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Changes in blood pressure in the rat induced by the venom extract from a sea anemone-*Bunodosoma cavernata*

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Summary

The effect of graded doses of crude sea anemone extract from *Bunodosoma cavernata* on rat blood pressure was investigated with a view to accessing its effects on the status of the cardiovascular system. From the results, the extract (2-8 ug protein/kg. i.v.) caused only transient hypotension. With higher doses of the extract (10-20 ug protein/kg, i.v.), the transient hypotension was accompanied by a dose-dependent increase in blood pressure. Atropine, mepyramine and propranolol failed to affect the transient hypotension. Of these drugs, only propranolol decreased the extract - induced hypertension. Doses of the extract above 20 ug protein/kg. i.v. produced a decrease in pulse rate and an increase in pulse pressure. We suggest that the extract - induced transient hypotension could be due to the presence of a less potent agent in the extract with acetylcholine - like action, and the hypertensive action of the extract was probably due to the stimulation of the sympathetic division of the autonomic nervous system by agent(s) in the extract.

Keywords: *Bunodosoma cavernata. extract, transient-hypotension, prolonged-hypertension.*

Résumé

L'effet de differentes does de l'extrait anemone brute Bunodoso,a cavernata sur la tension arterielle de souris avait ete investique avec pur vue de mesureron effect sur le systeme cardiovasculaire. Les resultats montrai tent que l'extrait de (2-8 ug protein/kg. i.v.) ont cause une hypotension passagere hypotension variant avec cette la dose qui s'augmentait avec le perssion sanquine (10-20llg. protein/kg. I/V). L'atropine, le me pyramine et le propranold n'affectaont pas l'hypotension passagere. De ces medicaments, seul le propranolol reduit l'hypertension induit par l'extrait les does de l'extrait superieur a 20 ug/protein/kg i.v. reduit poule et augmente la pression du poule. Nous suggerons que l'hypotension passagere induit par l'extrait pourrait etre due a la presence dun agent moins efficace dans l'extrait et l'action similaire a l'acetylcholine et l'action hypertensive de l'extrait devrait etre probablement due a stimulation de division sympathetique du systeme reduite autonomique par less agents de l'extrait.

Introduction

Sea anemones contain a variety of interesting biological active compounds, including potent toxins [1]. Proteins and peptides figure prominently amongst the various classes of sea anemone toxins isolated and characterized to date. The proteins were first isolated as cardiac stimulants [2] and neurotoxins [3] and these two activities remain the primary focus of attention.

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With the availability of the three dimensional structure of several anemone proteins, some interesting new findings have been made about the roles of the individual amino acids residues in their biological activities. The best characterized of these, Anthopleura xanthogrammica (AP - A), is a potent positive inotropic agent [4]. Its activity is not associated with any significant effects on heart rate or blood pressure in vivo [5] and it is also able to stimulate ischaemic myocardium [6]. Compared with the cardiac glycoside, digoxin, which is still used in wide spread clinical use for the treatment of congestive heart failure, AP - A is more potent and has a higher therapeutic index in dogs [7]. Therefore, it may serve as a valuable lead in the development of new therapeutic agents for treatment of the failing heart.

Many potentially useful therapeutic application of sea anemone toxins are currently being unfolded. The number of anemone toxin amino acid sequences has continued to grow and further information on the identity and role of residues essential for activity has come to light as a result of recent chemical modification and proteolysis studies [8,9]. Unfortunately, information about the biological activity of *B. cavernata* is hardly documented, although it is reported to contain agent(s) that inhibit gastric acid secretion [10]. Therefore, the main objective of this paper is to investigate the effects of this crude animal extract on mammalian blood pressure with a view to understanding its cardiovascular activities compared with toxins of the English species such as A. xanthogrammica already reported to contain a cardiac stimulant [4,9] and able to stimulate ischaemic myocardium [5,6,7].

Materials and methods

(a) Location and collection of animal specimens

The sea anemone used in this study was *Bunodosoma cavernata*. It is a jelly fish in the Phyllum Coelenterata and is found in abundance along the shores of an estuary in Port Harcourt, River State Capital, Nigeria. The exact location is "Opuaduakiri" fishing port (7°000E:4°20 N) which is quite close to Bonny town and is about 5 km to the Atlantic Ocean. The animals are found buried in the mud along the shores with only the oral disc exposed. Usually, inside the mud, their substratum is attached firmly to pieces of rotten wood, molluscan shells and other solid materials found in the mud. Some of the animals were caught with tiny dead fishes buried in their enteron. There was no evidence of any symbiotic relationship between these animals and other marine creatures.

