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A study on the membrane depolarization of skeletal muscles caused by a scorpion toxin, sea anemone toxin II and crotamine and the interaction between toxins

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1 Quinquestriatus toxin (QTX) isolated from the venom of a scorpion (*Leiurus quinquestriatus*) and sea anemone (*Anemonia sulcata*) toxin II enhanced the twitch response of the rat and mouse diaphragms and like crotamine (isolated from the venom of *Crotalus durissus terrificus*) caused spontaneous fasciculation of the muscle.

2 Trains of action potentials in muscles at 70–250 Hz, which could not be antagonized by (+)-tubocurarine, were triggered by single stimulation or occurred spontaneously after treatment with these toxins.

3 QTX and toxin II prolonged the rat muscle action potential 3 to 4 fold whereas crotamine prolonged the action potential by only 30%.

4 The membrane potential was depolarized from about −82 mV to −55 mV by crotamine 2 $\mu g\,ml^{-1}$, −41 mV by toxin II 5 $\mu g\,ml^{-1}$ and to −50 mV by QTX 1 $\mu g\,ml^{-1}$. The concentrations to induce 50% maximal depolarization ($K_{0.5}$) were 0.07, 0.15 and $> 0.4\,\mu g\,ml^{-1}$, respectively, for QTX, crotamine and toxin II, whereas the rates of depolarization were in the order toxin II > crotamine > QTX. The depolarizing effects of crotamine and QTX, but not of toxin II, were saturable.

5 The depolarizing effects of all three toxins were irreversible whereas the membrane potential could be restored by tetrodotoxin non-competitively.

6 Simultaneous treatment with crotamine and QTX or crotamine and toxin II at concentrations below $K_{0.5}$ caused only additive effects on depolarization.

7 When the muscle was depolarized by pretreating with a saturating concentration of crotamine, the onset of depolarization by QTX was greatly retarded whereas that by toxin II was unaffected. Action potentials were further prolonged in both cases.

8 It is inferred that all three peptide toxins act at sites on the sodium channel and the binding sites for QTX and crotamine overlap to a considerable extent. On the other hand, the site for toxin II appears not to overlap with that of crotamine but may overlap with that of QTX.

Introduction

The voltage-dependent sodium channels which generate action potentials in nerve, heart and skeletal muscle are vulnerable to a vast number of naturally occurring toxins. Catterall (1980) classified some of these toxins into three groups each binding to a separate receptor site associated with the sodium channel. The first receptor site situated on the external channel inlet binds the channel blockers, tetrodotoxin and saxitoxin. These group I toxins block the flux of sodium ion through the channel (Ritchie & Rogart, 1977). The second receptor site binds the lipid-soluble polycyclic compounds, batrachotoxin, grayanotoxin, veratridine and aconitine. These group II toxins alter the voltage-dependent activation and inactivation processes and cause persistent activation of sodium channel (Ulbricht, 1969; Schmidt & Schmitt, 1974; Khodorov & Revenko, 1979; Seyama & Narahashi, 1981). The effect is usually more profound with intracellular than with extracellular application of these toxins. The third receptor site, located

most probably on the external surface of the membrane, binds some polypeptide toxins isolated from scorpion venoms and sea anemone nematocysts. These group III toxins alter the inactivation process (Koppenhofer & Schmidt, 1968; Bergman, Dubois, Rojas & Rathmayer, 1976; Gillespie & Meves, 1980) and interact allosterically with group II toxins so that the persistent activation of the sodium channel is markedly enhanced (Catterall, 1977b; Catterall & Beress, 1978; Jacques, Fosset & Lazdunski, 1978; Tamkun & Catterall, 1981).

