POTENT VASOCONSTRICTION OF THE MESENTERIC VESSELS BY THE VENOM EXTRACT OF THE SEA ANEMONE, _TEALIA FELINA_

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SUMMARY Partially purified extract (extract IV) of the sea anemone, _Tealia felina_ was shown to have a vasoconstrictor effect on the mesenteric vessels of the rat. The potent constriction by the extract was found to be dose-related. Extract IV-induced responses on the mesenteric vessels were slow and long-lasting in contrast to the brief and short-lasting response of noradrenaline. Extract IV-induced responses rose slowly to a peak and lasted much longer after (time to peak = 2.5 min; duration = 12 min). This response of the extract was not blocked by α—blockers, as perfusion with indoramin (10⁻⁶ M) and; phenotamine (10⁻⁷ M) blocked the responses of noradrenaline (NA) but had no effect on the extract IV—induced vasoconstrictor responses. However, indomethacin (60 µg/ml) blocked the responses to extract IV.

KEYWORDS: _Tealia felina_, potent vasoconstriction, mesenteric vessels

Introduction
Cnidarian species found in the three main classes, Anthozoa, Scyphozoa, and Hydrozoa have been reported to have various effects in animals. They have been known to be toxic to animals in various tissues and under different conditions. (Russell Findlay 1967; Baxter and Marr 1969; Carielo and D'aniello 1975; Bernheimer and Avigad 1976; Walker 1977 a & b; Elliott et al 1986; Rottini et al 1995; Konya and Elliott, 1996). In this study, the investigation of the effect of extract IV of the sea anemone, _Tealia felina_ on the mesenteric vessels were investigated, as the extract was shown to cause arrhythmia when the isolated heart of the guinea-pig was perfused with the extract (Elliott and Konya 1984a). It was concluded from further investigations that the cardiotoxicity might be due to the constriction of the coronary vessels of the heart. (Konya and Elliott 1996). Its vasoconstrictor actions were therefore tested on the mesenteries. A cursory account had earlier been reported by Elliott and Konya (1984b). Moreover, other researchers had earlier shown that some sea anemone extracts have vasoconstrictor effects on the mesenteries. Prominent among them is Walker (1977).

Materials and Methods

Preparation of the extract
Extract IV is a partially purified toxin isolated from the sea anemone, _Tealia felina_ (Elliott et al, 1986). Sea anemones were freeze-dried, ground, and homogenates made in 0.9% saline.

It was centrifuged at 4°C. The supernatant (crude extract) was purified by successively passing it through agarose column (extract I); sephadex column (extract II); dye-matrix Blue B affinity column (extract III); and carboxy methyl cellulose cation exchanger (extract IV).

The active ingredients of the _Tealia felina_ extract was earlier defined by Aldeen et al (1981a). They described an assay for the toxin based on its histaminolytic (anti histaminic) action in the guinea—pig ileum. In this assay, an activity unit (AU) was defined such that a solution of 0.05 AU/ml in Krebs solution produced a 50% inhibition of the contractile response to histamine under specified conditions. Extract IV was preserved in a deep-freezer in aliquots and used when needed.

Animals
Female Wistar rats (250 – 270g) were used in all the experiments.

Perfused mesenteric vessels preparations
The McGregor (1965) method was used in the experiment. The female Wistar rats were anaesthetized by intraperitoneal injection with pentobarbitone sodium (60µg/kg). The superior mesenteric artery accompanying the vein in adipose tissue was identified and cannulated with the perfusion pump in operation. The perfusion apparatus consisted of a heating coil maintained at 37°C, a Watson – Marlow H.R. flow inducer pump and “Y” junction plastic tube, one
arm of which led to a Bell and Howell 4-122-0001 pressure transducer and the other via a short length of rubber tubing (for injection of drugs) to a portex cannula O.D 0.75mm. The perfused area of the mesenteries was identified by blanching and included an area of ileum and caecum. Since the ileal mesentery was the only area to be used, the other branches of the artery (caecal, ileo-colic, colic and pancreatico – duodenal) were tied off. The mesentery was severed close to the ileum so that only arteries and arterioles were perfused obviating possible interference from the activity of the intestinal smooth muscle.

The perfused mesenteric vessels were placed in a water-jacketed glass cup maintained at 37°C and given 1 hr to equilibrate.

