Pharmacological Study on Angusticeps-Type Toxins from Mamba Snake Venoms

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ABSTRACT
Five angusticeps-type toxins, F1, F2 and \( C_{10}S_2C_2 \) from Dendroaspis angusticeps and F and FS2 from D. polyelpis polyelpis, were tested for action on the chick biventer cervicis nerve-muscle, the frog rectus abdominis muscle and the mouse phrenic nerve-diaphragm preparations. In the chick muscle, none of these toxins exhibited any stimulatory effect up to 100 \( \mu \)g/ml. In the frog muscle, the response to acetylcholine, but not to carbachol, was enhanced dose dependently by F1 and C. No appreciable effect was observed with the other three toxins. In the mouse diaphragm, also only F1 and C augmented responses to indirect stimulation and produced spontaneous fasciculations. On tetanic stimulation, a marked Wedensky inhibition was observed. Their stimulatory effect was abolished by \( d \)-tubocurarine. In the presence of \( d \)-tubocurarine as well as in the denervated mouse diaphragm, neither toxin increased responses to direct stimulation. In low-calcium (0.6 mM) or high magnesium (4.2 mM) medium, the stimulatory effect of both toxins was markedly attenuated. The resting membrane potential of the mouse diaphragm was not changed. The amplitude and frequency of MEPPs and the quantal content and the half-decay time of EPPs was increased. Both toxins also produced a stimulatory effect on the isolated guinea-pig ileum, which was abolished by atropine. In the rat atrial preparation, both toxins caused negative inotropic and chronotropic effects, which were reversed by atropine. If pretreated with atropine, these effects were completely prevented. Both F1 and C markedly inhibited the cholinesterase activity of the homogenized mouse diaphragm and frog rectus abdominis muscle but not that of the chick biventer cervicis muscle. From these results, it is concluded that the angusticeps-type subgroup I toxins F1 and C stimulate both skeletal and intestinal smooth muscles and inhibit cardiac muscle by their anticholinesterase activity, as well as possibly by facilitating acetylcholine release from cholinergic nerve terminals.

The genus Dendroaspis (mamba) has so far yielded a variety of types of toxins. From D. angusticeps (eastern green mamba) venom, Viljoen and Botes (1973, 1974) purified and sequenced two major polypeptides, F1 and F2 (TA2), of low toxicity, which are structurally homologous to the short postynaptic neurotoxins but of completely distinct immunochemical properties. These polypeptides are conveniently called "angusticeps-type" toxins (Strydom, 1976). From the same venom, Joubert and Taljaard (1980a,b) sequenced two more angusticeps-type toxins \( C_{10}S_2C_2 \) and \( C_{10}S_2C_2 \) (TA2) of low toxicity, which are structurally homologous to the short postynaptic neurotoxins (Viljoen and Botes, 1973). From D. polyelpis polyelpis (black mamba) venom, two angusticeps-type toxins, FS2 (Strydom, 1977) and C (Joubert and Taljaard, 1978), a protease inhibitor E (Joubert and Strydom, 1978) and two protease inhibitor homologues, I and K (Strydom, 1973b) have been sequenced besides a short and a long postynaptic neurotoxin (Strydom, 1972). From D. jamesoni kaimosae (Jameson's mamba) venom, Joubert et al. (1978) also sequenced an angusticeps-type toxin \( S_9C_2 \) and a synergistic type protein besides a short and a long postynaptic neurotoxin (Strydom, 1973a). Shipolini et al. (1973) isolated 12 toxic components from D. viridis (western green mamba) venom. They subsequently sequenced four of these components, viz., a short and two long postynaptic neurotoxins (Banks et al., 1974) and an angusticeps-type toxin (Shipolini and Banks, 1974).