Crotamine, a basic polypetide with 42 amino acid residues contained in the venom of South American rattlesnake (*Crotalus durissus terrificus*) (Laure, 1975), acts on the sodium channel at a site different from that for group I and group II toxins and causes irreversible membrane depolarization in murine skeletal muscles (Chang & Tseng, 1978; Tsai, Peng & Chang, 1981). Occupancy of this site by crotamine potentiates the depolarizing action of group II toxins allosterically by increasing their affinity with the second receptor sites (Hong & Chang, 1983). Since both crotamine and group III toxins, such as toxin II isolated from a sea anemone (*Anemonia sulcata*) and the scorpion toxin isolated from the venom of *Leiurus quinquestriatus* (later referred to as quinquestriatus toxin), potentiate the action of group II toxins, the question arises as to whether the binding site for crotamine is common to that for group III toxins. Although sea anemone toxin II caused depolarization of rat diaphragm (Alsen, Harris & Tesseraux, 1981), quinquestriatus toxin had no substantial depolarizing action on frog skeletal muscle (Catterall, 1979). On the other hand, the crude venom was found to induce muscle contracture in diaphragm (Adam & Weiss, 1959; Cheymol, Bourillef, Roch-Arveiller & Heckle, 1973). Another scorpion toxin, tityus toxin induced depolarization on the rat diaphragm (Warnick, Albuquerque & Diniz, 1976). The present experiments were designed to study the effect of quinquestriatus toxin and sea anemone toxin II on diaphragms of the mouse and rat and the interaction between crotamine and these peptide toxins on the rate and extent of membrane depolarization to see whether there is any synergistic or antagonistic action and whether they bind to the same receptor (in order to shed more light on the binding sites of the sodium channel).

Methods

The diaphragm preparations (Bülbring, 1946) were isolated either from Long Evans rats weighing 180–300 g or from NIH strain mice weighing 20–25 g. The organ bath contained 8–15 ml Tyrode solution (composition mM: NaCl 137, KCl 2.8, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.33, $NaHCO_3$ 11.9 and glucose 11.2) maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. Contractile responses were induced by supramaximal pulses delivered to the phrenic nerve (pulse width, 0.05 ms) each followed 5 s later by a pulse (0.5 ms) delivered directly to the muscle at 0.1 Hz and the twitch tension was recorded isometrically with a Grass transducer (FT. 03).

Intracellular recordings of membrane potentials and action potentials were performed according to Fatt & Katz (1951) with glass capillary microelectrodes filled with 3 M KCl having resistance in the range of 6–20 megohms and were displayed on a cathode-ray oscilloscope. The data (mean ± s.e.) were calculated from values obtained from about 30 muscle fibres in three similar experiments unless otherwise indicated.

Crotamine was purified from the venom of the Brazilian rattlesnake, *Crotalus durissus terrificus* (supplied by Dr K.H. Slotta) according to Chang & Tseng (1978) to electrophoretic homogeneity on sodium dodecyl sulphate polyacrylamide gel. The quinquestriatus toxin isolated from *Leiurus quinquestriatus* was a gift from Dr W.A. Catterall (University of Washington, USA). Sea anemone toxin II isolated from *Anemonia sulcata* was a generous gift from Dr U. Ravens (University of Christian-Albrechts, Kiel, West Germany).

Results

Effect of quinquestriatus toxin on rat diaphragm

The effects of this scorpion toxin on mouse diaphragms were rather similar to those on rat diaphragms. The contractile force of twitches, either directly or indirectly induced, was increased by the toxin (0.25 $\mu g\,ml^{-1}$). The response was characteristic in that the duration of each single twitch was prolonged and the twitch was associated with a transient increase in resting muscle tension and spontaneous twitches. Quinquestriatus toxin induced significant membrane depolarization on both rat and mouse diaphragms. On incubation of the rat diaphragm with 0.05, 0.25 and 1.0 $\mu g\,ml^{-1}$ quinquestriatus toxin for 70 min, the muscles were depolarized, respectively, from about −82 mV to -70.4 ± 0.8, -59.7 ± 1.3 and -50.3 ± 2.2 mV (Figure 1). It seems that the depolarizing action of quinquestriatus toxin was beginning to saturate at 1 $\mu g\,ml^{-1}$ (cf. Figure 2). Unfortunately, the effect of still higher concentrations could not be tested because of the limited amount of the toxin. It appeared to be more potent than crotamine as judged from the concentration needed to cause half maximal depolarization (quinquestriatus toxin,