Experimental Procedure

The temperature of the perfused area was maintained at 37°C, and the extract and drugs were injected as a bolus of 1-100μl through the rubber tubing proximal to the cannula. Kreb’s solution bubbled with 5% CO₂ in oxygen was perfused continuously into the tissue at a rate of 2ml per min. The basal pressure in the mesenteric vessels was between 25-35 mm Hg in all the experiments. Before each injection, the perfusion pressure was brought back to baseline to allow room for pen incursions.

Various doses of the extract 0.079AU to 0.96AU) were injected to find out if the extract exhibited a dose-response relationship. The effect of α – blockers on the extract was investigated by injecting indoramin (10⁻⁶ M) and phentolamine (10⁻⁷ M) into the tissue after perfusion of the antagonists through the tissue for 1 hr. Extract IV was, however, injected into the tissues to confirm the vasoconstrictor action of the sample (aliquot) under test for each antagonist before equilibration with the α – blocker as control. Doses of noradrenaline, an α– agonist, was also injected into the tissue to confirm its effect before equilibration with the antagonist.

Indomethacin (60μg/ml) was also tested on the effect of extract IV, by its perfusion into the preparation for 1 hr before injection of the extract. Doses of NA and the extract were tested on the tissues before equilibrating with the antagonist for 1 hr in all cases to serve as controls.

Results

Dose – responsiveness

Extract IV was found to constrict isolated mesenteric vessels of the rat. Injection of the extract into the mesenteric vessels caused a dose-related increase in perfusion pressure over a range of 0.079 AU (1 μl) to 0.954 AU (12 μl). This effect was almost linear from 0.079 AU to 0.636 (correlation coefficient = 0.76). Doses above 0.63 AU produced smaller increases in perfusion pressure (n = 4) (fig 1). The dose that produced 50% of the maximum response (ED₅₀) was 0.32AU ± 5.8 mmHg. The duration of response depended on the dose (fig 2). The larger a response, the longer it took to get back to basal perfusion pressure.

Effect of α – blockers, indoramin and phentolamine.

Indoramin (10⁻⁶ M), a postsynaptic α₁-adrenocceptor blocker, blocked the effect of NA (0.5μg and 1 μg) as expected, but did not block the vasoconstrictor effect of extract IV (fig 3). It blocked 94% of the effect of 0.5μg, which produced 58% (ED₅₀) of the maximum response of the mesenteric vessels to NA, but had no effect on the response to 0.636 AU (ED₃₀) which produced maximum response to the extract. It also did not block the effect of 0.079 AU, which produced 14% (ED₅₀) of the maximum response to the extract.

Phentolamine (10⁻⁷ M), an α – adrenocceptor blocker, after perfusion into the preparation for 1 hr, blocked 90% of the response to 0.05μg NA which produced 51.0% (ED₅₀) of the maximum response of the tissue to NA. The effect of 0.1μg NA (ED₅₀) was also blocked by phentolamine. In contrast, it did not have any effect on the vasoconstrictor response of the tissue to 0.64 AU extract IV which produced 100% (ED₅₀), the maximum response to the extract (fig 4). The response to 0.31 AU (ED₅₀) which exerted 50% effect on the preparation, was also not blocked by phentolamine.

Effect of indomethacin and others.

Indomethacin (60μg/ml) after perfusion into the preparation for 1 hr, blocked 90% of the response to 0.05μg NA, which exerted 66% (ED₅₀) of the maximum response of the tissue to NA. It also completely blocked the response to 0.318 AU extract IV. The doses, 0.64 AU (ED₅₀) and 0.80 AU, of extract IV were also blocked by this concentration of indomethacin (fig 5).

When vasopressin (0.001 unit = 35mmHg, n = 2) and serotonin (0.05μg = 82.5 mmHg n = 3) were tested on the preparation, indomethacin also blocked their vasoconstrictor actions.

Bradykinin (0.05μg - 10μg) and arachidonic acid (10μg - 2mg) has no effect on the preparation. Acetyl choline (0.05μg - 100μg) also had no effect on the preparation. Angiotensin II constricted the vessels, but a second dose failed to reproduce the effect of the first.
Fig 1. Dose-responsiveness of extract IV of *Tealia helina* on the mesenteric vessels of rat. Values are mean ± SEM n=4. See definition of AU in text.

