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EFFECT OF CROTAMINE, A TOXIN OF SOUTH AMERICAN RATTLESNAKE VENOM, ON THE SODIUM CHANNEL OF MURINE SKELETAL MUSCLE

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1 Crotamine (0.5 μg/ml) augmented the single twitch response of the rat and mouse isolated diaphragm to direct stimulation and prolonged the time course of contraction. At higher doses (10 to 50 μg/ml), contracture was observed with spontaneous fibrillation.

2 The resting membrane potential of diaphragm was rapidly depolarized to about −50 mV within 5 minutes. No increase of depolarization occurred on prolongation of the incubation time or increase of crotamine concentration from 0.5 μg/ml to 50 μg/ml. The effect was not reversed by washing.

3 Tetrodotoxin, low Na^+ (12 mM), Ca^{2+} (10 mM) and procaine (1 mM) prevented the crotamine-depolarization. However, depolarization resumed when crotamine and the antagonists were removed.

4 Low Cl^- (8.5 mM) and pretreatment with ouabain enhanced depolarization by crotamine.

5 High K^+ (25 to 50 mM) prevented the further depolarization by crotamine and the membrane potential was restored to normal on washout of crotamine with normal Tyrode solution.

6 Effective membrane resistance was decreased by about 50% by crotamine.

7 ^{24}Na-influx of the rat diaphragm was increased by crotamine. ^{42}K-influx was slightly increased if tetrodotoxin was also present but was decreased in the absence of tetrodotoxin.

8 No effect on the miniature and evoked endplate potential of the rat diaphragm was observed. Skeletal muscles from frog and chick were not affected.

9 It is inferred that crotamine acts on a molecule regulating the Na^+- permeability of the Na^+ channel of murine muscles. It is proposed that extracellular K^+ depresses the permeability of the Na^+ channel by acting on the same regulator molecule.

Introduction

Crotamine in the venom of South American rattlesnake (*Crotalus durissus terrificus*) is strongly basic polypeptide toxin composed of 42 amino acid residues (Laure, 1975). It appears to produce spasmodic seizure and tonic convulsion in animals (Barrio & Vital Brazil, 1951; Cheymol, Bourillet, Roch-Arveiller & Toan, 1969; Cheymol, Gonçalves, Bourillet & Roch-Arveiller, 1971 a,b). Crotamine induces contracture of skeletal muscle, either *in situ* or isolated, of cats, rats and mice but not of adult chickens (Moussatché, Gonçalves, Vieira & Hasson, 1956; Gonçalves, 1956; Cheymol *et al.*, 1969; 1971 a,b). On the rat diaphragm, crotamine elicits an immediate contracture followed by spontaneous irregular contraction (Cheymol *et al.*, 1971 b) and depolarizes the resting membrane potential by about 25% (Pellegrini Filho, Vital Brazil & Laure, 1976). These effects are antagonized by tetrodotoxin, Ca^{2+}, Mg^{2+} and K^+.

In the present experiments, the depolarizing action of crotamine on the diaphragm muscle of the mouse and rat was studied further. The results suggest that crotamine acts on the Na^+ channel or its modulator, on which extra-cellular K^+ has a regulatory effect for controlling the Na^+-permeability of the sarcolemma. Since the effect of crotamine is not easily reversible by washing it should be useful as a tool for both biochemical and physiological studies of the Na^+ channel of sarcolemma and for the K^+-control of Na^+-permeability in mammalian skeletal muscle.

Methods

Purification of crotamine

Crotamine was isolated from the venom of *Crotalus durissus terrificus* purchased from Sigma Chem. Co.

(Lot. No. 65C-0008). Some lots of the venom were found not to contain crotamine since it has been shown that the venom constituents differ according to their geographical distribution in South America (Vellard, 1939; Moussatché *et al.*, 1956; Schenberg, 1959). The venom was first chromatographed on a CM-Sephadex C-25 column and eluted with increasing concentrations (0.05 to 2.5 M) of ammonium acetate. Crotamine was eluted only after the acidic components including crotoxin had been eluted out because of its basic nature. The crotamine fraction was identified by its effect on the skeletal muscle and further purified by gel filtration on Sephadex G-75. The crotamine eluted from the CM-Sephadex column was highly homogeneous and only one peak was obtained from Sephadex G-75 having a molecular weight of 5000 daltons. The homogeneity was also proved by electrophoresis on sodium dodecyl sulphate polyacrylamide gel. In addition to the major crotamine fraction, a more basic fraction was also isolated. This fraction was found to affect the skeletal muscle like crotamine. Only the major crotamine fraction was used in the present experiments.

