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Blocking Actions of the Sea Anemone, *Bunodosoma cavernata* Extract on Histamine and Acetylcholine – Induced Contractile Actions of the Wild Grasscutter and Rabbit Ilea

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ABSTRACT: Extract from the sea anemone, *Bunodosoma cavernata* was prepared by homogenising 100 of the sea anemones in 300ml of 0.9% saline in a Moulinex blender. This was centrifuged at 4000 revolutions per minute for 10 minutes. The supernatant was re – centrifuged to obtain the crude extract. The extract (1ml/l) was found to elicit long lasting contractions on the ilea of wild rabbit and grass cutter. It was also found to block acetylcholine (ACh) – induced contractions of the ileum of grass cutter (90 ± 6.2% inhibition ± SEM) and the contractile action induced by ACh (0.2µg and 0.4µg) (89 ± 5.8% inhibition ± SEM) and histamine (0.075µg and 0.15µg) on the rabbit ileum (80 ± 4.6% inhibition ± SEM). The antagonism by the extract was non – selective. © *JASEM*

The phylum Cnidaria, comprises about 9,000 species in three classes: Hydrozoa, Scyphozoa and Anthozoa. Sea anemones belong to the class, Anthozoa that constitute the largest class with some 6,000 species. Some sea anemones have been reported to have a wide spectrum of pharmacological activities, which affects man and mammals alike (Lane and Dodge 1960, Barnes 1967; Russell 1967; Baxter and Marr 1969; Moore and Scheuer 1971; Lin and Hessinger 1979). These activities have been attributed to various compounds identified in the sea anemone extracts. Some of such compounds include histamine, homarine, 5 –hydroxytryptamine, tetramethylammonium (TMA) (Mathias *et al*; 1960). Indeed TMA is said to be more abundant in the tentacles than in the rest of the body (Anthoni *et al*; 1989)

Cnidarians sting. The stinging unit of the Cnidarian is the nematocyst which is formed within an interstitial cell, the cnidoblast. While all Cnidarians are potentially dangerous, only a few have nematocysts capable of penetrating the skin and poisoning humans. Those that do are sometimes fatal. The wide range of the properties of their extracts include their toxic effects on tissues of mammals and man. Their contractile effects on the gut and other smooth muscles are some of those properties (Aldeen *et al*; 1981; Konya and Elliott; 1996).

Results of acute toxicity studies of *Bunodosoma cavernata* showed a dose – mortality relationship, which was apparently sigmoidal. The LD_{50} was 40µg protein/kg mice i.p (Eno *et al.* 1998). In the same study, it was found that the extract produced spontaneous contractions of the guineapig ileum, and that it blocked the contractile effect of histamine. The blocking actions of the extract of another sea anemone, *Tealia felina* on histamine – induced contractions of the guinea pig ileum had also been described by Aldeen *et al* (1981) *Bunodosoma cavernata* extract has also been found to have dermonecrotic effect on mammals. (Konya, in press)

This current study describes the effect of *Bunodosoma cavernata* obtained from Bonny Creeks of Rivers State, Nigeria on the ilea of wild rabbit and grass cutter. It also describes the effect of the extract on ACh – and histamine – induced contractions of these ilea. More studies of toxins appear to have been carried out on guinea pig and rat ilea by toxinologists than those of the rabbit and grass cutter. The grass cutter species of the family used in this study is known as the largest species of rodent in Nigeria after the crested porcupine (Happold 1987).

MATERIALS AND METHODS

Animals: Ilea of rabbits and grass cutters captured from the wild in Azumini village Isiokpo in Rivers State , Nigeria, were used. The sea anemone, *Bunodosoma cavernata* were collected from the

creeks of Opuaduakiri, a fishing port (7°00E, 4° 20'N) close to Bonny town in Rivers State, Nigeria.