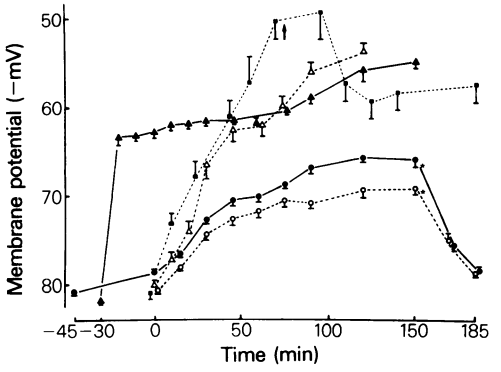

Figure 1 Time-course of the depolarizing action of quinquestriatus toxin (QTX) on the resting membrane potentials of the rat diaphragm. QTX was added at 0 min at 0.05 $\mu g\,ml^{-1}$, (○, ●), 0.25 $\mu g\,ml^{-1}$ (△, ▲) or 1 $\mu g\,ml^{-1}$ (■) in the absence (○····○, △····△, ■····■) or in the presence of either 0.1 $\mu g\,ml^{-1}$ (●—●) or 2 $\mu g\,ml^{-1}$ (▲—▲) crotamine added at −45 or −30 min. Arrow indicates repetitive washing with toxin-free Tyrode solution. Asterisks denote the addition of 0.5 $\mu g\,ml^{-1}$ tetrodotoxin at 155 min (■: from 13 fibres of one preparation).

ca 0.07 $\mu g\,ml^{-1}$ vs. crotamine, 0.15 $\mu g\,ml^{-1}$). However, the rate of depolarization by the scorpion toxin was very much slower than that exhibited by crotamine. Even for the highest concentration used (1 $\mu g\,ml^{-1}$), the time to attain 50% steady state depolarization was about 30 min whereas it was only 5 to 10 min for crotamine and sea anemone toxin II. When the membrane potential was still above

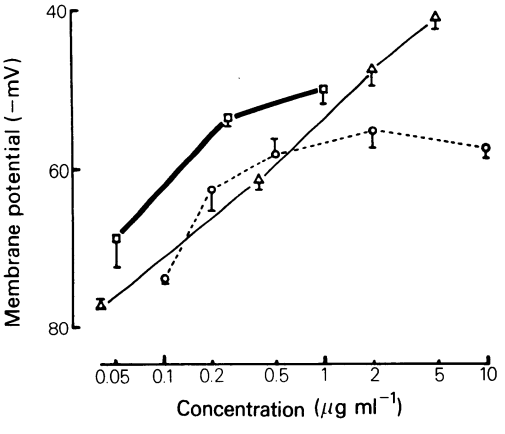

Figure 2 Relation between the concentrations of polypeptide toxins and the steady-state depolarizations of membrane potentials. (○) Crotamine on the mouse diaphragm; (△) sea anemone toxin II on the mouse diaphragm; (□) quinquestriatus toxin on the rat diaphragm. The membrane potentials prior to the application of toxins were −80 ~ −84 mV.