Nerve-muscle preparations

The phrenic nerve-diaphragm preparations (Bülbring, 1946) were isolated from Long Evans rats (about 200 g) and NIH mice (about 20 g) of either sex. The organ bath contained either 15 ml (rat) or 10 ml (mouse) Tyrode solution (composition mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4, 0.33 and glucose, 11.2) maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. The contraction was induced by a supramaximal pulse (0.1 ms) delivered to the phrenic nerve, followed 5 s later by a pulse (0.5 ms) delivered directly on the muscle, at 0.1 Hz unless otherwise indicated, and the tension was recorded isometrically with a transducer. Membrane potentials and action potentials of the muscle were recorded by an intracellular glass microelectrode filled with 3 M KCl by the conventional method (Fatt & Katz, 1951) and displayed on a cathode-ray oscilloscope. Differential of the action potential (dv/dt) was obtained by a CR circuit (50 pf, 100 kΩ). The capacitance of the microelectrode was not compensated. The effective membrane resistance was measured by the transmembrane voltage change induced by passing inward current through a Wheatstone bridge as described by Araki & Otani (1955). The quantal contents of endplate potentials were measured by the method of coefficient of variance in the presence of (+)-tubocurarine as described by Castillo & Katz (1954).

Other preparations studied include the biventer cervicis nerve-muscle preparation (Ginsborg & Warriner, 1960) isolated from 4 to 9 day old male leghorn and the sartorius muscle isolated from *Rana tigrina* in March and April.

All of the data shown are typical of at least three similar experiments; means ± standard error were calculated for values obtained from at least 10 fibres for each muscle preparation.

Ion flux studies

Rat diaphragms were used. Both left and right hemidiaphragms were cut into dorsal and ventral halves and grouped into two pairs so that both control and test groups contained diaphragm strips similar in respect of left–right or dorsal–ventral. ^{24}Na Cl (0.02 Ci/g) and ^{42}K Cl (0.2 Ci/g) were obtained from the National Tsing-Hua University, Taiwan. The amount of ^{42}K used was adjusted so that the final concentration would not exceed 2.7 mM and the unlabelled K^+ was reduced to maintain the total K^+ concentration at 2.7 mM. The test groups of diaphragm strips were first treated with crotamine 10 μg/ml for 30 min at 37°C and then washed repeatedly. The control and test diaphragms together were then incubated with ^{24}Na for 15 min or ^{42}K for 30 minutes. After repeatedly washed for 30 min, the radioactivity of each diaphragm strip was counted with a Packard gamma-counter and expressed as tissue-medium ratio (counts/g tissue to counts/ml medium). Paired t test was used for the calculation of significance in the ion-flux experiments.

Other toxins

Tetrodotoxin was purchased from the Sankyo Pharm. Co. and the grayanotoxin I was kindly supplied by Dr Junkichi Iwasa, Okayama University, Japan.

Results

Effect on contractile force

When injected intravenously to mice (5 mg/kg) crotamine caused marked depression of movement after transient asynchronous spasm, and the animals were severely paralysed at 20 minutes. Thereafter the animals took a seal-like posture extending the hind-legs as reported by Habermann & Cheng-Raude (1975). Respiratory paralysis ensued in about 40 min.

When added to the isolated diaphragm preparations of rats and mice, the contractile force of twitches, induced either indirectly or directly, was considerably increased at 0.5 μg/ml (Figure 1). In addition to the augmentation of the twitch response on application of higher concentrations, an immediate contracture and later fluctuating resting tension were observed (Figure 1). As reported previously (Chey-