Collection of the animals was easy after repeated trials. At the exact location, it was possible to collect over 500 animals within 10m radius in 1 hr. Collection was done by hand but wearing hand gloves. To collect each animal, the hand was pushed deep into the mud, far enough to exhume the supporting material (rotten wood, molluscan shell, etc.), in which the substratum of the animal was attached. The

animal was then detached from it and dropped into large plastic troughs containing the brackish water. A large quantity was usually collected each time. The animals were then taken life to the laboratory and washed one by one with clean brackish water, to remove all accompanying debris and mud. Each animal weighed 5-20g. They were freeze-dried and stored in sealed containers before keeping in the refrigerator at - 20°C.

(b) Preparation of the crude extract

The extract was prepared by the method of Walker (1977). One hundred grams (100g) of the freeze-dried animal specimen was homogenized in an electrically driven tissue grinder/ blender for 5 min. The homogenate was then dissolved in 100 ml normal saline (0.9%NaCl) and then centrifuged (10,000g) for 10min. The supernatant was collected into small tubes. The desired test concentrations of the extract were always prepared from this stock by serial dilutions with normal saline.

(c) Estimation of the protein content of the crude extract

The estimation of the protein content of the crude extract was performed as described by Lowry, *et al.* (1951).

(d) Toxicity test

Male white Wistar mice (15-20g) were randomly assigned to 10 cages of 12 animals per cage. Each group was injected intraperitoneally with one of the following: 20,25,30,35,40,45,55,60 and 65 ug protein/kg of the crude sea anemone extract. The maximum volume injected was 0.2ml per dose. The last group (control group) received 0.2 ml of normal saline (i.p.) per mice. The groups were returned to their home cages after injection and given free access to food and water. The mortality in each group (cage) was assessed 24 hr. after the administration of the extract. Percentage mortalities were converted to probits and plotted against the $\log_{10}$ of the dose of the extract. The results were subjected to statistical analysis of the regression line.

(e) Blood pressure studies

Normal male white wistar rats (250-320g) were anesthetized with 25% urethane at a dose of 6 ml/kg body weight. The trachea was intubated and the femoral vein and carotid artery cannulated (Portex cannulae, external diameter 1.02mm, internal diameter 0.75mm). The cannulation of the femoral vein and the carotid artery were for drug administration and blood pressure (BP) recordings, respectively. The BP was recorded on the Washington recording machine (Model 400 MD/2C) using the BP transducer (Washsington F.T. 400). The temperature was maintained at 37 1.°C by means of a rat table heater. Various doses of the extract or drugs were injected (i.v.) via the femoral vein. The maximum volume of injected fluid was 0.2ml. Each injected dose was followed by a flushing injection of 0.2ml saline. Blood pressure responses were measured as change in mean arterial pressure (MAP in mmHg), from the pretreated levels. The MAP was calculated by using the following formula:

MAP= DP + (SP - DP) according to Friedman (1976), where SP and DP are the systolic and diastolic pressures, respectively. Rats treated with extract or drugs were considered hypertensive (elevated blood pressure) or hypotensive (reduced blood pressure) when their MAPs were significantly higher or lower than the controls (untreated group) as the case may be. Atropine (1 mg/kg), mepyramine (2 mg/kg), propranolol (0.5 mg/kg) and hexamethonium (2.5 mg/kg) were used to influence the action of the extract on the blood pressure.

Results

(a) Toxicity text

The results of the toxicity studies in mice are summarized in Fig. 1. The dose-mortality relationship was apparently sigmoidal. A plot of probit values (% mortality) versus log-dose gave a straight line. From the straight line graph, the LD_{50} of the extract in mice was extrapolated and this was about 40 Fg protein/kg ip.

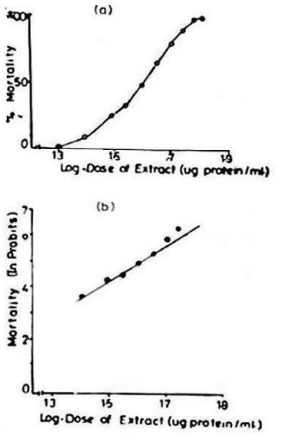

Fig. 1: Lethality studies in mice. The percentage mortality, (a); and the mortality in probits, (b), within 24 hr. following the administration of graded doses (20-65 Fg protein/Kg.i.p.) of the crude extract to male mice.

(b) Blood pressure studies

The control mean arterial pressure (MAP) measured from urethane anaesthetized rat was about 70.25" 1.60mm Hg (SEM, n = 8). Slow intravenous (i.v) administration of various concentrations (8-20 Fg protein/kg) of the sea anemone (*B. cavernata*) extract caused transient (2-5 sec.) hypotension which was immediately followed by a hypertensive state (Fig. 2). The initial transient hypotension markedly showed a fall in both the systolic and diastolic pressures (Fig. 2a).

