# 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 = 12min). This response of the extract was not blocked by $\alpha$ –blockers, as perfusion with indoramin ($10^{-6}$ M) and; phentolamine ($10^{-7}$ M) blocked the responses of noradrenaline (NA) but had no effect on the extract IV – induced vasoconstrictor responses. However, indomethacin (60μg/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 0.05AU/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 (60μgkg$^{-1}$). 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 4-422-0001 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. Krebs solution bubbled with 5% $CO_2$ 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 0.636 AU produced smaller increases in perfusion pressure (n = 4) (fig 1). The dose that produced 50% of the maximum response ($ED_{50}$) 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 $\alpha_1$–adrenoceptor 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% ($ED_{58}$) of the maximum response of the mesenteric vessels to NA, but had no effect on the response to 0.636 AU ($ED_{100}$) which produced maximum response to the extract. It also did not block the effect of 0.079 AU, which produced 14% ($ED_{14}$) of the maximum response to the extract.

Phentolamine ($10^{-7}$ M), an α – adrenoceptor blocker, after perfusion into the preparation for 1 hr, blocked 90% of the response to 0.05µg NA which produced 51.0% ($ED_{51}$) of the maximum response of the tissue to NA. The effect of 0.1µg NA ($ED_{88}$) 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% ($ED_{100}$), the maximum response to the extract (fig 4). The response to 0.318 AU ($ED_{50}$) 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% ($ED_{66}$) 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 ($ED_{100}$) 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 arachidonicacid (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.

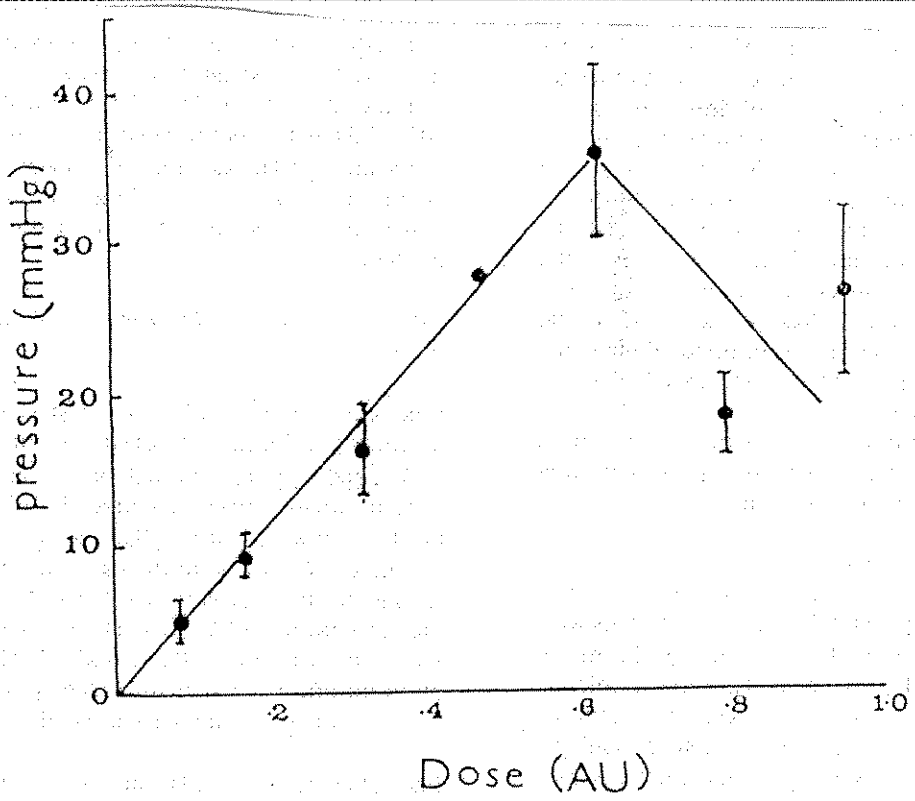


Fig 1. Dose-responsiveness of extract IV of *Tealia felina* on the mesenteric vessels of rat. Values are mean ± SEM n=4. See definition of AU in text.

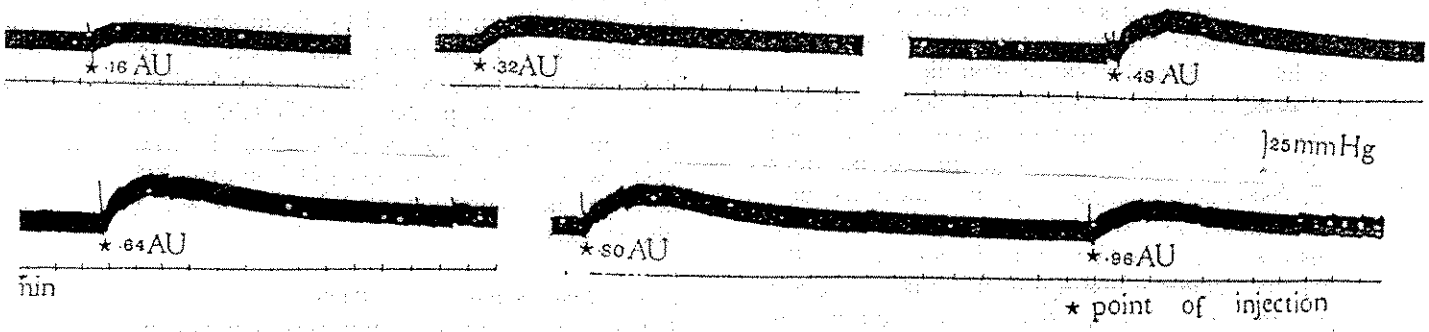


Fig 2 Effect of extract IV according to its doses (dose-responsiveness). Time scale is minutes.