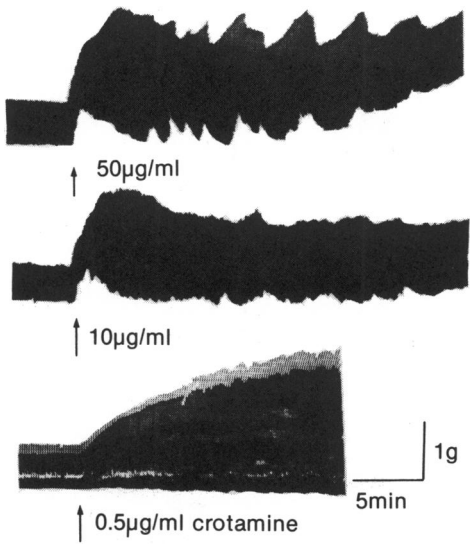

Figure 1 Effect of crotamine on the twitch contraction of the mouse phrenic nerve-diaphragm preparation. The twitch contraction was induced alternately with direct and indirect stimulation.

mol *et al.*, 1971b) these effects were not antagonized by (+)-tubocurarine. The contractility was finally depressed after 30 to 60 min treatment with crotamine 50 μg/ml. The augmentation of twitch response was acompanied by an increase of the time course of contraction (Figure 2). These effects of crotamine were not reversed even 60 min after repetitive washings of the diaphragm muscle, indicating that the effect is practically irreversible. In contrast to the murine muscle, no augmentation of contraction was observed in the chick biventer muscle treated with (+)-tubocurarine or in the frog sartorius muscle.

The crotamine-induced contracture was effectively antagonized by $MgCl_2$ (12 mM), $CaCl_2$ (10 mM) or tetrodotoxin (1 μg/ml). However, on washout of the crotamine with normal Tyrode solution, the augmentation and prolongation of twitch contraction all reappeared. It is likely therefore that no real competitive antagonism occurred between these agents and crotamine.

Effect on resting membrane potential

The resting transmembrane potential of both rat and mouse diaphragms was rapidly depolarized on application of crotamine. As illustrated in Figure 3, the membrane potential was reduced from about −80 mV to −50 or −55 mV within 5 min for the majority of superficial muscle fibres. However, no further reduction was observed even after treatment for as long as 60 minutes. In about 20% of the rat diaphragm preparations, however, the depolarization was brief and 10 to 20 mV restoration of membrane potential was observed within 10 min after addition of crotamine. The reason for this is unknown and these experiments have not been included. Interestingly the maximum depolarization was the same even when the concentration of crotamine was increased by a factor of 100 from 0.5 μg/ml to 50 μg/ml (Figure 4). It is evident that the site of action on the sarcolemma for crotamine was saturated at 0.5 μg/ml as far as the superficial fibres were concerned. The membrane potential of the depolarized muscle was not restored significantly after removal of crotamine by repetitive washings for 60 min, indicating the irreversible nature of the action of crotamine under the experimental conditions employed.

In contrast to the murine muscle, the resting potential of chick biventer cervicis and frog sartorius muscles was not changed appreciably by crotamine up to 30 μg/ml.

Effect of tetrodotoxin and low Na^+

When tetrodotoxin (1 μg/ml) was added to the rat diaphragm preparation in the presence of crotamine, the resting membrane potential was restored almost to normal within 10 min (Figures 5, 7 and 8). Depolarization occurred again, however, when both crotamine and tetrodotoxin were simultaneously washed off. These results suggest that the depolarization was induced mainly through an increase of the Na^+ permeability and that the antagonistic effect of tetrodotoxin was not by competition.

The depolarizing effect of crotamine was also studied in a medium in which 137 mM NaCl was replaced either with isotonic sucrose or Tris-chloride at pH 7.4. Crotamine-induced depolarization was found to be almost completely abolished (Figure 6). As with tetrodotoxin, depolarization to about −50 mV ensued when crotamine was removed and the concentration of Na^+ restored (Figure 6).

Effect of low Cl^-

The possible contribution of inhibition of Cl^- permeability to the depolarization caused by crotamine was tested by substituting NaCl (137 mM) by Na_2SO_4 (68.5 mM) and sucrose (68.5 mM). The membrane potential was not changed by this substitution although there were transient spontaneous fibrillations. Addition of crotamine (10 μg/ml) now caused an even more marked depolarization to about −30 mV (Figure 7) a level never attained by any concentration of crotamine in normal Tyrode solution. Tetrodotoxin was still effective in restoring the mem-

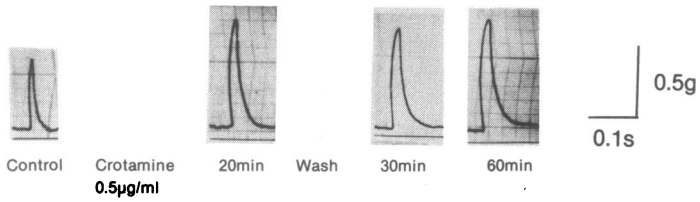

Figure 2 Prolongation of the time course of single twitch contraction by crotamine in the mouse diaphragm and the effect of washings. Time in min after addition of crotamine or after washout.