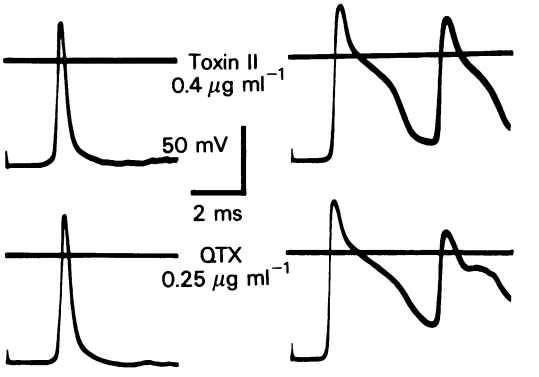

Figure 3 Prolongation by QTX and toxin II of the action potential of the rat diaphragm. Action potentials were evoked by single pulses on the phrenic nerve (note the stimulus artifact). Left panels show the control and right panels the action potentials obtained 20 min after toxin application from fibres which were not yet markedly depolarized. Note the repetitive firings on single stimulations after toxin treatment.

−60 mV, a train of repetitive muscle action potentials at 70–250 Hz, either spontaneously occurring or evoked by a single stimulation, was frequently observed (Figure 3). These effects were not antagonized by (+)-tubocurarine. The membrane potential was only partially restored (from −50 to −57 mV) when the toxin (1.0 $\mu g\,ml^{-1}$) was washed out repeatedly, indicating that the effect of toxin is largely irreversible. On the other hand, tetrodotoxin (0.5 $\mu g\,ml^{-1}$) effectively restored the membrane potential of the muscle depolarized after incubation with quinquestriatus toxin (0.05 $\mu g\,ml^{-1}$) to nearly normal levels without washing out the toxin (Figure 1).

The duration of the muscle action potential after treatment with this scorpion toxin was prolonged by 3–4 times as illustrated in Figure 3. Sometimes, durations longer than 200 ms were observed. Only the recovery phase of the action potential was significantly affected, resulting in a plateau at about +10 ~ −30 mV. Crotamine, however, prolonged the duration by only 30% as previously reported (Chang & Tseng, 1978). At a high concentration of quinquestriatus toxin (1 $\mu g\,ml^{-1}$), very marked fasiculations of the muscle were always observed which could be largely antagonized by 5 $\mu g\,ml^{-1}$ (+)-tubocurarine. This indicates that the scorpion toxin is able to trigger spontaneous action potentials in the phrenic nerve as well as in the diaphragm muscle.

Interaction between crotamine and quinquestriatus toxin on rat diaphragm

Crotamine has been found to potentiate the depolarizing action of group II toxins (Hong & Chang),

at a concentration ($0.1\,\mu g\,ml^{-1}$) which is far below the saturating one ($2\,\mu g\,ml^{-1}$) and causes only minimal depolarization by itself. Quinquestriatus toxin at $0.05\,\mu g\,ml^{-1}$ depolarized the muscle to -69.3 ± 0.8 mV after 120 min incubation and the time required to reach 50% depolarization was about 26 min (Figure 1). In the rat diaphragm pretreated with crotamine $0.1\,\mu g\,ml^{-1}$ for 45 min, which depolarized the muscle by no more than 3 mV, further treatment with quinquestriatus toxin ($0.05\,\mu g\,ml^{-1}$) now depolarized the muscle to -65.7 ± 0.9 mV in 120 min and the time required to attain 50% depolarization was about 27 min (Figure 1), almost the same as in the absence of crotamine. In the control group the membrane potentials were -79.4 ± 0.4 mV 4 h after isolation. This result indicates that crotamine does not potentiate scorpion toxin but acts additively with it although it markedly enhances the rate of action of group II toxin (Hong & Chang, 1983).

