Histaminolytic Agents in a Crude Venom Extract from the Sea Anemone *Bunodosoma cavernata*

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Abstract

A crude extract from the sea anemone *Bunodosoma cavernata* antagonized histamine-induced contractions of the ileum in a dose-related fashion and shifted the histamine dose-response curve to the right. Doubling the dose of histamine failed to surmount the antagonism. Similar results were obtained when ACh was used as the agonist. While the extract appeared to have no effect on 5-HT-induced contractions, it potentiated KCl-induced contraction of the ileum. Therefore, we suggest that the crude extract from *B. cavernata* contains histaminolytic agent(s) which inhibited histamine-induced contractions of the ileum. The action of the extract was probably non-selective since ACh-induced contractions were similarly inhibited. Furthermore, the histamine antagonism was probably of a non-competitive type since it was not surmounted by doubling the concentration of histamine. Finally, that the extract failed to inhibit 5-HT- and KCl-induced contractions appear to exclude a general toxic or local anaesthetic action on the tissue, and a receptor action is favoured.

Keywords: *Bunodosoma cavernata*, extract, histaminolytic, non-selective, non-competitive.

Introduction

As with other members of the phylum Cnidaria (Coelenterata), sea anemones possess numerous tentacles containing specialized stinging cells or cnidocytes. Within these small stinging cells, are organelles known as nematocysts that contain small threads which can be ejected forcefully following chemical or mechanical stimulation (Watson & Hessinger, 1989). This venom apparatus is used by these creatures (anemones) for defence against predators and in aggression, as well as in the capture of prey such as small fish and crustaceans (Ayre, 1982). It is not surprising to find, therefore, that sea anemones contain a variety of interesting biologically active compounds, including some potent toxins (Beress, 1982). Proteins and peptides figure prominently among the various classes of sea anemone toxins isolated and characterized to date. The proteins were first isolated as cardiac stimulants (Norton et al., 1976) and neurotoxins (Beress & Wunderer, 1975) and these two activities remain the primary focus of attention.

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, *Anthopeura xanthogrammica* (AP-A), is a potent positive inotropic agent (Shibata et al., 1976). Its activity is not associated with any significant effects on heart rate or blood pressure in vivo (Blair et al., 1978) and it is also able to stimulate ischaemic myocardium (Gross et al., 1985). Compared with the cardiac glycoside, digoxin, which is still in widespread clinical use for the treatment of congestive heart failure, AP-A is more potent and has a higher therapeutic index in dogs (Norton, 1981). Therefore, it may serve as a valuable lead in the development of a new therapeutic agent 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 (Kem et al., 1990; Gould et al., 1990). Unfortunately, information about the biological activities of B. cavernata toxin is hardly documented, although it is reported to be highly toxic with an LD50 value of about 40μg protein/kg in mice when given ip. (Eno et al., 1998a). A recent report suggested that B. cavernata toxin contained agents which possess antihistamine action, based on gastric acid secretion studies (Eno et al., 1998b). An earlier report suggested that an extract from the sea anemone (Tealia felina) contained a compound which antagonized the contractile action of histamine on the guinea pig ileum (Aldeen et al., 1981).

Since sea anemones are toxicologically and biochemically similar (Toom et al., 1975), the main objective of this study, was to investigate the effect of B. cavernata extract on the guinea pig ileum.

Materials and methods

(a) Preparation of crude extract

The location and collection of the animal specimen was as described by Eno et al. (1998a). The extract was prepared by the method of Walker (1977). Freeze-dried animal specimens (100g) were homogenized in an electrically driven grinder/blender for 5 min. The homogenate was then dissolved in 100 ml of saline (0.9% NaCl) and centrifuged (10,000 g) for 10 min. The supernatant was collected in small tubes. The desired test concentrations of the extract were prepared from this stock by serial dilutions with saline.

(b) Measurement of antihistaminic action of the extract on guinea pig ileum

(i) Animal preparation

Guinea pigs of either sex were fasted for 24 hr to ensure complete emptying of the small intestine. They were killed by a single blow at the head and a midline incision was made at the abdomen to expose the small intestine. The ileum was isolated, cut into short pieces (3–4 cm long), and mounted vertically in a 25 ml organ bath containing Tyrode solution of the following composition (mM concentration): NaCl, 140; KCl, 2.7; NaHCO3, 12.0; MgCl2, 0.5; Na2HPO4, 0.3; CaCl2, 0.9; glucose 5.5. The solution was bubbled with air and maintained at 37 ± 1°C. One end of the tissue was tied to a fixed support inside the organ bath, and the other end was connected to a polygraph (Grass Model 7D) via an isotonic tension transducer (FT 0.03). A resting tension of 1 g was maintained throughout the experiments. The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (1 g = 25 mm). The tissue preparations were allowed to equilibrate in the bath for 30 min before the commencement of the experiments.