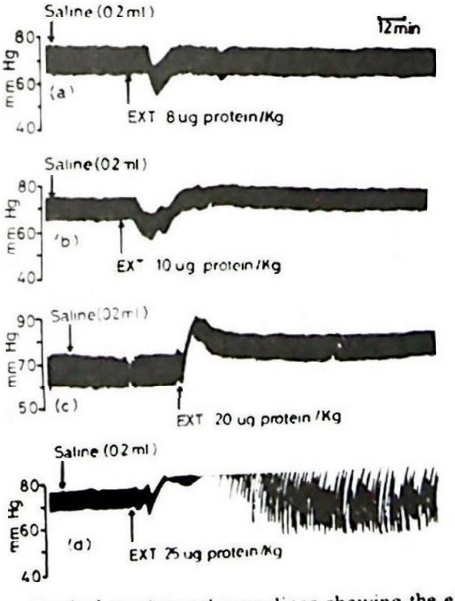

Fig. 2: Typical mechanical recordings showing the effect of the crude extract from *B. cavernata* (8-25 Fg protein/kg. i.v.) on the arterial blood

The hypertension that followed, rapidly reached peak heights (initial level) before returning to the steady state levels. In preliminary studies, intravenous injection of atropine (1mg/kg), mepyramaine (2mg/kg) and propranolol (0.5 mg/kg) to another group of rats, without the administration of the extract, produced either hypotension or hypertension depending on the drug. Both atropine and mepyramine caused the MAP to rise significantly from the mean control level to a level of about 82.9 ± 8.5 mmHg and 98.6 ± 7.4mmHg, respectively (SEM, n=5, $P < 0.05$) (Table 1). On the other hand, propranolol (0.5mg/kg) caused a fall in blood pressure (BP) from the mean control level to about 39.8 ± 9.3 mmHg. This represents about 42% decrease in BP by propranolol (0.5mg/kg). In another group of rats, these drugs (atropine, mepyramine and propranolol) were administered in combination with the extract (20 ug protein/kg). The results showed that the transient hypotension persisted and was not affected by any of the three drugs. Also, the accompanying hypertension was not affected by the extract combination with either atropine or mepyramine even after injection of a second dose of the drugs (not shown). However, a second dose of propranolol (0.5mg/kg) given about 20 sec. after the first injection significantly reduced the height of the extract-induced hypertension from 78.8 ± 5.8 mmHg to about 54.5 ± 3.2 mmHg (S.E.M., n=5, $P < 0.01$) (Fig. 3a). This value represents about 31.4% depression of BP by propranolol (0.5mg/kg) in the presence of the extract (20 ug protein/kg. i.v.).

An attempt was made to determine whether the BP changes seen after extract injection were central or peripheral in origin by blocking the autonomic ganglia with hexamethonium (2.5 mg/kg. i.v.). From the results obtained from 5 experiments (Figs. 3b & 3c), hexamethonium caused about 23.3"9.5% reduction in the extract (20 ug protein/kg) - evoked hypertension. This result was significant ($P < 0.05$).

The hypertensive responses evoked by various doses of the extract are summarized in Fig. 2a-c. These BP changes were dose-dependent. The latencies of responses were remarkably short (1-3 sec.). Previous experiments have revealed that the extract, between the concentration range of 1-8 ug protein/kg did not change the BP of rats to levels significantly different from controls. However, a concentration of 8 ug protein/kg of the extract produced only the transient hypotension without the accompanying hypertension (Fig. 2a). The duration of the hypertensive action of the extract of doses higher than 8Fg protein/kg.iv. were also dose-dependent although two animals showed wide variations. The recovery from the hypertensive state was slow and incomplete, taking between 1-311/2hr depending on the dose of extract.

Fig. 2d shows the effect of administering 25 ug protein/kg iv. of extract on the rat BP. Four of these experiments were carried out and the results were similar.

Table 1: Dose-response relationship

Agents injected (i.v.)	Concentration	Mean arterial blood pressure (MAP)		
		Pre-injection (control) values (mm Hg)	Post-injection (treated) values (mm Hg)	
Extact	8 µg proten/kg	70.25 ± 1.60	71.13 ± 4.8	(8)
,,	10 ,,	69.70 ± 3.4	78.45 ± 3.2	(6)
,,	15 ,,	70.15 ± 2.8	85.45 ± 4.8	(6)
,,	20 ,,	72.14 ± 3.6	98.64 ± 5.4	(5)
,,	25 ,,	68.16 ± 1.8	112.35 ± 6.6	(4)
,,	1 mg/kg	71.41 ± 4.2	82.91 ± 8.5	(4)
,,	2 mg/kg	69.32 ± 3.5	98.63 ± 7.4	(4)
,,	0.5 mg/kg	68.53 ± 1.6	39.84 ± 9.3	(4)

Effects of graded doses (8-25 µg Protein/kg) of the crude extract from B. cavernata and some pharmacological agents (atr opine, 1 mg/kg; mepyramine, 2 mg/kg; propranolol, 0.5 mg/kg) on the mean arterial blood pressure (MAP) in the rat. Data shown are means ± S.E.M. and the number of experiments in parentheses.