Among the elapid family, toxins from cobra and krait venoms have been studied extensively (for reviews, see Lee, 1972; Chang, 1979), but relatively little attention has been given to the pharmacological properties of toxins from mamba venoms. Recently, the venom of D. angusticeps was shown to have marked effects on skeletal muscle preparations, increasing ACh release in response to nerve stimulation, blocking ACh receptors and, in high concentrations, inhibiting muscle contractility (Barrett and Harvey, 1979). The facilitation of ACh release was

ABBREVIATIONS: ACh, acetylcholine; C(SCAMS), S-carboxamidomethyl sulphonium derivative of toxin C; DFP, diisopropyl fluorophosphatase; ChE, cholinesterase.
attributed to one polypeptide called dendrotoxin, a protease inhibitor homologue (Harvey and Karlsson, 1980; Harvey and Gage, 1981). The amino acid sequence of dendrotoxin (E. Karlsson, unpublished observations) appears to be identical to that of C13S2C3 isolated from the same venom (Joubert and Taljaard, 1980b). Despite extensive chemical characterization of the angusticeps-type toxins, so far no pharmacological study on the toxins of this type has been reported. In the present investigation, the pharmacological properties of five angusticeps-type toxins, F1, F2, and C13S2C3 from D. angusticeps venom and C and FS1 from D. polyplepis polyplepis venom, were compared and their mode of action was studied. Some of these results were presented to the IUBS International Symposium on Toxins and Lectins, Pretoria, South Africa (Lee, 1982), and the Seventh European Neuroscience Congress, Hamburg, Germany (Lee et al., 1983).

**Materials and Methods**

Isolation and purification of toxins. Five angusticeps-type toxins, F1, F2, and C13S2C3 from D. angusticeps venom and C and FS1 from D. polyplepis polyplepis venom, were isolated and purified as described (Viljoen et al., 1978; Strydom, 1976). The purity of these toxins was ascertained by amino acid composition, amino-terminal sequence analysis and, additionally, in the case of F2, immunodiffusion.

In order to eliminate trace contamination by postnystic neurotoxin(s) in toxin C, Met-33 (Joubert and Taljaard, 1978) was alkylated with iodoacetamide to give C(SCAMS) according to the method described by Vithayasil and Richards (1960). Purification of the derivative was achieved by ion-exchange chromatography on CM-Sephrose, eluting with a linear gradient of ammonium acetate buffer, pH 5.0, from 0.15 to 0.5 M over 500 ml at 10 ml/h. The derivative C(SCAMS) eluted later than toxin C because it carried an extra positive charge. The purity of the derivative was assayed by amino acid analysis following performic acid oxidation and hydrolysis in 6 M hydrochloric acid. The absence of methionine sulfone is an indication of complete derivatization.

Chick biventer cervicis nerve-muscle preparation. Biventer cervicis nerve-muscle preparation (Ginsborg and Warriner, 1960) was isolated from male Leghorns aged 4 to 7 days and mounted in a 10-ml organ bath with a resting tension of approximately 0.5 g in Krebs solution (composition in millimolar: NaCl, 118.4; KCl, 4.7; CaCl2, 2.5; MgSO4, 2.4; NaHCO3, 25.0; glucose, 11.2) that was maintained at 37 ± 0.5°C and bubbled with a mixture of 95% O2 and 5% CO2. The preparation was stimulated indirectly through the tendon with supramaximal rectangular pulses 0.5 msec in duration at a frequency of 0.2 Hz. To test the ACh response, a contracture was induced by application of ACh (20 μg/ml) for 60 sec in the absence of nerve stimulation. The tension was recorded isometrically on a Grass 78D polygraph using Grass FT.03 force displacement transducer (Grass Instruments Co., Quincy, MA).

"Toxin C was found to have neuromuscular blocking action on the chick muscle at 1 μg/ml as described in "Results" owing to trace contamination by postnystic neurotoxin(s) that are present in D. polyplepis polyplepis venom (Strydom, 1972, 1976). Repeated attempts at purification by chromatography failed to eliminate the contamination. In preliminary experiments on chemical modification of the single methionine Met-33, it was found that this neuromuscular blocking activity was abolished upon formation of the C(SCAMS). The other properties associated with angusticeps-type toxin were retained. The possibility that toxin C might have inherent neuromuscular blocking properties was excluded because those functionally inactive amino acid residues found in postnystic neurotoxins, such as Thr-29, Arg-37 and Gly-36 (see Karlsson, 1979), are lacking in the primary structure of toxin C (Joubert and Taljaard, 1978) and, moreover, Met-33 is not an essential residue for postnystic neurotoxic action. For F1 has no neuromuscular blocking activity, although a very close homologue of toxin C. Hence, it was concluded that the modification had inactivated the contaminating neurotoxin(s) and/or resulted in their being eluted as derivatives in positions different from the SCAMS derivative of toxin C when it was purified by chromatography as described above.