To test whether quinquestriatus toxin competes with crotamine for the same binding site, the depolarizing action of scorpion toxin was tested in the presence of a saturating concentration of crotamine ($2\,\mu g\,ml^{-1}$). Crotamine alone depolarized the muscle membrane potential to a maximal steady level ($-61 \sim -63$ mV) within 10 min. In diaphragms treated with quinquestriatus toxin ($0.25\,\mu g\,ml^{-1}$) alone, the membrane potentials were -61.7 ± 1.5 mV and -53.4 ± 1.0 mV after 60 and 120 min incubation, respectively (Figure 1). When diaphragms were pretreated with crotamine $2\,\mu g\,ml^{-1}$ for 30 min, scorpion toxin ($0.25\,\mu g\,ml^{-1}$) now produced little further depolarization within 60 min (-61.6 ± 0.5 mV) and it took another 60 min to depolarize the muscle to -55.8 ± 1.0 mV (Figure 1), indicating that the effect of quinquestriatus toxin is markedly retarded when the muscle is saturated with crotamine. Interestingly, spontaneous action potentials with prolonged duration and plateau were observed after the addition of the scorpion toxin. When the rat diaphragm was first treated with a high concentration ($1\,\mu g\,ml^{-1}$) of quinquestriatus toxin for 45 min and depolarized to -60.9 ± 2.0 mV, further treatment with $0.5\,\mu g\,ml^{-1}$ crotamine for 20 min decreased the membrane potential to -50.2 ± 2.0 mV, a value similar to that obtained with $1\,\mu g\,ml^{-1}$ of quinquestriatus toxin alone for 70 min.

Effect of sea anemone toxin II on the rat and mouse diaphragms

Toxin II at $2\,\mu g\,ml^{-1}$ caused a marked augmentation of the twitch response of the mouse diaphragm stimulated either directly or indirectly. Both the amplitude and the duration of the twitch contraction to single stimulation were increased. The augmentation by

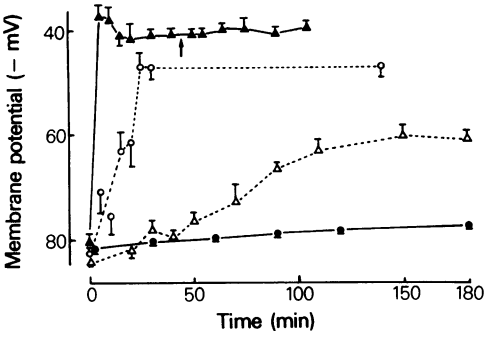

Figure 4 Time-course of the depolarizing effect of toxin II on the resting membrane potentials of the mouse diaphragm. The concentrations ($\mu g\,ml^{-1}$) of toxin II added at 0 min, were (●) 0.4; (△) 0.4; (○) 2; (▲) 5. Arrow indicates the further addition of $10\,\mu g\,ml^{-1}$ crotamine at 45 min (▲: from 13 fibres of one preparation).

toxin II in the directly stimulated preparation was not antagonized by (+)-tubocurarine. The resting membrane potential was also rapidly decreased in a concentration-dependent manner between 0.04 to $5\,\mu g\,ml^{-1}$ (Figures 2 and 4). Unlike crotamine, no sign of saturation could be observed for toxin II up to $5\,\mu g\,ml^{-1}$ which was the highest concentration we could use (Figure 2). The steady state potential attained with $5\,\mu g\,ml^{-1}$ toxin II was as high as -40.6 ± 1.3 mV, which is much higher than those attained with a saturating concentration of crotamine (-55.4 ± 2.1 mV at $2\,\mu g\,ml^{-1}$) or with a high concentration of quinquestriatus toxin (-50.3 ± 2.2 mV at $1\,\mu g\,ml^{-1}$). However, the concentration for toxin II ($> 0.4\,\mu g\,ml^{-1}$) needed to evoke half maximal depolarization appears higher than those for quinquestriatus toxin ($0.07\,\mu g\,ml^{-1}$) and crotamine ($0.15\,\mu g\,ml^{-1}$). Washing out of the toxin did not restore the membrane potential appreciably whereas tetrodotoxin was effective in restoring the membrane potential of the affected muscle. Interestingly, about one fourth of the surface muscle fibres were relatively unresponsive to the depolarizing action of toxin II and their membrane potential stayed at -74 to -84 mV 120 min after incubation with $0.4\,\mu g\,ml^{-1}$ of toxin II. The same treatment depolarized the remaining muscle fibres to $-60 \sim -64$ mV.