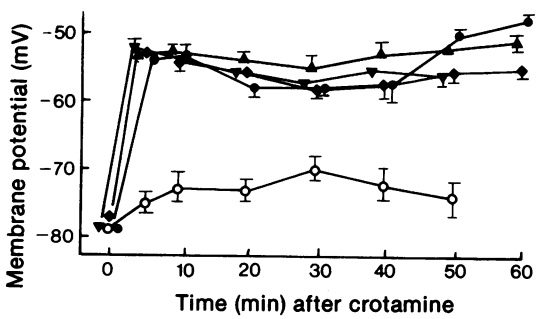

Figure 3 Effect of crotamine on the resting membrane potential of the rat diaphragm. The control transmembrane potential before addition of crotamine is shown at 0 min. The concentrations of crotamine added were: 0.1 μg/ml (○), 0.5 μg/ml (▼), 2 μg/ml (▲), 10 μg/ml (●) and 50 μg/ml (◆).

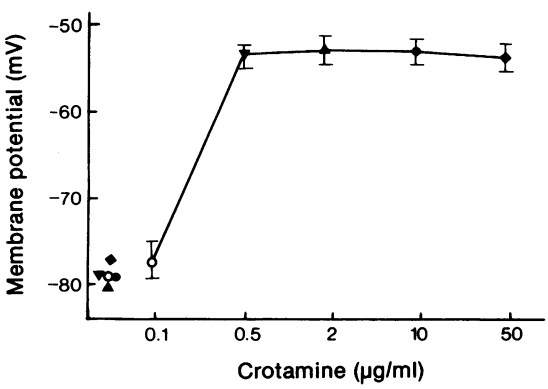

Figure 4 The relation between the concentration of crotamine and the maximum depolarization of membrane potential of rat diaphragm.

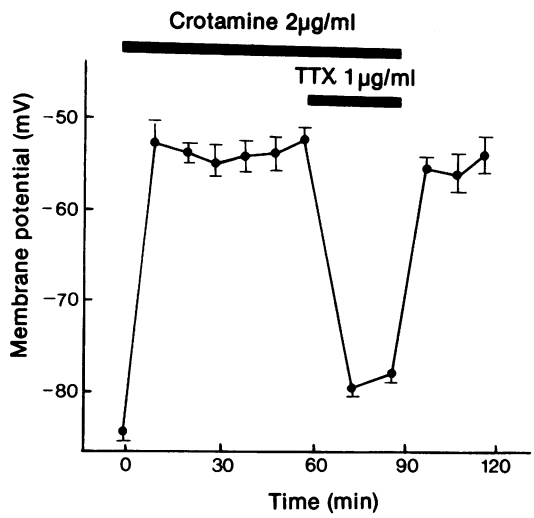

Figure 5 Effect of tetrodotoxin (TTX) on the membrane potential of the rat diaphragm depolarized by crotamine. Horizontal bars indicate the duration of addition of crotamine or tetrodotoxin.

brane potential. Inhibition of Cl^--permeability by crotamine is thus unlikely.

Effects of Ca^{2+}, Mg^{2+} and procaine

In the presence of $CaCl_2$ 10 mM, the depolarizing effect of crotamine was almost completely abolished (Figure 8). However, depolarization ensued when $CaCl_2$ was reduced to normal and crotamine removed even when a minimum saturating concentration (0.5 μg/ml) of crotamine was used. When Ca^{2+} was increased to 10 mM in the muscle pretreated with crotamine, the membrane potential could be only slowly and partially restored.