Protein Estimation: Protein content of the extract was estimated using the method of Waddell (1956). The absorbance of the extract was measured at 215nm and 225nm. Normal saline (0.9% NaCl) was used as blank. The absorbance at 225nm was subtracted from that at 215nm. If the absorbance at 215nm exceeded 1.5, the extract was further diluted. The difference between the absorbance at 225nm and 215nm was multiplied by a factor, 144. This gave the concentration of protein in the extract in μg/ml. The factor 144 is identical on different spectrophotometers rendering recalibration unnecessary. The use of the difference rather than the absorbance at a single wavelength minimizes the error from non – protein constituents

Preparation of Bunodosoma cavernata extract : About 100 cleaned sea anemones were homogenised in 300ml of 0.9% saline in a Moulinex blender. It was centrifuged. The supernatant was re – centrifuged at 4,000 revolutions per minute for 10mins and the resultant supernatant tested on the ilea.

Preparation of Ilea

Each rabbit or grass cutter was killed by placing it in a covered container containing a few drops of chloroform for a few minutes. The abdomen was opened , and pieces of ileum (3cm long) separated, cleaned and mounted vertically in a 20ml organ bath containing Tyrode solution of the following composition in g/l: NaCl 8; KCl 0.2; $NaHC0_3$ 1.0; $MgCl_2$ 0.1; NaH_2P0_4 0.05; Glucose 2.0; $CaCl_2$, $6H_20$. 0.2. The solution was bubbled with oxygen (95% O_2, 5%CO_2) and the temperature maintained at 37 ± 1.5°C. One end of the ileum was knotted to the support in the tissue bath while the other end was knotted to the kymograph using a chart speed of 0.4mm / sec. Care was taken not to close the lumen of the ileum as that would interfere with the circulation of solution in the hollow of the tissue.

Effect of Extract on grass cutter and rabbit ilea:

To test the effect of the extract on the ileum, the Tyrode solution in the tissue bath was exchanged for the extract (Img/l) and the ileum left to contract spontaneoulsy. Contractions of the ileum to ACh and histamine were recorded and a dose – response curve for both drugs were obtained. In each case, two doses, which produced approximately 25% and 75% maximum responses, were selected and the two doses alternated at 5 min interval until 3 pairs of fairly consistent responses were obtained. The bathing fluid was then exchanged for Tyrode solution containing *Bunodosoma cavernata* extract and left to stand for 25 mins to equilibrate.

Alternate doses of ACh were added to the bath containing grass cutter and rabbit ilea to determine the effect of the extract on ACh – induced (0.2μg and 0.4μg) contraction of the grass cutter ileum (n = 14) and those of the rabbit ileum (n = 14; 0.075μg and 0.15μg). Its effect on histamine – induced (0.05μg and o.1μg) contractions of the rabbit ileum were also tested.

RESULTS AND DISCUSSION

The extract was found to be proteinaceous, Img/l. Peptides and proteins have been reported over the period as the main constituent of Cnidarian extracts (Platou et al; 1986; Norton 1991). This has made researchers focus attention on the biological activities of the protein molecules of various Cnidarian species, sometimes attempting to elucidate their components by analysing their amino acid residues. Tealiatoxin from the sea anemone, *Tealia felina* was investigated, for example, and found to contain amino acids, with a molecular weight of about 7,800 (Elliott et al; 1986). It is therefore not surprising that enzymes have been identified in some Cnidarian extracts. *Chrysaora quinquecirrha*_ was reported to contain at least seven enzymes (Burnett and Calton 1974). This explains the loss of biological activities (denaturation) of Cnidarian extracts over time.

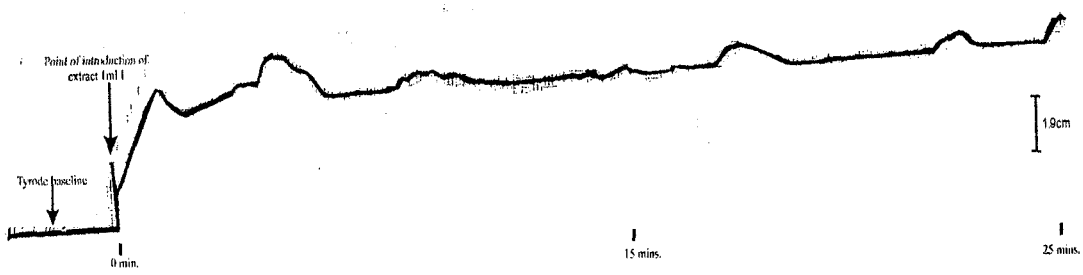

Fig. 1. A typical record showing effect of *Bunodosoma cavernata* on the rabbit ileum