Fig 2. Effect of extract IV according to its doses (dose-responsiveness). Time scale is minutes.

Fig 3. Effect of the α-blocker, Indoramin, on the vasoconstrictor action of extract IV. The initial responses are "controls" before perfusion with the antagonist for 1 hr. (gap in fig). Indoramin $10^{-6}$ M had no effect on the extract.
**Discussion**

The results of these experiments definitely show that extract IV has a potent vasoconstrictor action on the mesenteric vessels of rat. The effect is dose-related. Previous experiments on the same extract had indicated that its antiarrhythmic effect in the heart was due to its constrictor effect on the coronary vessels (Konya and Elliott 1996). Similarly, Walker (1977) had reported that a jellyfish, *Cyanea capillata* toxin had constricted the mesenteric vessels of the rat. The dose responsiveness of extract IV reveals the fact that the receptors on which the extract acts possibly get maximally occupied at the dose, 0.636 AU.

The vasoconstrictor action of extract IV on the mesenteric vessels was found to be reversible. This was not surprising as earlier work on the extract had revealed that its molecular weight is only 7,500 (Konya 1984, Elliott et al 1986), indicating that the molecule is relatively small. Its size therefore may afford it move freely in and out of the mesenteric tissue, if there is no limitation. Its action was completely abolished by continuous perfusion with Krebs solution.

Indomethacin, a potent $\alpha_1$-blocker, did not have any effect on the vasoconstrictor action of the extract, showing that postsynaptic $\alpha_1$-receptors do not mediate the action of the extract.

The vasoconstrictor action of the extract was also not affected by the $\alpha_-$ adrenoceptor blocker, phenolamine. It is therefore not out of place to state that the vasoconstrictor action of the extract may not involve $\alpha_-$.

**Adrenoceptors.** Isolated rat mesenteric vessels are known not to possess any postsynaptic vasoconstrictor $\alpha_2$-adrenoceptors (Fiotakis and Pipili 1983). Intact mesenteric arterial bed of rat in vivo have also been reported not to contain a significant population of postsynaptic vasoconstrictor $\alpha_2$-adrenoceptors (Hiley and Nichols, 1983). $\alpha_-$-receptors are therefore not involved in the vasoconstrictor action of the extract on the mesenteric vessels of the rat.

Indomethacin blocked the effect of extract IV on the mesenteric vasculature of the rat. Although indomethacin blocks prostaglandin synthesis, it blocks the action of a variety of other compounds e.g. bradykinin (Northover, 1967; Starr and West, 1966).
Vasoconstriction of mesenteric vessel by sea anemone

In this work, indomethacin blocked the constrictor effect of vasopressin, serotonin, and noradrenaline. The action of indomethacin therefore is non-specific. It is possible that the blocking effect of indomethacin on the action of the extract may be at the point of Ca²⁺ influx into the tissue, since Ca²⁺ influx has been reported to be blocked by it in smooth muscles (Northover, 1971). Bunodosoma granulifera and stichodactyla helianthus have also been reported to act on potassium channels (Karisson et al, 1993).

Sheardown (1980) had also shown that indomethacin blocked the contractile effect of extract II of Tealia felina on the guinea-pig ileum. He concluded that the action of extract II was kinin-like; but with the mesentery preparation, no effect was observed with bradykinin (0.05µg to 10µg). This was in support of the findings of Northover (1967), when he reported that very large amounts of bradykinin were necessary to constrict rat mesenteric arteries and that its effects were weak and inconsistent. From the response of extract IV on the mesenteries, there is surely no resemblance, either in the dose or in the force of response. Extract IV’s response was very strong. The action of extract IV, therefore, may not be kinin-like.

This vasoconstrictor action of the extract is more likely to be implicated in the rise in the blood pressure of rats caused by the extract and its toxicity in the heart of guinea-pigs (Konya and Elliott, 1996).

Extract IV’s vasoconstrictor action may not be mediated by α-receptors from the results obtained in this work. However, this does not exclude the fact that its actions are receptor-based. More work with other antagonists will be carried out in an attempt to identify the receptors mediating the action of the extract.

These results have increased the inclination towards the conclusion that the arrhythmia caused by the extract in the isolated heart of the guinea-pig, and the increase in the blood pressure in the rat are consequences of its potent vasoconstrictor action.

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References


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