Rectus abdominis muscle of the frog. The rectus abdominis muscle of Rana marina was excised and mounted in a 5-ml bath containing frog Ringer solution (composition in millimolar: NaCl, 111.0; KCl, 2.0; CaCl2, 1.8; NaHCO3, 6.0; glucose, 11.2) at room temperature (20–25°C). Dose-response curves were obtained by applying ACh in various concentrations from 0.1 to 4.0 μg/ml and 60 min after toxin application. The dose-response curves to carbamol were produced similarly.

Mouse phrenic nerve-hemidiaphragm preparation. Albino mice (NIH-ICR strain) of either sex, fed on Lab Chows 5001 (Ralston Purina Co., St. Louis, MO), weighing 18 to 25 g were used throughout the experiment. The isolated phrenic nerve-hemidiaphragm preparation (Büllbring, 1946) was mounted in a 10-ml organ bath with a resting tension of 1.0 g in modified Tyrode solution (composition in millimolar: NaCl, 135.0; KCl, 5.0; CaCl2, 2.0; MgCl2, 1.0; Na2HPO4, 1.0; NaH2PO4, 15.0; glucose, 11.2) at 37 ± 0.5°C unless otherwise stated and bubbled with 95% O2 and 5% CO2. The phrenic nerve was stimulated at a frequency of 0.2 Hz with supramaximal rectangular pulses 0.05 msec in duration to elicit maximal twitches of the hemidiaphragm. For direct muscle stimulation, neuromuscular transmission was abolished by d-tubocurarine (1–3 μg/ml). The hemidiaphragm was then stimulated directly through a hook electrode inserted into the rib tissue with supramaximal rectangular pulses 0.5 msec in duration at a frequency of 0.2 Hz. Contractions were recorded isometrically in the same way as described above.

Denserved mouse hemidiaphragm. Denervation of the left phrenic nerve was performed on 20- to 25-g mice under ether anesthesia according to the method of Lüllmann and Muscholl (1964). Ten to 14 days after denervation, the denserved hemidiaphragm was isolated and mounted in Tyrode solution, and contractions elicited by direct stimulation were recorded in the same way as described above.

Electrophysiological study. For intracellular recording, the mouse phrenic nerve-hemidiaphragm preparation was fixed on a paraffin-lined Flexiglass plate with a planoconvex lens at its center, which was immersed in a 20-ml bath at 30 ± 0.5°C. Motor nerve was stimulated by a pair of platinum electrodes with rectangular pulses of 0.05 msec in duration delivered through a photoelectric isolation unit (Digitimer D4030 with DS2 stimulator, Digitimer Ltd., Welwyn Garden City, Hertfordshire, England). The stimulation frequency was once every 5 sec with either a single pulse or a train of five pulses at 200 Hz.

For recording the EPP, d-tubocurarine (2–4 μg/ml) was added to immobilize the preparation. All recordings were from surface fibers using glass microelectrodes filled with 3 M KCl (6–20 meghoms). The potentials were displayed on Tektronix oscilloscope (Type 5444; Tektronix, Inc., Beaverton, OR), recorded on film with Grass C4 camera and measured under magnification.

For measuring the quantal content, the preparation was pretreated with DFP at 1 nM for 30 min, followed by continuous washing for at least 30 min. The muscle was then cut according to the method described by Barstard and Lilliehall (1966) and the EPP was recorded by glass microelectrode as described above. The quantal content was calculated by the variance method (del Castillo and Katz, 1954).

Isolated guinea-pig ileum. A piece of the guinea-pig ileum, 2 to 3 cm long, was mounted in an organ bath that contained 10 ml Tyrode solution at 37 ± 0.5°C and was bubbled with 95% O2 and 5% CO2. Longitudinal contractions were recorded isometrically in the same way as described above.

Isolated rat atrial preparation. Rats (Sprague-Dawley strain) of either sex, weighing 200 to 250 g, were used. The atrial preparation was prepared according to the method described by Burn (1962) and mounted in 20 ml of well-oxygenated Locke's solution at 30°C. The spontaneous contractions of the auricle were recorded isometrically in the same way as described above.