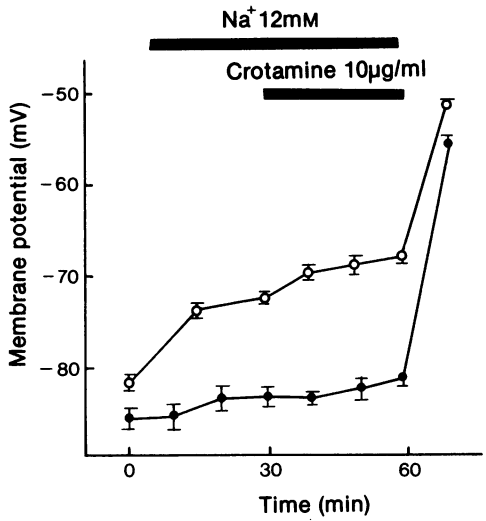

Figure 6 Effect on the rat diaphragm membrane potential of crotamine in a medium with reduced Na^+. The concentration of Na^+ was reduced from 149 mM to 12 mM by substituting sucrose (○) or Tris-chloride (●) for NaCl.

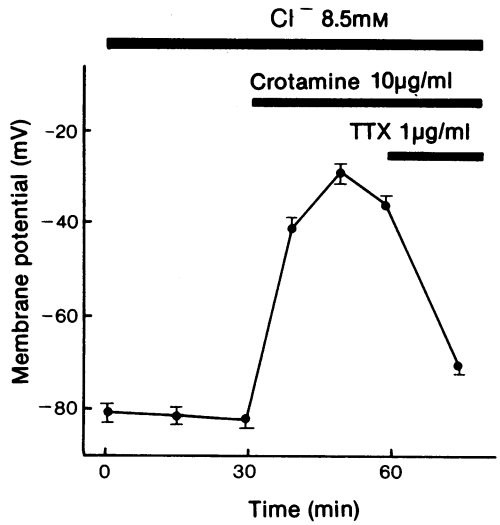

Figure 7 Effect on the rat diaphragm membrane potential of crotamine in the medium with reduced Cl^-. NaCl (137 mM) was replaced with Na_2SO_4 (68.5 mM) and sucrose (68.5 mM).

A high concentration of $MgCl_2$ (12 mM) was found to antagonize the depolarization only to a slight extent although the contracture could be abolished. Procaine at 1 mM was also effective in preventing the crotamine-depolarization. However, depolarization resumed on removal of both agents.

Effects of K^+ and grayanotoxin I

Crotamine (10 μg/ml) did not cause further depolarization of rat diaphragm muscle which had been depolarized to −30 mV by incubation with KCl 50 mM (NaCl reduced in proportion) (Figure 9). In contrast to all other antagonists tested, removal of crotamine and reduction of K^+ restored the membrane potential to normal. Moreover, K^+ (25 mM) was also able to restore the membrane potential of the diaphragm which had been previously depolarized with crotamine (10 μg/ml), suggesting that crotamine bound on sarcolemma could be removed in the presence of high K^+.

In order to see whether the antagonistic effect of high concentrations of K^+ was due simply to the reduced membrane potential, the effect of grayanotoxin I (Seyama, 1970; Narahashi & Seyama, 1974), was studied. In confirmation of previous reports, grayanotoxin I at 5 μg/ml depolarized the rat diaphragm muscle from −83 mV to about −50 mV in about 20 min and the effect did not increase. In contrast to crotamine, the membrane potential was rapidly restored to −73 mV in 15 min on wash-out of grayanotoxin I. When crotamine (1 μg/ml) was added in the presence of grayanotoxin I, the membrane potential was further depolarized from -50.3 ± 0.45 mV to -42.6 ± 0.79 mV in 10 min and wash-out of both crotamine and grayanotoxin restored the potential only to -48.9 ± 0.35 mV in 30 min, a level usually attained after treatment only with crotamine and subsequent washout. The results thus indicate that depolarization by grayanotoxin has no antagonistic effect on crotamine induced depolarization.

Effect of ouabain

The part played by inhibition of Na^+, K^+-pump in the depolarization induced by crotamine was tested. In the presence of ouabain 5×10^{-5} M, the membrane potential decreased to about −65 mV. Crotamine (10 μg/ml) further decreased the potential to about −45 mV. It is apparent that the depolarization was more marked than in the absence of an inhibition of the Na^+, K^+-pump by ouabain, suggesting that the enzyme has some compensatory effect against crotamine-depolarization.

