonstrated.

Histological examination of lungs

In the post-mortem examinations of 17 severely intoxicated mice, areas of dense red colouration on the surface of the lungs were observed in 13 animals. All other visceral organs appeared normal and no haemorrhages were seen. There was considerable variation between animals as to the number, size and distribution of these patches; 5 animals showed only mild involvement with a few small scattered areas, and in 4 animals very large areas of discolouration were observed in 3 or more lobes, particularly at the hilus. These lesions were not seen in control animals and were not related to the mode of death by decapitation. The lungs of 7 mice with varying degrees of gross abnormality were examined by light microscopy. The only significant pathology was the presence of areas of interstitial and intra-alveolar haemorrhage (Figure 4a); oedema was mild. Platelet thrombi were seen occasionally blocking the capillaries but no fibrin thrombi were observed. In confirmation, the toxin (1 mg/ml) showed no clotting activity on either citrated plasma or fibrinogen solution and did not enhance the recalcification time of plasma. Indications that several factors may contribute to the clinical condition of the animal, particularly if intoxication is of slow onset, were apparent in 4 mice which received Mojave toxin (0.4 to 2 µg/g) either subcutaneously or intramuscularly. The animals appeared to be in a state of shock which was not apparently related to respiratory difficulty. When the animals were killed at 2.5 to 3.5 h and the innervated hemidiaphragm isolated, the twitch tension elicited by nerve stimulation was well maintained in recording periods which ranged from 60 min (1) to 2 to 3 h (3). When the lungs of 3 of these animals were visually examined, two showed gross abnormalities (Figure 4a) and one appeared normal.

Neutralization of Mojave toxin by antiserum produced against crotoxin

As the similarities in the biological as well as biochemical properties of crotoxin became apparent, it was of considerable interest to determine if Mojave toxin showed antigenic similarities to the lethal component of South American rattlesnake venom. The presence of antibodies to both crotoxin and Mojave toxin in the serum raised against crotoxin was shown by the single precipitating line which the serum formed against both toxins during gel immunodiffusion. No reaction was observed when serum from a non-immunized rabbit was used. The intravenous LD_{50} in mice of the sample of Mojave toxin was 0.08 µg/g mouse and 0.1 ml of antiserum at 1:4 dilution completely neutralized a dose of $2LD_{50}$. On this basis, 1 ml of crude antiserum would be capable of neutralising 80 (i.v.) LD_{50} in mice. The sample of crotoxin used to raise the antibodies had an (i.v.) LD_{50} of 0.23 µg/g mouse and the neutralizing capacity of 1 ml of antiserum was determined as 160 (i.v.) LD_{50} in mice.

Discussion

The results of the present study suggest that Mojave toxin interferes with neuromuscular transmission both *in vitro* and *in vivo*. A presynaptic site of action is indicated by the finding that spontaneous release of transmitter could be recorded at severely intoxicated endplates in which the neurally evoked response was absent or confined to the release of a few quanta only, and that spontaneous release of transmitter was increased to only a small extent by potassium depolarization of the nerve terminal. In this respect its action is similar to that of crotoxin, the lethal toxin from the venom of the South American rattlesnake, which is considered to interfere with depolarization-secretion coupling at the motor nerve terminal (Chang & Lee, 1977; Hawgood & Smith, 1977). However, certain features of crotoxin poisoning, such as an alteration in m.e.p.p. frequency and the appearance of a high proportion of large m.e.p.ps were not observed in Mojave intoxication. In addition the failure of a 50 Hz tetanus to increase m.e.p.p. frequency at a late stage of Mojave intoxication suggests that impulses may no longer be able to invade the motor nerve terminal. Depolarization of the nerve terminal is contraindicated by the unchanged nature of m.e.p.p. frequency and the normal contractile response to direct muscle stimulation indicates that there are no major changes in the properties of the sarcolemmal membrane. *In vitro* the smooth progression to blockade of the contractile response to nervous stimulation suggests that the defect resides in or very close to the