Toxin II frequently induced a train of spontaneous repetitive (100–250 Hz) action potentials in the muscle fibres which were not yet markedly depolarized as did quinquestriatus toxin and crotamine. Single stimulation often evoked repetitive firings (Figure 3). This effect again was not antagonized by (+)-tubocurarine.

In mice the duration of the evoked action potential was only slightly prolonged ($< 100\%$) and no

plateau was observed. In contrast, the action potential in the rat was markedly prolonged more than 3 fold with a plateau occurring at $+10 \sim -30$ mV (Figure 3).

Interaction between crotamine and sea anemone toxin II on mouse diaphragm

The interaction between crotamine and sea anemone toxin II was carried out on the rationale that when a certain concentration of crotamine and toxin II produces quantitatively the same effect, one half concentration of each toxin used simultaneously should elicit the same effect if their actions are additive. This occurs when both toxins act on the same site or by a similar mechanism. If a greater than additive effect, i.e. potentiation, is obtained after combined use of the two toxins, this may imply that the two toxins act at different sites and/or by different mechanisms. The steady state potentials in diaphragms treated with 0.4 $\mu g\,ml^{-1}$ sea anemone toxin II for 120 min (-60.6 ± 1.7 mV) were found to be very close to those treated with 0.2 $\mu g\,ml^{-1}$ crotamine (-61.2 ± 1.8 mV) (Figure 5). The time required to attain 50% depolarization was 70 and 17 min, respectively, for sea anemone toxin II and crotamine. As shown in Figure 5, when diaphragms were treated simultaneously with toxin II 0.2 $\mu g\,ml^{-1}$ and crotamine 0.1 $\mu g\,ml^{-1}$, i.e., both at half the concentration described above, the steady state potentials after 120 min incubation were -63.1 ± 2.0 mV which is not different from those treated with either toxin II 0.4 $\mu g\,ml^{-1}$ or crotamine 0.2 $\mu g\,ml^{-1}$ alone and the time required to attain 50% depolarization was 40 min, a value between those obtained with either sea anemone toxin II or crotamine.

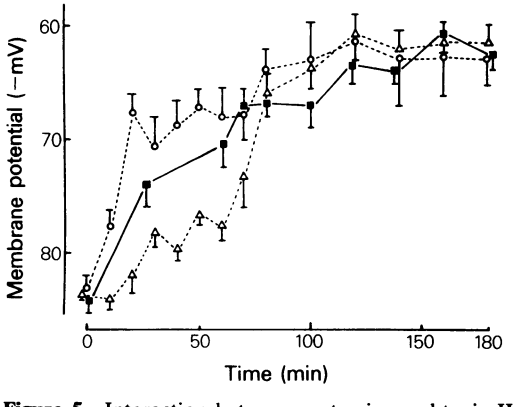

Figure 5 Interaction between crotamine and toxin II on the rate and extent of membrane depolarization. Mouse diaphragms were treated with crotamine 0.2 $\mu g\,ml^{-1}$ (○) or toxin II 0.4 $\mu g\,ml^{-1}$ (△) alone or treated simultaneously with crotamine 0.1 $\mu g\,ml^{-1}$ and toxin II 0.2 $\mu g\,ml^{-1}$ (■).

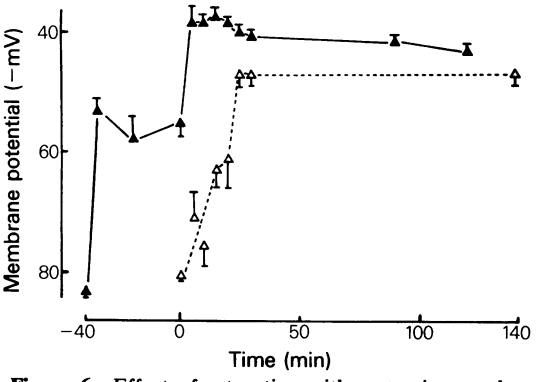

Figure 6 Effect of saturation with crotamine on the rate and extent of membrane depolarization induced by toxin II. Mouse diaphragms were treated with toxin II 2 $\mu g\,ml^{-1}$ at 0 min in the absence (△) or presence (▲) of crotamine 2 $\mu g\,ml^{-1}$.