(c) Experimental procedures

(i) Dose-effect relationship

Various concentrations (100–1000 ng/ml) of histamine were added to the bathing solution containing the tissue preparation, and the responses of the tissue to each added concentration was recorded. Using the same doses of histamine (1.5 and 3 μg/ml), the experiment was repeated, but in a bathing solution containing the extract (5.2 and 10.4 μg protein/ml).

(ii) Inhibition of histamine-induced contraction of the ileum by the extract

A concentration-response curve to histamine was first obtained from which two concentrations were selected which gave approximately 25% and 75% maximal responses. These two concentrations were applied alternately at 5 min interval (without the extract) until consistent responses of the ileum were obtained. The bathing fluid was then exchanged for atropinized (2.9 × 10^-6 M) Tyrode solution containing the extract to be assayed. The application of the two selected doses of histamine alternately to the tissue preparation was repeated in the presence of the extract using the same time interval. Further studies were conducted employing the same procedure described above, but with double the concentration of histamine or applying other pharmacological agents (agonists) such as ACh, 5-HT, KCl, and with ACh and histamine, alternated in the same experiment.

For studies on the histamine blocking action of the extract, atropine (2.9 × 10^-6 M) was added to the Tyrode solution, and when acetylcholine (ACh) was studied, mepyramine (2.8 × 10^-6 M) was added to block histamine (H1 subtype) receptors. When ACh and histamine were used in the same experiment, no antagonist was added. The experiments with 5-HT and KCl were carried out in the presence of both atropine (2.9 × 10^-6 M) and mepyramine (2.8 × 10^-6 M) (Aldeen et al., 1981).

(iii) Maintenance of extract-induced inhibition of responses of the ileum to histamine

The crude extract (10.4 μg protein/ml) was applied to the tissue preparation at zero time, to antagonize histamine-induced contraction of the ileum. In the continued presence of the extract, the amplitude of contractions were measured at 30 min intervals for 180 min. The measurements were also made when the tissue preparation was washed at 30 min intervals for 300 min with extract-free Tyrode solution.

Drugs

5-Hydroxytryptamine, acetylcholine chloride and atropine sulphate were from BDH; mepyramine maleate and histamine acid phosphate from Evans; indomethacin from Sigma.
Results

(a) Isolated guinea pig ileum experiments

(i) Extract-induced spontaneous contractions

The crude sea anemone extract from *B. cavernata* evoked a spasmogenic effect on the isolated guinea pig ileum (not shown). The contractions were slow to develop and had a latency of about 15–23 sec; reached maximum heights in about 5 min and then declined slowly over a period of about 20 min in the continued presence of the extract. Superimposed on these slow contractions were some fast twitch-like contractions. If the tissue preparation was washed with Tyrode solution after about 5 min exposure to the extract, partial recovery resulted and further contractions could be elicited with the extract, but these contractions varied in amplitude. Atropine sulphate (1.5–2.9 × 10⁻⁶ M) reduced and/or abolished the extract-induced spontaneous contractions dose-dependently, whereas both methysergide (2.5 × 10⁻⁵ M) and indomethacin (2.8 × 10⁻⁵ M) did not affect the spontaneous contractions.

(b) Antagonist action of the crude extract

(i) Dose-effect relationship

The dose-response relationships were apparently sigmoidal (Fig. 1) and, for each curve, a straight line regression was fitted for the linear portions of the curves, from which the ED₅₀ values were determined. The ED₅₀ values for histamine and histamine in the presence of extract 5.2 and 10.4 μg protein/ml were 281.8, 398.1 and 630.9 ng/ml histamine, respectively. The crude extract (5.2 and 10.4 μg protein/ml) inhibited histamine-induced contractions of the ileum dose-dependently and shifted the histamine dose-response curve to the right. The maximum inhibition produced by the extract at 5.2 and 10.4 μg protein/ml were 13.4 and 29.6%, respectively.

