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BIPHASIC MECHANICAL RESPONSES OF THE GUINEA-PIG ISOLATED ILEUM TO THE VENOM OF THE MARINE SNAIL *Conus striatus*

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Venom extract of *Conus striatus* elicited a rhythmic, transient contraction of the guinea-pig isolated ileum followed by a relaxation at concentrations greater than 1 μg/ml, which was abolished by tetrodotoxin and a low-Na medium. The contraction induced by the venom was inhibited by atropine but not mecamylamine, whereas the relaxation was not affected by bretylium, guanethidine or phentolamine. These results suggest that the contraction of the ileum induced by the venom is due to the excitation of cholinergic nerves, while the relaxation is mediated through non-adrenergic inhibitory nerves.

Introduction The venom of the piscivorous Conidae, *Conus striatus* Linné quickly immobilizes fish and is lethal to mice (Endean & Rudkin, 1965). The pharmacological properties of the *C.striatus* venom have been extensively studied in amphibian and mammalian skeletal muscles (Endean, Izatt & McColm, 1967; Freeman, Turner & Silva, 1974; Endean, Williams, Gyr & Surridge, 1976) and in guinea-pig cardiac musculature (Endean, Surridge & Gyr, 1977b; Endean, Gyr & Surridge, 1979). It has been reported that in the guinea-pig ileum the venom causes a rhythmic contraction (Endean, Gyr & Surridge, 1977a). In the present work, we have shown that this venom elicits rhythmic, transient contractions of the guinea-pig ileum followed by relaxations. The chemical properties of the active principle in the venom suggest a protein (mol. wt. $\gtrsim$ 10,000).

Methods Male guinea-pigs, weighing 300–350 g, were killed by cervical dislocation. The procedure of preparing the ileum and the technique for measurement of the response were carried out as described previously (Ohizumi & Shibata, 1981). The strip was suspended in a 20 ml organ bath containing Krebs-Ringer solution of the following composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4 \cdot 7H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.2 and glucose 5.8; pH 7.4. A low-Na^+ (36 mM) solution was made by the replacement of 109 mM NaCl with isotonic sucrose. Specimens of *Conus striatus* were obtained from the tidal reefs in Okinawa waters. The gastropods were immediately frozen, shipped via air to Tokyo and stored at −20°C until required. The venom was stripped from the venom duct of each specimen, weighed and taken up with 0.9% w/v NaCl solution (saline). The following drugs were used: tetrodotoxin (Sankyo), phentolamine methanesulphonate (Rogitine, Ciba-Geigy), bretylium tosylate (Burroughs-Wellcome), guanethidine sulphate (Ishmeline, Ciba-Geigy), atropine sulphate (Tokyo Kasei), mecamylamine hydrochloride (Meiji Seika), chlorpheniramine maleate (Sankyo), methysergide (Sandoz), carbamylcholine chloride (Sigma) and noradrenaline bitartrate (Sigma).

Results The venom extract of *C.striatus* caused rhythmic, biphasic responses, consisting of a transient contraction followed by a relaxation, at a concentration of 1 μg/ml or more, which lasted for at least 1 h (Figure 1a). The contraction and relaxation induced by the venom (10 μg/ml) were comparable to the maximal response to carbamylcholine (10^{-6}M) and noradrenaline (10^{-6}M), respectively. Washing with fresh medium 3 times for 1 min could not remove effects of the venom. These responses induced by the venom were abolished in the presence of tetrodotoxin (TTX, 5×10^{-7}M) (Figure 1b) and by incubation in a low-Na^+ (36 mM) solution (Figure 1c). The inhibitory response of the ileum to the venom was not affected by bretylium (10^{-4}M) (Figure 1d), guanethidine (10^{-4}M) or phentolamine (2×10^{-6}M). The excitatory effect of the venom was markedly inhibited by atropine (2×10^{-6}M), but not affected by mecamylamine (3×10^{-5}M), chlorphreniramine (10^{-6}M) and methysergide (5×10^{-7}M).