When the mouse diaphragm was exposed to a high concentration of sea anemone toxin II (5 $\mu g\,ml^{-1}$) for 40 min the steady state membrane potentials were not changed by further treatment with a very high concentration of crotamine (10 $\mu g\,ml^{-1}$) (-40.6 ± 1.3 mV vs. -39.7 ± 1.2 mV, Figure 4). On the other hand, when diaphragms were first depolarized to about -55 mV with a saturating concentration of crotamine (2 $\mu g\,ml^{-1}$), sea anemone toxin II at 2 $\mu g\,ml^{-1}$ still exerted its depolarizing action without delay and depolarized the muscle further to -38.5 ± 1.2 mV within 10 min. Thereafter the muscle repolarized by about 5 mV and attained a steady state membrane potential of -43.6 ± 1.2 mV (Figure 6).

Discussion

The effects of quinquestriatus toxin and sea anemone toxin II on the twitch response, resting membrane potential and action potential of the mouse and rat diaphragm muscles observed in the present experiments are quite similar to those reported for tityus toxin isolated from the venom of another scorpion, *Tityus serrulatus* (Warnick *et al.*, 1976) and toxin II (Alsen *et al.*, 1981). In common with crotamine, both toxins enhanced the twitch response by increasing the amplitude and prolonging the time-course, and induced spontaneous fasciculation and contracture. Repetitive action potentials occurring spontaneously in the muscle or evoked by a stimulus can account for the above change of muscle contraction. All the three toxins also produced significant depolarization of the muscle membrane and this effect was always reversed by tetrodotoxin, indicating that they all act on the sodium channel. In addition, all the three toxins have

been shown to enhance the depolarizing effect, sodium influx promoting effect or binding of group II sodium channel toxins (Catterall, 1977b; Catterall & Coppersmith, 1981; Hong & Chang, 1983).

Crotamine, however, is different from quinquestriatus toxin and toxin II in the following aspects. Crotamine acts mainly on the mammalian skeletal muscle leaving other excitable organs, nerve and heart, mostly unaffected (Chang & Tseng, 1978) whereas nerve and heart tissues are affected by many scorpion and sea anemone toxins (Ravens, 1976; Romey, Abita, Schweitz, Wunderer & Lazdunski, 1976; Zlotkin, Miranda & Rochat, 1978; Romey, Renaud, Fosset & Lazdunski, 1980). Quinquestriatus toxin and toxin II greatly retarded the falling phase of the action potential of the rat diaphragm as previously reported for other nerve and muscles (Coraboeuf, Deroubaix & Tazieff-Depierre, 1975; Warnick *et al.*, 1976; Bernard, Couraud & Lissitzky, 1977; Catterall, 1979; Alsen *et al.*, 1981) and prolonged the 80% repolarization time 3 to 4 fold, whereas crotamine prolonged the time-course by only 30%. It has been concluded that scorpion and sea anemone toxins act mainly on the inactivation mechanism of the sodium channel (Koppenhofer & Schmidt, 1968; Bergman *et al.*, 1976). Since crotamine causes less depolarization of the muscle than the other two toxins, this difference might be due to its smaller intrinsic activity on the inactivation. A comparison of the depolarizing effect of these toxins reveals that the rate of association of toxin to its binding site, as judged from the time to attain 50% depolarization of the steady state level, is in the order toxin II (<5 min) $>$ crotamine (<10 min) $>$ quinquestriatus toxin (30 min), whereas the affinity as judged from the toxin concentration for causing 50% maximal depolarization ($K_{0.5}$) is quinquestriatus toxin ($0.07\,\mu g\,ml^{-1}$) $>$ crotamine ($0.15\,\mu g\,ml^{-1}$) $>$ toxin II ($>0.4\,\mu g\,ml^{-1}$). On the other hand, the maximal depolarization induced in the diaphragm muscle with high concentrations of toxins is in the order toxin II $>$ quinquestriatus toxin $>$ crotamine. Toxin II also caused a greater sodium influx than did quinquestriatus toxin in cultured rat muscle cells (Lawrence & Catterall, 1981a). Interestingly, the site of action for the depolarizing effect of crotamine was easily saturable whereas that for toxin II showed no sign of saturation in the range $0.04-5\,\mu g\,ml^{-1}$, suggesting that there are more binding sites for toxin II than for crotamine and quinquestriatus toxin. The site for quinquestriatus toxin also appears to be saturable.