Assay of ChE activity. The ChE activity was assayed by pH-stat titration at pH 7.4.7. The mouse diaphragm and the frog (Rana marina) rectus abdominis muscle were homogenized with Tyrode or frog Ringer solution (NaHCO3 and Na2HPO4 omitted), respectively, to a final concentration of 20 mg/ml. The chick biventer cervicis was homoge-
nized with Krebs solution (NaHCO₃ and KH₂PO₄ omitted) to a final concentration of 10 mg/ml. These homogenates were incubated for 20 min with or without toxin either at 37 ± 0.2°C or at room temperature (for the frog muscle), and then ACh was added as substrate. The ACh concentration was 1 mM. The acetic acid liberated was titrated with 2 mM NaOH.

**Results**

**Chick biventer cervicis nerve-muscle preparation.** The three angusticeps-type toxins (F₂, F₃ and C₁₀S₂C₂) from *D. angusticeps* venom did not exhibit any appreciable effect on the chick biventer cervicis muscle preparation even at a concentration as high as 100 μg/ml. ACh response of the muscle tested 2 to 3 h after toxin application also remained unaffected. By contrast, toxin C from *D. polyplepis polyplepis* venom produced a neuromuscular block at a concentration as low as 1 μg/ml and abolished the ACh response of muscle completely. The 100% neuromuscular blocking time (mean ± S.E.M., n = 3) was 141 ± 14 min at 1 μg/ml and 38 ± 4 min at 10 μg/ml. On the other hand, toxin C(SCAMS), in which Met-33 was alkylated with iodoacetamide, did not cause neuromuscular block even at a concentration of 10 μg/ml, suggesting that toxin C may be contaminated by some postsynaptic neurotoxin(s) present in *D. polyplepis polyplepis* venom. The neuromuscular blocking activity of toxin FS₂ was much weaker than toxin C. No complete neuromuscular block was observed within 3 h at 100 μg/ml (about 90% block after 3 h).

**Mouse phrenic nerve-hemidiaphragm preparation.** Toxin F₂ augmented contractile responses to indirect stimulation dose dependently at concentrations of 0.1 to 10 μg/ml and also caused spontaneous fasciculations in the absence of electric stimulation at concentrations of 1.0 μg/ml and above in this preparation (fig. 1a), whereas toxins F₃ and C₁₀S₂C₂ were without any effect at a concentration as high as 100 μg/ml. Similarly, no stimulatory effect was observed with toxin FS₂ at 100 μg/ml; it produced complete neuromuscular block about 2 h after toxin application. By contrast, toxin C exhibited similar stimulatory effects as those of toxin F₂ at first, followed by gradual neuromuscular block (94 ± 12 min at 10 μg/ml, 204 ± 22 min at 3 μg/ml and 323 ± 39 min at 1 μg/ml, n = 3 each), again indicating possible contamination of some postsynaptic neurotoxin(s) in toxin C. Thus, toxin C(SCAMS) did not produce neuromuscular block even at higher concentrations (10–100 μg/ml); it augmented contractile responses to indirect stimulation at a concentration as low as 0.05 μg/ml and caused spontaneous fasciculations at concentrations of 0.3 μg/ml and above (fig. 1b), indicating that the stimulatory effect of toxin C was unaffected whereas the contamination of postsynaptic neurotoxin(s) was mostly eliminated after alkylation of Met-33 of the toxin molecule with iodoacetamide, followed by purification with CM-Sepharose chromatography.

The height of tetanic contraction evoked by repetitive stimulation was also increased by both toxins F₂ and C(SCAMS) but the tetanic contraction did not sustain, exhibiting typical Wedensky inhibition (fig. 1, a and b). Augmentation of contractile responses to indirect stimulation as well as the spontaneous fasciculations produced by these toxins was antagonized by d-tubocurarine. In d-tubocurarine-pretreated preparations, both toxins did not affect contractile responses to direct stimulation but exhibited a decuraring effect on responses to indirect stimulation if the concentration of d-tubocurarine was low (fig. 2, a and b). Similarly, there was no increase in twitch height in the denervated hemidiaphragm preparations that were stimulated directly.