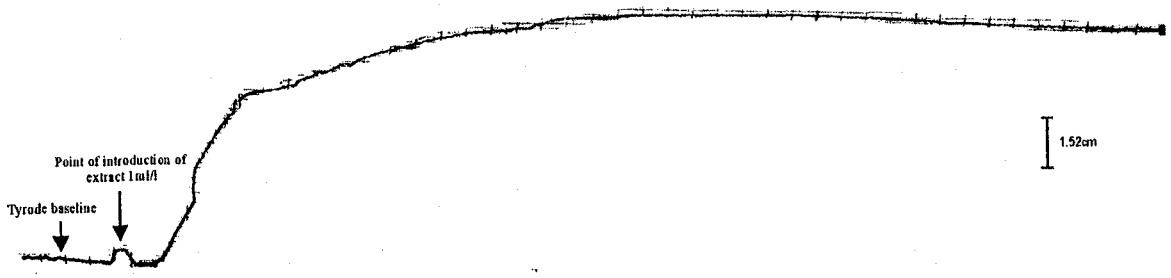

Fig. 2. A typical record showing effect of *Bunodosoma cavernata* on the grass cutter ileum

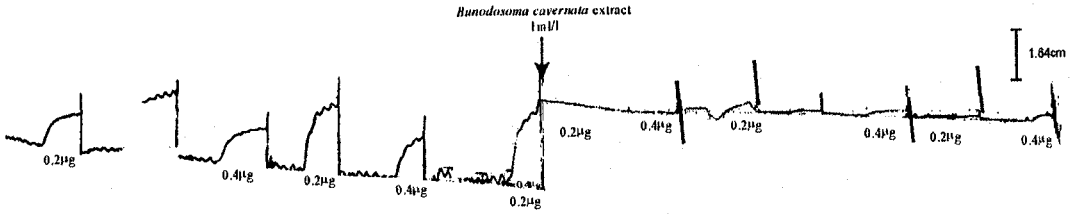

Fig. 3. A typical record showing inhibition of Ach-induced contractions of the grass cutter ileum by *Bunodosoma cavernata* extract

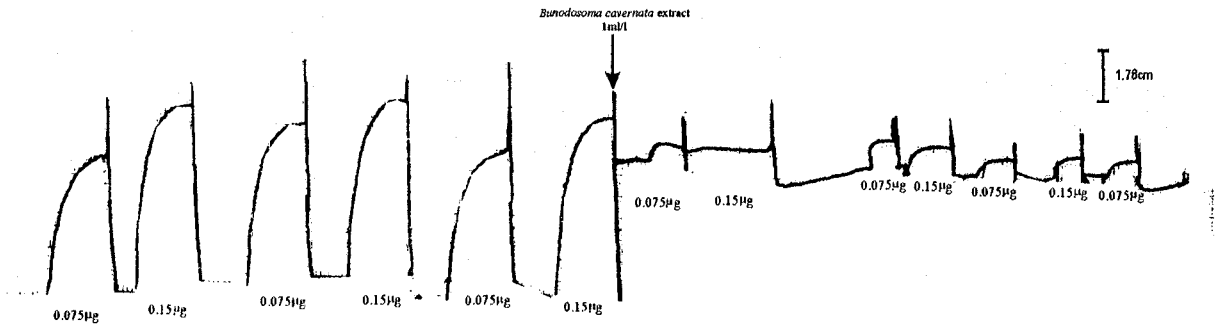

Fig. 4. A typical record showing inhibition of Ach-induced contractions of the rabbit ileum by *Bunodosoma cavernata* extract

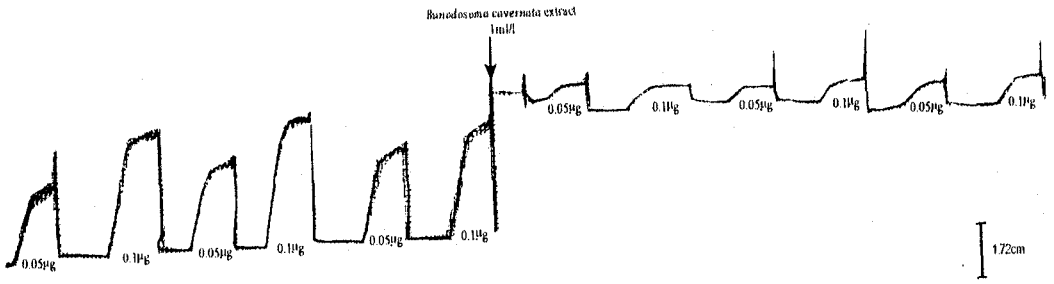

Fig. 5. A typical record showing inhibition of Histamine-induced contractions of the rabbit ileum by *Bunodosoma cavernata* extract