The results obtained with interaction between crotamine and the other two toxins provide further information concerning the binding sites of these toxins. When concentrations lower than $K_{0.5}$ of each toxin were simultaneously applied, crotamine plus toxin II or crotamine plus quinquestriatus toxin caused neither potentiation of each other's depolarizing effect nor antagonism, indicating that these three toxins are acting on the same site or by a similar mechanism at non-allosteric sites. It is interesting in this respect that, when the muscle was first saturated with crotamine, toxin II acted differently from quinquestriatus toxin. The depolarizing effect of quinquestriatus toxin was greatly retarded while that of toxin II appeared unaffected. This result suggests that the binding sites for quinquestriatus toxin and crotamine could be the same or overlap each other, whereas the site for toxin II is probably different from that for crotamine. This inference is in accordance with the observations showing that the site for toxin II, but not that for the other two toxins, is not easily saturable (Figure 2) and that the number of binding sites for toxin II is in excess of that for scorpion toxin (Vincent, Balerna, Barhanin, Fosset & Lazdunski, 1980). Vincent *et al.* (1980) reported that the binding of scorpion toxins to synaptosomes could be completely prevented by sea anemone toxin II, while the binding of the latter could not be prevented by scorpion toxins. However, according to Catterall & Beress (1978), Lawrence & Catterall, (1981b) and Habermann & Beress (1979), the scorpion toxins can prevent the binding of sea anemone toxin II in neuroblastoma cells, cultured rat muscle cells and mouse diaphragm muscles.

The retardation by crotamine of the depolarizing effect of quinquestriatus toxin but not of toxin II might be due, at least partly, to the higher dependency of binding on the membrane potential; the more the membrane is depolarized, the lower the affinity (Catterall, 1977a; 1980; Lawrence & Catterall, 1981b). The observation that the depolarizing effect of quinquestriatus toxin on the muscle depolarized with a saturating concentration of crotamine was not completely abolished and that the prolongation of the muscle action potential by quinquestriatus toxin was unaffected, suggest that these two toxins may not act on exactly the same site. They may share some common binding sites, but quinquestriatus toxin may bind to other additional sites.

It may be inferred from the above that crotamine shares some binding sites with quinquestriatus toxin which in turn shares other binding sites with sea anemone toxin II. On the other hand, crotamine appears not to share common binding sites with toxin II. These binding sites, however, could be closely located and strategically related to the gating mechanism of the sodium channel and the binding site for the group II toxins. Interestingly, toxin T_{46} from *Ptychodiscus brevis* also enhanced the group II toxin-induced ^{22}Na-uptake in neuroblastoma cells without affecting the specific binding of the scorpion toxin (Catterall & Risk, 1981). It is likely that in the

region of group III toxin-binding sites on the sodium channel there could be many sites binding peptides or other toxins and the occupation of these sites with various basic polypeptide toxins may result in a change of gating mechanism of the sodium channel.

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