In Ca²⁺-free medium, both toxins did not exhibit any effect but upon washing with Tyrode solution, the maximal stimulatory effect was observed within 2 min, indicating that binding of these toxins to the target site occurred in Ca²⁺-free medium. In low-Ca⁺⁺ (0.6 mM) or high-Mg⁺⁺ (4.2 mM) medium, the stimulatory effect of both toxins was attenuated but restored maximally within 2 min upon washing with Tyrode solution. In figure 3, only experiments with toxin F₂ are shown and toxin C(SCAMS) behaved similarly.

**Frog rectus abdominis muscle.** The response of the frog rectus abdominis muscle to ACh was enhanced by toxins F₂ and C(SCAMS) dose dependently at concentrations from 0.3 to 10 μg/ml. The ACh dose-response curves were shifted to left in parallel with the control curve obtained before the application of toxin. The effects of toxin C(SCAMS) at various concentrations are shown in figure 4. In contrast, no appreciable effect was observed on the carbachol response. Toxins F₂, C₁₀S₂C₂ and FS₂ were without effect on the ACh response up to 10 μg/ml.

**Effect on resting membrane potential and muscle action potential.** Both toxins F₂ and C(SCAMS) did not affect the resting membrane potential of muscle fibers of the mouse diaphragm at 10 μg/ml.

At concentrations of 1 μg/ml and above both toxins caused repetitive firings of muscle action potentials to single nerve stimulation. In figure 5, only the effect of toxin F₂ is shown and toxin C(SCAMS) behaved similarly. Spontaneous action potentials were also observed in the absence of nerve stimulation.

**Effect on MEPPs.** The frequency of MEPPs increased up to 2 and 3 times control within 60 to 90 min after toxin F₂ (1 μg/ml) application. During this period, spontaneous giant potentials (i.e., potentials with amplitudes greater than twice the pretoxin value) were frequently observed (figs. 6 and 7). Similar
changes in the frequency and amplitude of MEPPs were also observed with toxin C(SCAMS) (0.3 μg/ml) in the tetrodotoxin-(1 μg/ml) treated preparation.

**Effect on EPPs.** Twenty minutes after F7 (1 μg/ml) treatment of the hemidiaphragm preparation paralyzed by d-tubocurarine (2 μg/ml), the amplitude of EPP was increased whereas the half-decay time of EPP was not appreciably altered (table 1). Thereafter, the EPP amplitude was increased to such an extent that the recording electrode was dislodged by muscle twitching in response to nerve stimulation. In this case, a higher d-tubocurarine concentration (4 μg/ml) was added to immobilize the muscle. Sixty and 110 minutes after F7 treatment, not only EPP amplitude was increased but also the half-decay time was significantly prolonged (table 1).

Figure 8 illustrates the pattern of five successive EPPs elicited by a train of five pulses at 100 Hz. The train of EPPs...
obtained in the \( F_1 \)-treated preparation showed a pattern of sustained depolarization.

**Effect on quantal content of EPP.** In order to find whether toxins \( F_1 \) and C(SCAMS) have presynaptic effects in addition to their anti-ChE activity, the effects of \( F_1 \) (1 \( \mu \)g/ml) and C(SCAMS) (0.5 \( \mu \)g/ml) on the quantal content of EPP were tested on the mouse hemidiaphragms pretreated with DFP (1 mM). As shown in table 2, both toxins \( F_1 \) and C(SCAMS) significantly increased the quantal content of the EPP even after the cholinesterase activity of the diaphragm had been inhibited by DFP.

**Effect on the isolated guinea-pig ileum.** At concentrations of 0.3 \( \mu \)g/ml and above, both toxins \( F_1 \) and C(SCAMS) increased gradually the tone and motility (amplitude of longitudinal muscle contractions) of the isolated guinea-pig ileum, which was antagonized by low concentrations (3–10 ng/ml) of atropine (fig. 9, a and b). In the atropine-pretreated preparation, no stimulatory effect of these toxins was observed. After washout of both atropine and toxin, however, the stimulatory effect of toxin reappeared (fig. 9c).

**Effect on the rat atrial preparation.** At concentrations of 10 to 30 \( \mu \)g/ml, toxin \( F_1 \) exhibited negative inotropic and chronotropic effects, which were antagonized by 0.1 \( \mu \)g/ml of atropine (fig. 10a). In the atropine-pretreated preparations, the toxin effects were completely prevented (fig. 10b). Toxin C(SCAMS) behaved similarly.