Effect on membrane resistance and action potential

The effective membrane resistance of rat diaphragm muscles before and after treatment with crotamine (10

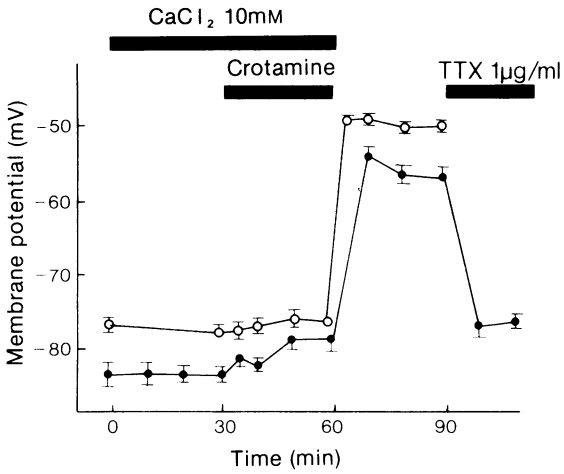

Figure 8 Effect of Ca^{2+} on the crotamine-induced depolarization in the rat diaphragm. The concentration of crotamine was 0.5 μg/ml (○) or 10 μg/ml (●).

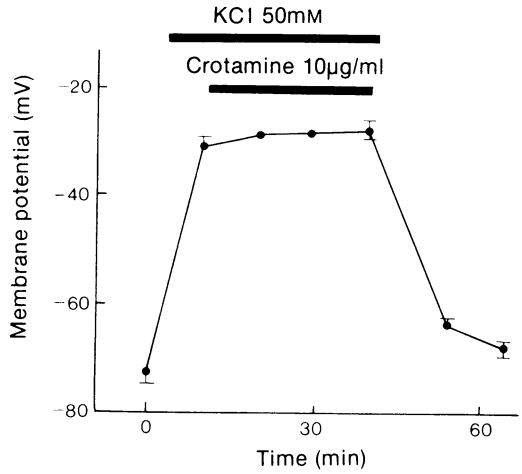

Figure 9 Effect of K^+ on the crotamine-induced depolarization in the rat diaphragm.

μg/ml) is compared in Table 1. Crotamine consistently decreased the resistance by about 50%, indicating the conductance was nearly doubled.

Action potentials could not be elicited when the membrane potential was decreased to about −50 mV by crotamine in both the rat and mouse diaphragms. In preparations treated with small doses (0.5 μg/ml) of crotamine, action potentials could be elicited in some muscle fibres which were not yet appreciably depolarized. The spike amplitude, rate of rise and rate of fall of the action potentials were decreased by 13, 25 and 30%, respectively, and the duration from the peak to 70% repolarization increased by 30%. No marked delay of repolarization was observed even in muscles which were significantly depolarized and going to fail to elicit the action potential. In many of the muscles whose membrane potential was not yet fully affected, spontaneous action potentials firing at a rate of 50–70 Hz (Figure 10) were frequently

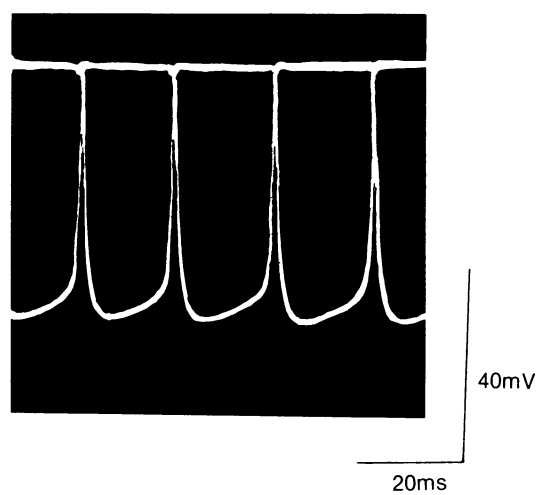

Figure 10 Spontaneous repetitive action potentials of a mouse diaphragm treated with crotamine (10 μg/ml).

Table 1 Effect of crotamine on the effective membrane resistance of the rat diaphragm muscle fibre

Expt. No.	Effective membrane resistance ($\times 10^5$ Ω) Control	Crotamine (10 μg/ml)	%
1	8.23 ± 0.62 (10)	3.36 ± 0.34 (10)	40.8
2	8.57 ± 0.34 (10)	4.87 ± 0.45 (19)	56.8
3	8.18 ± 0.34 (10)	5.26 ± 0.22 (14)	64.3
4	6.38 ± 0.45 (11)	3.25 ± 0.22 (10)	50.9

Mean ± s.e. = 53.20 ± 4.96%

observed and this fibrillation often resulted in rapid depolarization.