The extract (25 ug protein/kg) caused a drastic fall in the pulse rate but inceased the pulse pressure (Fig.2d), and the animals died in less than 10 min post injection. For this resason, higher doses of the extract were not tested. Hexamethonium (2.5 mg/kg. i.v.) blocked this increase in pulse pressure, although the action was transient (Fig. 3c).

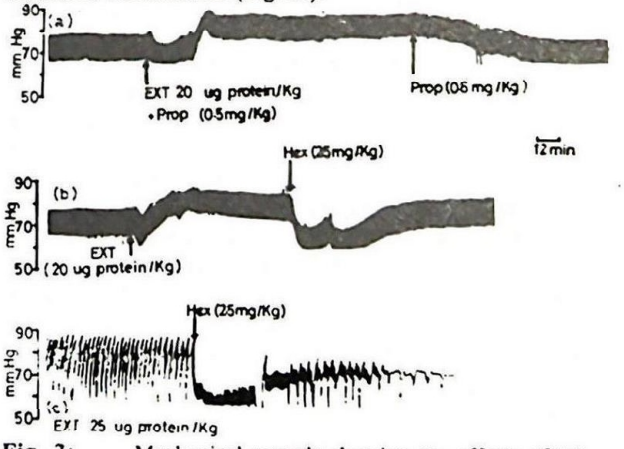

Fig. 3: Mechanical records showing the effects of (a) propranolol (0.5mg/kg. i.v.) and (b) hexamethonium (2.5 mg/kg. i.v.) on the extract (20 Fg protein/kg, i.v.) - induced increase in arterial blood pressure of the rat. The effect of hexamethonium (2.5 mg/kg. i.v.) on the extract (20 ug protein/kg. i.v.) - induced changes in pulse rate and pulse pressure of the rat is shown in (c).

Discussion

The crude sea anemone extract, with an LD_{50} of 40 Fg protein/ml, was probably not highly toxic to mice. Other jelly fish classified as highly toxic are *Physalia physalia* with an LD_{50}

of 0.75 mg/Kg mice [13] and *Stomolophus meleagris* having 0.85mg/kg mice [14]. Although lethality to prey species of the jellyfish was not examined, but the mammalian species tested suggests that the crude extract from B. cavernata was not highly toxic.

Blood pressure (BP) measurements reflect the status of the cardiovascular system (CVS) and the maintenance of an adequate BP in the aorta depends on the product of two factors, the cardiac output and the total peripheral resistance of the vessels. Therefore, the present study was focussed on the effects of the crude extract from *B. cavernata* on the rat blood pressure.

The experiments performed in this study clearly points to the hypertensive effect of the sea anemone (*B. cavernata*) extract. This effect was probably due to some interference with the sympathetic transmission by agent(s) present in the crude extract, leaving the parasympathetic component unopposed. The evidence supporting this view is that propranolol, a beta adrenoceptor blocker, reduced the extract-induced increase in blood pressure whereas removing the vagal effect on the heart with atropine sulphate failed to aggravate the hypertensive condition. However, a parasympathetic-like effect of the extract may not be completely ruled out. Also, the hypertension was significantly blocked by the autonomic ganglion blocker, hexamethonium. This indicates the possibility of the CNS contribution to the hypertensive state.

Figure 2(a-c) shows that the hypertensive effect of the extract was preceded by a transient hypotension. Several reports have been documented about the biphasic effect of some sea anemone toxins. These include the *Anthopleura xanthogrammica* [15]; *Stomophus meleagris* [16], *Anemonia sulcata* [17] and even the sea wasp *Chironex fleckerii* [18]. It has been suggested that coelenterate toxins resemble one another both toxicologically and biochemically [14]. Therefore, the biphasic action (transient hypotension and prolonged hypertension) of the sea anemone (*B. cavernata*) extract is not surprising.

The failure to abolish or influence the transient hypotension with atropine, mepyramine and propranolol, excludes the possibility that it was due to the involvement of cholinergic, histaminergic and adrenergic transmissions, respectively. A direct effect of the extract on the heart might therefore be suggested. This could be possible because a large number of jelly-fish toxins possess membrane-active properties [16,19,20] and the regulation of Ca^{2+} ions in the extracellular environment is suggested to be one of the actions of jelly-fish toxins [21]. Alternatively, the transient hypotension could be due to a compensatory reflex decrease in peripheral vascular resistance that resulted from the extract-induced hypertension. Also, it could be due to the presence of a less potent agent in the extract with acetylcholine-like action.

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