Discussion Recently, Endean *et al.* (1977a) found that venom extract of *Conus striatus* elicited a rhythmic contraction of the guinea-pig isolated ileum, which was blocked by a specific Na^+ channel blocker (TTX), a muscarinic receptor blocker (atropine) and the 5-hydroxytryptamine antagonist BOL-148. However, in the present experiments the venom caused rhythmic biphasic responses of the ileum which consisted not only of an excitatory component but also involved an inhibitory component. These

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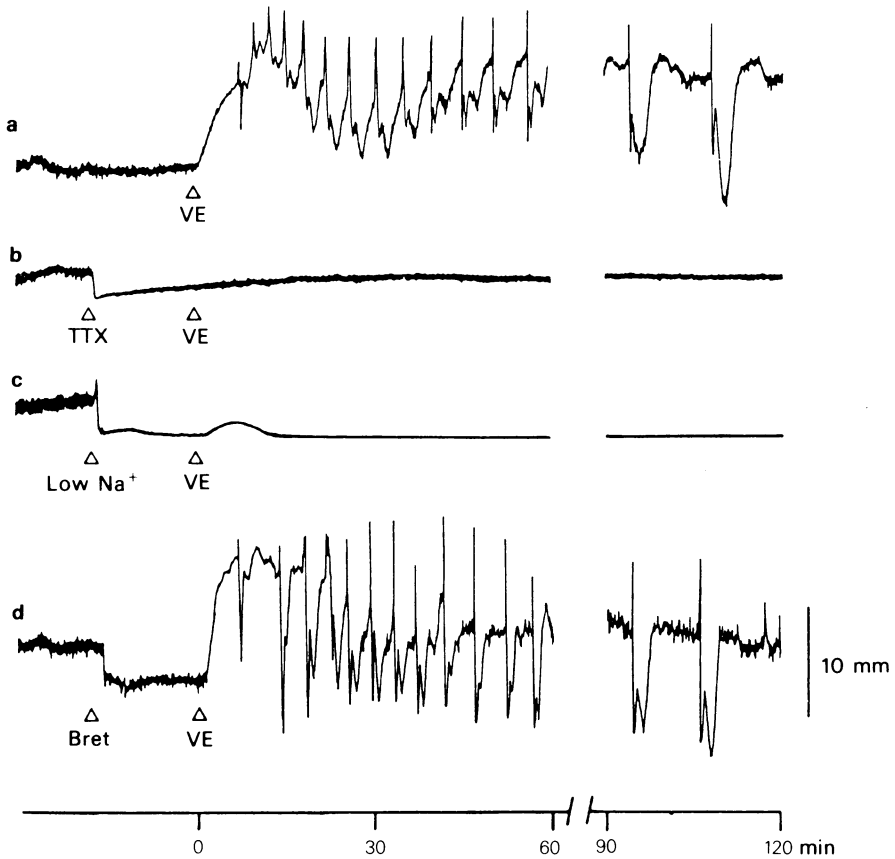

Figure 1 Effect of tetrodotoxin (TTX), low Na^+-sucrose medium (Low Na^+) and bretylium (Bret) on the contraction and relaxation induced by venom extract (VE) of *Conus striatus* in the guinea-pig isolated ileum. (a) Control; (b) TTX (5×10^{-7}M); (c) low Na^+ (36 mM); (d) bretylium (10^{-4}M). Venom extract was applied 15 min after treatment with TTX, low Na^+ and bretylium. Venom extract, TTX, low Na^+ and bretylium were added at Δ.

responses were abolished by TTX or the low-Na (36 mM) solution. These data suggest that biphasic responses induced by the venom were caused by increasing Na^+ permeability across the nerve cell membrane, which could play an important role in both responses. The relaxation induced by the venom was not affected by an α-adrenoceptor blocker (phentolamine) or adrenergic neurone blocking agents (bretylium and guanethidine). It is well known that non-adrenergic inhibitory nerves, as well as adrenergic inhibitory mechanisms, are present throughout mammalian gastrointestinal smooth muscle preparations (Burnstock, 1972). These results suggest that the relaxation induced by the venom may be mediated through non-adrenergic inhibitory mechanisms. On the other hand, the venom-induced contraction was markedly inhibited by atropine, but not by mecamylamine, methysergide and an H_1-receptor blocker (chlorphreniramine). These results suggest that this contraction is mainly caused by acetylcholine released from cholinergic nerve endings.

Cardiotonic polypeptides, anthopleurin A (mol. wt. 5,183), B (mol. wt. 4,590) and C (mol. wt. 4,875) have been isolated from sea anemones (Norton, Kashiwagi & Shibata, 1978) and have been found to cause relaxation of the guinea-pig ileum following a transient contraction (Ohizumi & Shibata 1981). The shape and pharmacological nature of these responses were very similar to those produced by the venom of *C.striatus*. The active substance in the *C.striatus* venom is considered to be a protein having a molecular weight of 10,000 or more, judging from our results with protease and gel-filtration experiments with the venom extract (unpublished data). Further clarification of the pharmacological and chemical properties of this protein is now under way.

Reprint requests to J.K.

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