Effect on miniature and evoked endplate potentials

No appreciable effect was observed on the frequency and amplitude of the spontaneous miniature endplate potentials in the rat diaphragm after treatment with 10 μg/ml crotamine. The quantal content of evoked endplate potentials in the presence of (+)-tubocurarine 1.5 μg/ml was also not affected. These results

suggest that the nerve of the diaphragm was not affected by crotamine.

Effect on ion fluxes

The effect of crotamine on the influx of ^{24}Na and ^{42}K, calculated as tissue-medium ratio, is compared in Table 2. The net Na^+ influx was found to be increased by 67%. However, this value is far below the actual increase of the rate of influx because, under the conditions used, there was already 50% or more saturation of the intracellular Na^+ pool in the control preparations. On the other hand, the K^+ influx was decreased by about 30%. However, in the presence of tetrodotoxin, which restored the membrane potential of crotamine-treated muscle to normal, crotamine appeared to increase the net K^+ influx by 24%. This increase could be regarded as reflecting the increase of the rate of influx since the net ^{42}K influx was only about 1% of the intracellular K^+ pool.

Discussion

The present experiments demonstrate that crotamine causes a limited decrease of murine skeletal muscle membrane potential. The degree of depolarization was only about 35% even at full saturation of the site of action by crotamine. The effective prevention of crotamine-depolarization by tetrodotoxin and by decrease of Na^+-concentration clearly indicate that the resting permeability of sarcolemma to Na^+ is increased. The increase of ^{24}Na influx as well as the marked increase of membrane conductance also support this contention. That the depolarization is not due to an inhibition of Cl^- permeability or of the Na^+, K^+-pump is evident from the enhanced depolarization observed in experiments with low Cl^- concentration or with the enzyme inhibited by ouabain. A reduction of resting membrane potential by an inhibition of the K^+ permeability is unlikely although crotamine reduced the K^+ influx in deporized muscle since the K^+ influx was increased slightly rather than decreased by crotamine in the presence of tetrodotoxin. The decrease of K^+ influx in the absence of tetrodotoxin can be due to the decrease of membrane potential which results in the decrease of electric gradient. Moreover, there was an increase of conductance. It thus seems beyond doubt that crotamine reduces the membrane potential by an effect on the Na^+-channel. On the basis of the Goldman-Hodgkin-Katz equation and the change of membrane resistance, the increase of permeability to Na^+ could be roughly calculated to be between 10 to 30 fold.

Since the antagonism by tetrodotoxin or low Na^+ is not competitive, as revealed by the recurrence of depolarization, it is unlikely that crotamine acts on the same site of the Na^+-channel as tetrodotoxin. The antagonism by Ca^{2+} may suggest that the crotamine-depolarization involves a mobilization of membrane-bound Ca^{2+} in the action. It is evident, however, that this antagonism is not due to any competition as viewed from the recurrence of depolarization. Ca^{2+} as well as procaine may act by general stabilization of the membrane inhibiting the increase in Na^+ permeability. The antagonism by high concentrations of K^+ is of great interest since the effect of crotamine was permanently abolished and no recurrence of depolarization occurred after K^+-concentration was restored to normal. This result suggests that crotamine was removed from its binding site on the membrane. The antagonism by K^+ might have been due either to a direct effect of the ion on the crotamine binding site or to the depolarization induced. Recently, other depolarizing agents, such as batrachotoxin (Bartels-Barnal, Rosenberg & Daly, 1977) and scorpion toxin (Catterall, Ray & Morrow, 1976), which act on Na^+ channel, have been shown to interact with the membrane in a way depending on the membrane potential. However, in the case of crot-

Table 2 Effect of crotamine on ^{24}Na- and ^{42}K-influxes of the rat diaphragm.