Bunodosoma cavernata extract (1ml/l) was found to have a prolonged contractile effect on both the rabbit ileum and the grass cutter ileum (Figs 1 and 2). Eno et al; (1998) had earlier reported that the extract produced spontaneous contractions of the guinea pig ileum. This result therefore shows that it also has similar action on grass cutter and rabbit ilea.

The slow contractile action of the extract could be due to the size of the active compound in the extract as penetration into the tissues will be difficult and slow if molecule is large. Another possibility is that the active molecule in the crude extract may react with the membrane of the ileum forming a complex which may slowly contract the ileum. A third possibility could be a slow liberation of the active compound on contact with the tissues. It is also possible that the contractile effect may be Kinin – like (Burnett et al., 1975). It may also have an indirect action through receptors.

When the Tyrode solution in the organ bath was exchanged with the extract (1ml/l) after obtaining the dose – responses of the tissue to ACh, it was observed that the extract blocked the contractile action of ACh on the grass cutter ileum (Fig 3, n=14; 90±6.2% inhibition ±SEM) and the rabbit ileum (Fig 4, n = 14, 89 ± 5.8% inhibition ± SEM). The change in gain of the response recording at the point of extract introduction, may be due to the extract's spontaneous contractile effect on the preparations (Figs 3-5).

Bunodosoma cavernata crude extract has been reported by Eno et al; (1998) to block the histamine – induced contractions of guinea pig ileum and that the inhibitory response developed very slowly and reached a maximum in about 35 – 40mins. The result obtained in this work therefore reveals that the extract also blocks histamine – induced contractions of the rabbit ileum in similar duration (80 ± 4.6% inhibition ± SEM). The action of the extract suggests a non – specific mechanism. The extract is non – selective as an antagonist since the responses to ACh were blocked as well as those of histamine. Eno et al., (2001) had earlier reported that the blocking action of *Bunodosoma cavernata* on the guinea pig ileum is non – competitive as doubling the doses of histamine failed to abolish the inhibitory affect of the extract. The primary site of attachment of the extract in that preparation therefore may be different from that of histamine, which appears unable to protect its own H_1 receptor against irreversible blockade (Cook et al., 1988). It is possible that the inhibition of ileal responses to ACh and histamine by the extract could either result from a direct action of the active component(s) on the ileal tissue or an indirect action involving receptors.

Sea anemones have been found to have effects on the rabbit in other circumstances. One of such reports is that reported by Baxter *et al*; (1972) which stated that fatal intoxication of rabbits, sheep and monkeys occurred when the venom of the sea wasp, *Chironex fleckeri* was injected into them. Other sea anemones have also been reported to block the histamine - induced contractions of ilea of other animals. Aldeen *et al.*, (1981) reported irreversible blocking effect of *Tealia felina* extract on the guinea pig ileum.

Conclusion: *Bunodosoma cavernata* extract produced prolonged contractile action on the rabbit and the grass cutter ilea. That the extract antagonized ACh – induced contraction of the grass cutter ileum and ACh – and histamine - induced contractions of the rabbit ileum suggests that it is a non – selective antagonist. It is possible that the compound(s) in the extract contracting the ilea spontaneously may be different or the same as the antagonist. Only the purification of the crude extract can reveal this, as the extract may be a cocktail of compounds.

It is also difficult to pin down the origin of the potent compound at this stage since the extract from the whole body (not tentacular) was used. It may be premature to attempt to identify the active compound(s) of the potent component until the purification of crude extract is completed. This result is part of the evidence that the sea anemone, *Bunodosoma cavernata* from Bonny Creek in Rivers State , Nigeria contains potent compound (s) which is (are) physiologically / pharmacologically significant.

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