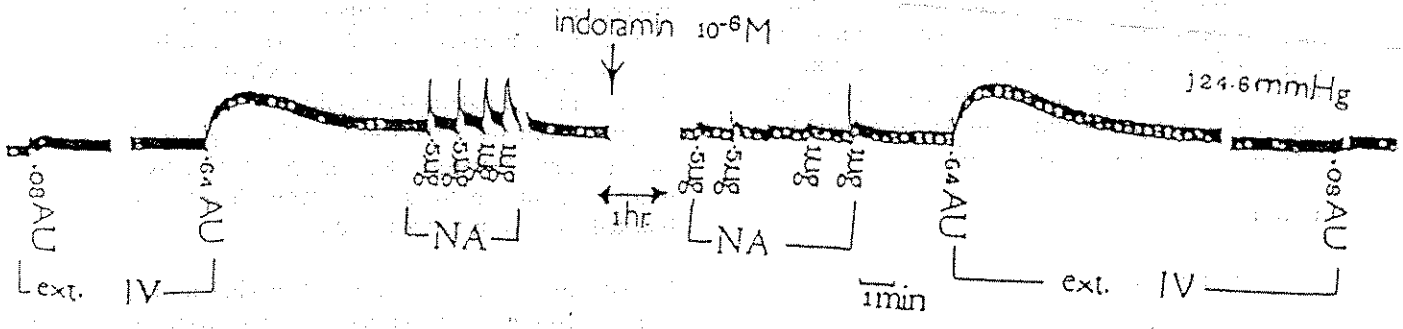


Fig 3 Effect of the α-blocker, Indoramin, on the vasoconstrictor action of extract IV. The initial responses are "controls" before perfusion with the antagonist for 1hr. (gap in fig). Indoramin $10^{-6}$ M) had no effect on the extract.

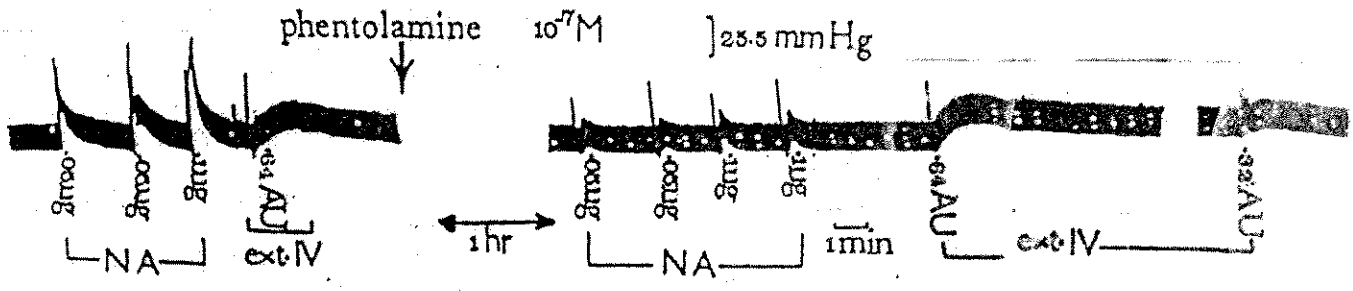


Fig 4. Effect of the α-blocker, phentolamine, on the vasoconstrictor action of extract IV. The initial responses are "controls" before perfusion with the antagonist for 1 hr. (gap in fig). Phentolamine ($10^{-7}$ M) had no effect on the extract.