Isotope	*Treatment*	*No. of expt.*	*Tissue: medium*	*% of paired control*
Na^{24}	Control	4	0.026 ± 0.0065	100
	Crotamine	4	0.043 ± 0.0035*	167.3 ± 16.8*
K^{42}	Control	7	0.79 ± 0.065	100
	Crotamine	7	0.55 ± 0.056*	71 ± 6.7*
	Tetrodotoxin	6	0.534 ± 0.033	100
	Crotamine + tetrodotoxin	6	0.656 ± 0.064	124.4 ± 8.1*

For the experimental details seen Methods. Crotamine, 10 μg/ml; tetrodotoxin, 1 μg/ml.
* $P < 0.05$ vs control.

amine the interaction with the membrane site appears not to be dependent on the membrane potential since depolarization of the diaphragm muscle by pretreatment with grayanotoxin I did not appreciably prevent the action of crotamine. It is likely then that K^+ interferes competitively with the specific binding of crotamine. It may be inferred that the crotamine binding site is not on the molecule of the Na^+ channel, but probably on another molecule adjacent to the channel; otherwise the crotamine-depolarization should be influenced by a change in the conformation of the channel molecule induced by depolarization, as reported for scorpion toxin and batrachotoxin.

From the results of the present work, it may be further inferred that there is a regulatory site near the Na^+ channel restricting its permeability to Na^+ and that the activity of this site is controlled by extracellular K^+. Through the binding with this regulatory site, crotamine may increase the Na^+ permeability by either displacing K^+ or antagonizing the regulatory effect of the latter ion. The reduction of resting membrane potential when extra-cellular K^+ was eliminated in skeletal muscle (Hodgkin & Horowicz, 1959) could be explained on the same basis. In line with this inference are the recent findings that external K^+ directly inhibits the inward Na^+ current flow in voltage-clamped squid axons (Cohen, Palti & Adelman, 1975) and in cultured rat skeletal muscles (Gartner, Land & Podleski, 1976). There are several possible reasons why the membrane potential was not further depolarized beyond -50 mV by crotamine. It can be accounted for partly, if crotamine does not act directly on the Na^+ channel as batrachotoxin does (Albuquerque & Daly, 1976) and partly by the compensation brought about by Cl^- permeability and presumably by an increase of Na^+, K^+-pump activity induced by increased Na^+ influx. The slight increase of K^+ permeability as observed in the K^+ influx study may also contribute to attenuate the crotamine depolarization. Since the binding of crotamine with this regulatory site of Na^+ channel appears to be rather firm as indicated by its irreversible depolarization in normal Tyrode solution, crotamine could be a useful tool in the physiological study of Na^+ channels and in the isolation of such modulator molecules.

Finally, the specificity of action of crotamine needs some description. This strongly basic polypeptide is active only on the mammalian skeletal muscle among the peripheral organs studied. Motor nerves were evidently unaffected, as judged from the unchanged frequency of miniature endplate potentials and unchanged quantal content of endplate potentials. The contractions of guinea-pig ileum induced indirectly by transmural stimulation were also unaffected. No effect was observed on the contraction and rhythmicity of guinea-pig atria, *in vitro* or *in vivo* (unpublished observation). By contrast, the skeletal muscle of chicks (Cheymol *et al.*, 1971a) and frogs (unpublished) are not sensitive to crotamine. On the other hand, the motor nerve of the young chick is highly sensitive (Lee, Huang & Bonilla, 1972). When injected directly in the brain or spinal cord, crotamine is much more toxic than when injected systemically (Habermann & Cheng-Raude, 1975), indicating the central nervous system is sensitive to crotamine. In contrast, batrachotoxin (Albuquerque & Daly, 1976) and scorpion toxin (Coraboeuf, Devoubaix & Tazieff-Depierre, 1975; Warnick, Albuquerque & Diniz, 1976) are active on almost all of the excitable membranes tested. Furthermore, the amino acid sequences of scorpion toxins (Rochat, Rochat, Sampieri & Miranda, 1972) are quite different from that of crotamine (Laure, 1975). However, some resemblances can be found in the amino acid sequence between crotamine and a toxin from *Anemonia sulcata* (Wunderer, Machleidt & Wachter, 1976), which is active on mammalian heart, augmenting its contraction without depolarization; an effect also antagonized by high K^+ concentration and tetrodotoxin (Ravens, 1976). The cardiac effect, however, is reversible. An extensive comparison of the physiological actions of these peptide toxins may shed more light on our understanding of the properties of the Na^+ channel.

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