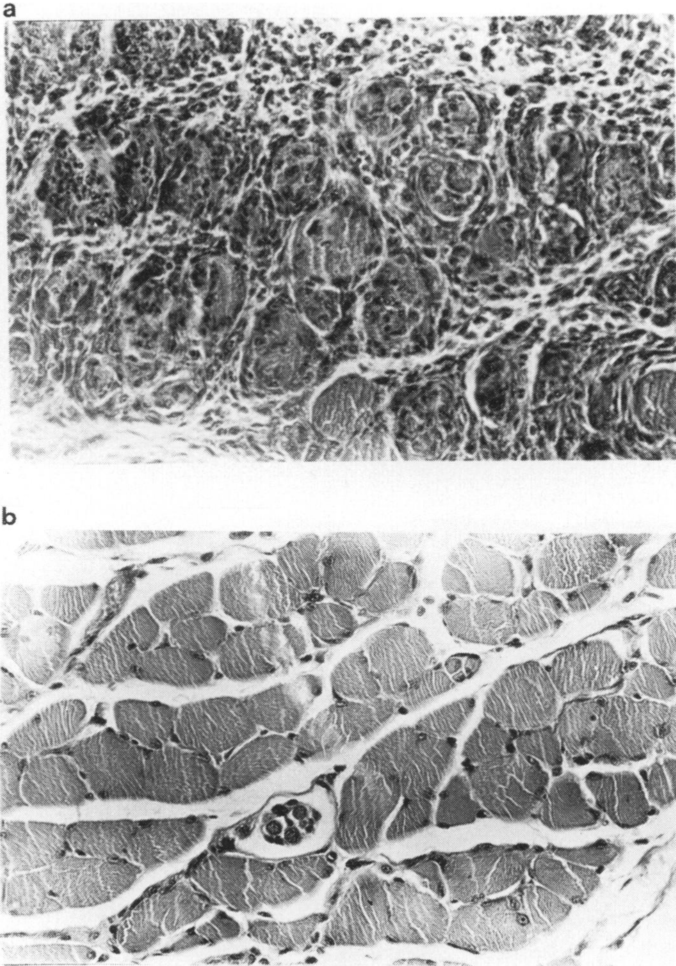

Figure 3 Transverse section of the soleus muscle of a mouse at 72 h after local injection of (a) 3 μg Mojave toxin and (b) saline. Notice the intense cellular infiltration in (a) indicative of myonecrosis. Magnification ×400, H.E.

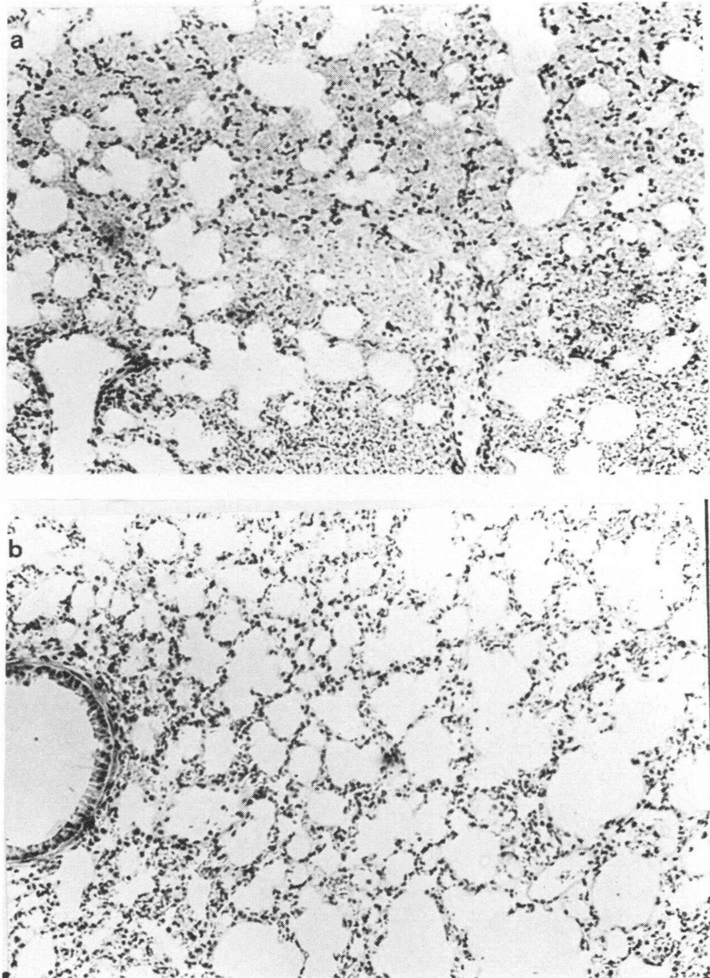

Figure 4 Transverse sections of lung (a) from a mouse intoxicated with 1 μg/g Mojave toxin given subcutaneously. The animal appeared moribund and was killed at 2 h. Three lobes were grossly affected. (b) Lung section from a control mouse. Magnification ×240, H.E.

nerve terminal rather than resulting from the loss of motor units. Potentials evoked at partially intoxicated endplates by a tetanus of 50 Hz showed an abnormal pattern of response which included transient facilitation in the presence of (+)-tubocurarine. Varying concentrations of (+)-tubocurarine can lead to different rates of initial decline of e.p.p. amplitudes within a train (Hubbard & Wilson, 1973) but transient facilitation of response due to high frequency stimulation was not observed in the presence of curare (Glavinović, 1979). These results also suggest that Mojave toxin interferes with transmitter release.

The myonecrotic activity shown by Mojave toxin following prolonged exposure suggests that it belongs to the group of toxins which include crotoxin, notexin from *Notechis scutatus scutatus* venom and taipoxin from *Oxyuranus scutellatus scutellatus* venom which show three biological activities viz. phospholipase A activity (Halpert & Eaker, 1975; Fohlman, Eaker, Karlsson & Thesleff, 1976; Cate & Bieber, 1978; Habermann & Breithaupt, 1978), a presynaptic blocking action at motor nerve terminals (Harris, Karlsson & Thesleff, 1973; Kamenskaya & Thesleff, 1974) and a more slowly developing myonecrosis of skeletal muscle (Harris, Johnson & Karlsson, 1975; Harris, Johnson & MacDonell, 1977; Gopalakrishnakone & Hawgood, 1979).