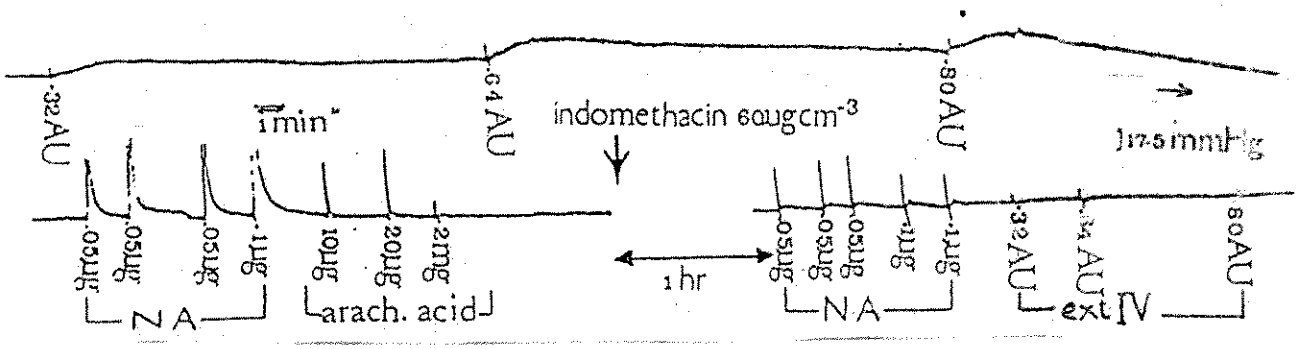


Fig 5. Effect of indomethacin on the vasoconstrictor action of extract IV. The initial responses of extract (10, 32 AU, 0.64 AU, 0.80 AU) (top panel) and noradrenaline (NA) 0.05μg and 0, 10μg) "controls" before perfusion with the antagonist for 1 hr. (gap in fig). Arachidonic acid 10μg, 20μg and 2mg) did not seem to have much effect on the preparation. Indomethacin (60μgcm$^{-3}$) blocked the vasoconstrictor action of extract IV. It is a continuous perfusion.

**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.

Indoramin, 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, phentolamine. 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 postjunctional 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 postjunctional 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).

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 $Ca^{2+}$ influx into the tissue, since $Ca^{2+}$ 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 (Karlsson *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**

Aldeen, S. I.; Elliott, R. C. and Sheardown, M (1981 a) The partial purification and bioassay of a toxin present in extracts of the sea anemone *Tealia felina* L ***Br. J. Pharmac*** 72:211 – 220

Baxter, E.H, and Marr, A.G.M (1969) – Sea wasp (*Chironex fleckeri)* Venom: Lethal, haemolytic and dermonecrotic properties–***Toxicon*** 7:195-210

Bernheimer, A.W. Avigad ,L.S (1976). Properties of a toxin from the sea anemone *Stoichaitis helianthus* including specific binding to sphingomyelin ***Proc Natl Acad Sci*** USA 73:467 – 471

Cariello, L and D' Aniello, A (1975). Isolation and characterization of four toxic protein fractions from the sea anemone *Anemonia sulcata* ***Toxicon*** 13:353 – 357

Elliott, R. C; Konya, R. S. (1984a) The cardiotoxic action of extract IV from *Tealia felina* ***Br. J. Pharmac*** 81, 162 p.

Elliott, R. C; Konya, R. S. (1984b) Vasoconstrictor effects of extract IV on the rat mesenteric vessels ***Proc. IXth Int. congr.*** Pharmac p. 633

Elliott, R. C; Konya, R. S; Vickneshwara, T (1986) The isolation of a toxin from the Dahlia sea anemone *Tealia felina.* ***Toxicon*** 24 (2):117-122

Fiotakis, D and Pipili, E. (1983). The effects of α2 – adrenoceptor agonism and antagonism and of PG – inhibition of the responsiveness of the rat mesenteric arterial bed. ***Br. J. Pharmac*** 79:234

Hiley, C.R and Nichols, A.J. (1983). α - adrenoceptors in the in-situ blood perfused superior mesenteric arterial bed of the rat. ***British J. Pharmac***. 80:615 p.

Karlsson, E.; Harvey, A. L; Aneiros, A and Castaneda, O (1993) Potassium channel toxins from marine animals. ***Toxicon*** 31; 497 – 540

Konya, R.S. (1984). The purification and pharmacological properties of an extract (IV) from the sea anemone, *Tealia felina.* PhD Thesis, Brunel University, Uxbridge, England.

Konya, R. S. and Elliott, R. C. (1996). The coronary vasoconstrictor action of extract IV from the Dahlia sea anemone *Tealia felina* ***Toxicon*** 34(2): 277 – 282.

McGregor, D.D (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat ***J. Physiol.*** 177: 21-30

Northover, B. J. (1967). The effect of anti-inflammatory drugs on vascular smooth muscle. ***Br. J. Pharm Chemother*** 31:483-493.

Northover, B. J. (1971). Mechanism of the inhibitory action of indomethacin on smooth muscle ***Br. J. Pharmac*** 41:540-551.

Rottini, G; Gusmani, L; Parovel, E; Avian, M and Patriarca, P (1995). Purification and properties of a cytolytic toxin in venom of the jellyfish *Carybdea marsupialis*. ***Toxicon*** 33 no 3; 315 – 326

Russell, Findlay, E. (1967) Comparative pharmacology of some animal toxins – ***Federation proceedings*** 26 (4): 1206 – 1224.

Sheardown, M. J (1980) The pharmacological activity of an extract from the sea anemone *Tealia felina*. PhD Thesis, Brunel University Uxbridge, England.

Starr, M. S. and West, W. B. (1966). The effect of bradykinin and anti-
inflammatory agents on isolated arteries. ***J. Pharm and Pharmacol*** 18:838-840.

Walker, M.J.A. (1977a). Pharmacological and biochemical properties of a
toxin containing material from the jelly fish, *Cyanea capillata* ***Toxicon*** 15:13-14.

Walker, M. J. A. (1977b). The cardiac actions of toxin – containing material from the jelly fish, *Cyanea capillata*. ***Toxicon*** 15:15-27.

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