**Effect on ChE activity of homogenized muscles.** As shown in table 3, both toxins \( F_1 \) and C(SCAMS) exhibited a potent anti-ChE activity comparable to that of neostigmine in the homogenized mouse diaphragm and frog rectus abdominis muscle but only a negligible effect in the chick biventer cervicis muscle.

**Discussion**

Among five \( angusticeps \)-type toxins, \( F_1 \), \( F_2 \) and \( C_{16} S_{1} C_{2} \) from \( D. angusticeps \) venom and \( F_{52} \) and \( C \) from \( D. polylepis \) \( polylepis \) venom examined in the present study, only toxins \( F_1 \) and \( C \), which belong to subgroup I of \( angusticeps \)-type toxins (Joubert et al., 1978; Joubert and Taljaard, 1980a), were found to have stimulatory effects on neuromuscular transmission of the mouse and amphibian skeletal muscles. Toxin \( C \) was apparently contaminated with some postsynaptic neurotoxin(s), present in \( D. polylepis \) \( polylepis \) venom, because toxin \( C \) produced neuromuscular block in both chick biventer cervicis muscle and mouse phrenic nerve-diaphragm preparations. After alkylation of Met-33 with iodoacetamide followed by purification by CM-Sepharose chromatography, toxin C(SCAMS) did not produce neuromuscular block even at a high concentration, although its stimulatory effect on neuromuscular transmission of the mouse diaphragm remained unaffected, indicating that Met-33 is not essential for the stimulatory effect of toxin \( C \).

Both toxins \( F_1 \) and C(SCAMS) produced marked augmentation of twitch responses to indirect stimulation and spontaneous fasciculations in the mouse phrenic nerve-hemidiaphragm preparation. This stimulatory effect was antagonized by \( d \)-tubocurarine and attenuated by low \( Ca^{2+} \) or high \( Mg^{2+} \). Because both toxins did not augment the contactions of directly stimulated preparations, either pretreated with \( d \)-tubocurarine or chronically denervated, the site of action would appear to be
TABLE 1
Effect of toxin F₁ (1 μg/ml) on the amplitude and the half decay time of EPPs in the mouse diaphragm
Results are presented as means ± S.E.M. from four preparations.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time after F₁ Treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Amplitude (mV)</td>
<td>20 min⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.47 ± 0.19</td>
<td>1.86 ± 0.19⁺</td>
</tr>
<tr>
<td></td>
<td>1.56 ± 0.07</td>
<td>1.59 ± 0.06</td>
</tr>
</tbody>
</table>

* In the presence of d-tubocurarine, 2 μg/ml.
⁺ In the presence of d-tubocurarine, 4 μg/ml.
* P < .05; ** P < .01 as compared with the control value.
⁺ P > .05. The value of EPP amplitude at 60 min should be much higher than 1.82 ± 0.24 mV if the concentration of d-tubocurarine were the same as that of control. In that case, the P value should be less than .05.

TABLE 2
Effects of toxins F₁ and C(SCAMS) on the quantal content of EPP in mouse hemidiaphragms pretreated with DFP (1 mM)
Values of quantal content are presented as means ± S.E.M., calculated from 13 to 21 endplates by the variance method.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time after F₁ Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 min</td>
</tr>
<tr>
<td>F₁ (1 μg/ml)</td>
<td>81.6 ± 20.1</td>
<td>243.8 ± 37.3*</td>
</tr>
<tr>
<td>C(SCAMS) (0.5 μg/ml)</td>
<td>76.4 ± 11.4</td>
<td>242.2 ± 59.0*</td>
</tr>
</tbody>
</table>

* P < .05 as compared with the control value.

TABLE 3
Anti-ChE activity of toxins F₁ and C(SCAMS) in homogenized muscles as compared with neostigmine
ChE activity of homogenized muscles was assayed by pH-stat titration at pH 7.4. For details see "Materials and Methods."