Systemic intoxication

Studies by Brazil (1972) and Breithaupt (1976) indicate respiratory paralysis of peripheral origin as the cause of death following intravenous crotoxin intoxication, and crotoxin failed to produce any prolonged effect on the cardiovascular system (Brazil, Farina, Yoshida & de Oliveira, 1966; Breithaupt, 1976). In Mojave intoxication of anaesthetized rabbits, cardiovascular collapse occurred at the stage when the diaphragm still responded to phrenic nerve stimulation and artificial respiration produced no improvement; this suggested a direct cardiovascular action by Mojave toxin (Bieber *et al.*, 1975). However, the present study has shown that, in the absence of a depressant action of anaesthetics, Mojave toxin can cause death by a direct neurotoxic action on the motor nerve terminals. This is based on the finding of interference with the contractile response of the innervated diaphragm to nervous but not to direct stimulation at the stage of full intoxication, as well as interference with neurally evoked release of transmitter at the motor endplate. Mojave toxin appears to have multiple sites of action and in addition to neurotoxic and myonecrotic activity, signs of cardiovascular involvement were observed in the present study. This was shown in the frequent but not invariable presence of localized lung lesions and in a lack of correlation of the clinical condition of the intoxicated mice with the functional state of the diaphragm following subcutaneous injection.

The observed type of lung pathology in which interstitial and some intra-alveolar haemorrhage was associated with mild oedema and some platelet thrombi was markedly different from the massive haemorrhages associated with fibrin thrombi formation shown by coagulative venoms such as *Echis carinatus* (Jerushalmy, Sanbank, Aschheim & de Vries, 1970). The absence of any signs of haemorrhagic lesions in organs other than the lungs including skin and muscle, indicates that Mojave toxin does not have a generalized action on capillaries but does suggest that the lungs may be a relatively sensitive index of circulatory changes induced by Mojave toxin. No pathological changes in the lungs were observed during crotoxin intoxication.

Mojave toxin (Bieber *et al.*, 1975) and *C. durissus terrificus* phospholipase A alone or in complex form as crotoxin (Breithaupt, 1976) induced acute hypotension of rapid onset following fast intravenous injection; Breithaupt considered this to be due to the release of histamine from mast cells. However, it is of interest that these substances all show phospholipase A_2 activity and that this enzyme is intimately involved in prostaglandin formation (Vogt, 1978). Exogenous phospholipase A_2 from *Naja* venom has been shown to release prostaglandin-like substances from perfused guinea-pig lung (Damerau, Lege, Oldigs & Vogt, 1975) but no histamine release or mast cell degranulation was observed. Prostaglandins of the E series lower arterial pressure in all species studied at least partly by a direct vasodilator action in resistance vessels (Malik & McGiff, 1976). Activation of prostaglandin synthesis is rapid (Blackwell, Flower, Nijkamp & Vane, 1977) as also is the disappearance of prostaglandins from the circulation (Ferreira & Vane, 1967) and it is feasible that the phospholipase A activity of Mojave toxin and of crotoxin releases prostaglandins into the circulation. With Mojave intoxication the prostaglandin concentration may rise sufficiently high to produce, in localized areas of the lungs, severe congestion and an increased vascular permeability leading to haemorrhage. There is no published information as to the role prostaglandin formation may play in the genesis of circulatory shock due to any snake venom and the possible role of prostaglandins in cardiovascular changes produced by Mojave toxin warrants detailed study. Its neurotoxic action would act synergistically to hasten the onset of circulatory failure. Mojave toxin had no direct effect on the isolated heart of the rat and such lack of effect has also been shown for crotoxin (Breithaupt, 1976). The ECG changes observed in the final stages of Mojave intoxication in the anaesthetized animal (Bieber *et al.*, 1975) may be a secondary

effect mediated by an increased concentration of circulating substances including K^+ ions.

In addition to the biological similarities of neurotoxic and myonecrotic activity, Mojave toxin and crotoxin have antigenic similarities as shown by the ability of antiserum produced against crotoxin to afford protection against Mojave intoxication in mice. The neutralization capacity of the antiserum was two fold less for Mojave toxin than for crotoxin. The commercially available antivenin (Wyeth Antivenin Crotalidae polyvalent) which was prepared with *C. durissus terrificus* (South American rattlesnake) venom as one of the 4 venoms, was less effective in neutralizing *C.s. scutulatus* (Mojave rattlesnake) venom than *C.d. terrificus* venom by a factor of 1.9. This antivenin poorly neutralized Mojave rattlesnake venom in comparison with the venom from other North American rattlesnakes (Glenn & Straight, 1977; 1978). As Mojave toxin has been shown to be a potent neurotoxin and circulatory failure due to rattlesnake envenomation may arise from the action of a variety of substances depending on the species, the preferred horse antiserum for use in Mojave rattlesnake venom poisoning should contain a high titre against Mojave toxin. This could be best provided by the preparation of species-specific (monospecific) antiserum but, in the future, antiserum raised against individual lethal toxins may prove the most effective means of combating envenomation.

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