<table>
<thead>
<tr>
<th>Homogenized Muscle</th>
<th>F₁ (μM⁻¹)</th>
<th>C(SCAMS) (μM⁻¹)</th>
<th>Neostigmine (μM⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse diaphragm</td>
<td>5.2 × 10⁻⁴</td>
<td>3.7 × 10⁻⁴</td>
<td>1.8 × 10⁻⁴</td>
</tr>
<tr>
<td>Frog rectus abdominis</td>
<td>3.3 × 10⁻⁴</td>
<td>N.D.</td>
<td>1.3 × 10⁻⁴</td>
</tr>
<tr>
<td>Chick biventer cervix</td>
<td>&gt;4.2 × 10⁻⁴</td>
<td>N.D.</td>
<td>7.7 × 10⁻⁴</td>
</tr>
</tbody>
</table>

* Only 22 ± 3% (n = 5) inhibition at 4.2 × 10⁻⁴ M.

at the neuromuscular junction rather than on the muscle fibers themselves.

Both toxins F₁ and C(SCAMS) augmented also the response of the frog rectus abdominis muscle to ACh but not to carbachol, although no augmentation of ACh response was observed in the chick biventer cervix muscle. These results are in accordance with the finding that both toxins F₁ and C(SCAMS) have a potent anti-ChE activity in the homogenized muscle diaphragm and frog rectus muscle but only a negligible effect on the chick muscle. Although Barrett and Harvey (1979) failed to find any anti-ChE activity in D. angusticeps venom, recently, Rodriguez-Ithurralde et al. (1983) have isolated two proteins, called fasciculins, that inhibit ChE, from the same venom, and one of them (fasciculin 2) was found to be identical to toxin F₁ (Rodriguez-Ithurralde and Karlsson, 1983). Therefore, the stimulatory effects of toxins F₁ and C(SCAMS) on skeletal and intestinal smooth muscles and their inhibitory effect on the atrial muscle could be attributed mostly, if not entirely, to their anti-ChE activity. Wedensky inhibition in the mouse diaphragm on tetanic stimulation, sustained depolarization of the train of EPPs, repetitive firings of muscle action potentials to single nerve stimulation, increases in the amplitude of EPPs and MEPPs and prolongation of the half-decay time of EPPs, all observed after the toxin treatment, also could be explained on the basis of anti-ChE activity of these toxins. On the other hand, the increase in the quantal content of EPPs in the DFP-pretreated muscle produced by these toxins is rather indicative of facilitation of ACh release from motor nerve terminals. The increase in the amplitude of EPPs without alteration in the time course at 20 min after F₁ treatment may be also indicative of earlier appearance of its presynaptic effect than its anti-ChE activity. All of these findings support the view that toxins F₁ and C(SCAMS) facilitate ACh release from cholinergic nerve terminals in addition to their anti-ChE activity.

Because of similarities in their amino acid sequences, these angusticeps-type toxins have been classified as either membrane toxin (cardiotoxin) homologues (Karlsson, 1979) or short neurotoxin homologues (Dufton and Hider, 1983). The present study indicates clearly that the angusticeps-type toxins belong to a group of novel snake toxins with distinct pharmacological properties.

Although toxins of this group have low lethality when tested alone, synergistic lethal effects have been found when used in combination with other components such as protease inhibitor homologues of the same or other mamba venoms (Strydom, 1976, 1977; Lee et al., 1982). Because some of the angusticeps-type toxins (e.g., F₁ and C) have potent anti-ChE activity and protease inhibitor homologues from mamba venoms have been shown to increase ACh release in response to motor nerve stimulation (Harvey and Karlsson, 1982; Lee, 1982; Lee et al., 1983), the synergistic lethal effects between these two group toxins could be easily attributable.
Fig. 9. Effect of toxins F7 and C(SCAMS) on the isolated guinea-pig ileum. A, toxin F7 (1 μg/ml) followed by atropine (10 ng/ml); B, toxin C(SCAMS) (0.3 μg/ml) followed by atropine (3 ng/ml); C, prevention of the stimulatory effect of toxin C(SCAMS) (1 μg/ml) by atropine (10 ng/ml); w, washout with Tyrode solution.

Fig. 10. Effects of toxin F7 on the isolated right atrial preparation of the rat. a, F7 (30 μg/ml) followed by atropine (0.1 μg/ml); b, atropine (0.1 μg/ml) pretreatment prevented the effects of F7.

References


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