(ii) Inhibition of histamine-induced contractions

The extract (10.4 μg protein/ml) inhibited histamine-induced contractile responses of the guinea pig ileum. The inhibition was maximal after 35–40 min of exposure to the extract and thereafter was sustained at the maximum level for at least 3 hr following continuous exposure of the tissue to the extract. For this reason, the percentage inhibition produced by the extract was measured after 35–40 min exposure of the tissue to the extract in the assay procedure. Two selected doses of histamine (1.5 and 3 μg/ml, based on the histamine dose-response curve) were given alternately. Results showed that the extract (10.4 μg protein/ml) inhibited twitch responses induced by histamine (1.5 μg/ml) by 59.8 ± 9.7%, and those induced by histamine (3 μg/ml) by 71.52 ± 11.3%. Both inhibitions were significant (P < 0.05 in both cases) (Fig. 2a).

Doubling the doses of histamine failed to abolish the inhibitory effect of the extract (Fig. 2b). Alternating both histamine (4 μg/ml) and acetylcholine (1.6 μg/ml) on the same piece of tissue caused contractions which were blocked by the extract at 10.4 μg protein/ml (Fig. 2c). Preliminary studies have shown that 4 μg/ml and 1.6 μg/ml of histamine and acetylcholine, respectively, produced about the same percentage of maximum responses. The percentage inhibition caused by the extract (10.4 μg protein/ml) on histamine- and acetylcholine-induced contractions were about 62.4 ± 6.9% and 57.5 ± 8.6% respectively, on the same tissue preparation (Fig. 2c).

(iii) Inhibition of acetylcholine-induced contractions

The inhibitory action of the extract (10.4 μg protein/ml) on acetylcholine-induced contraction of the ileum was further studied in five tissue preparations. From the dose-response curve of acetylcholine (ACh), two doses (0.5 and 1.0 μg/ml) that gave 25% and 75% of the maximum responses were selected. The extract (10.4 μg protein/ml), caused about 28.7 ± 8.9% and 53.3 ± 7.5% inhibition of the responses induced by acetylcholine 0.5 and 1.0 μg/ml, respectively (Fig. 3a).
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(iv) Effect on 5-hydroxytryptamine-induced contractions

The action of the crude extract (10.4 µg protein/ml) on 5-hydroxytryptamine (5-HT)-induced contractions of the ileum was difficult to determine. Figure 3b shows typical results from ten experiments carried out with ilea from four guinea pigs. The results were variable with different tissue preparations. Most results showed that the extract had no effect on 5-HT-induced contractions (n = 6). Others showed either an inhibitory or excitatory action. Thus, it was difficult to rely on these results.

(v) Effect on potassium chloride-induced contractions

Potassium chloride-induced contractions of the ileum were potentiated by the crude extract (10.4 µg protein/ml). Two doses (0.3 and 0.6 µg/ml) of potassium chloride (KCl) were selected from the dose-response curve (doses that gave 25% and 75% of maximum responses respectively), and were applied to the ileum alternately. The extract (10.4 µg protein/ml) increased the amplitude of KCl-induced responses (Fig. 3c). The percentage increases were 49.45 ± 7.2% and 71.43 ± 5.4% for KCl 0.3 µg/ml and 0.6 µg/ml respectively.

(vi) Effect of prolonged exposure of the tissue preparation to the extract

Figure 4 shows the result of eight experiments on the gradual development of inhibition of responses to histamine (3 µg/ml) produced by the extract (10.4 µg protein/ml). In about 35–40 min after application of the extract, about 92.8 ± 7.9% of histamine-induced contractions were inhibited. This inhibition was maintained for at least 3 hr if the preparation was not washed throughout the duration of the experiment. If the preparation was washed at 30 min intervals, there was partial recovery, but it was never complete even when washed at 30 min interval for 5 hr with a total volume of 600 ml of extract-free Tyrode solution.

**Discussion**

Many sea anemone extracts have a wide spectrum of pharmacological activities (Elliott & Sheardown, 1980; Aldeen...
et al., 1981). Tetramethylammonium, 5-hydroxytryptamine, histamine, homarine and a protein substance were found in sea anemone extracts. Each of these compounds has been implicated for the different biological activities of the extract (Mathias et al., 1960).

In the present study, the effects of the crude extract from *B. cavernata* on histamine-induced contraction of the guinea pig ileum was investigated. Since the extract dose-dependently evoked slow spontaneous contractions of the ileum which were not blocked by either mepyramine, indomethacin or methysergide, it may be suggested that this action was unrelated to the release of histamine, kinins and serotonin, respectively. Atropine sulphate, however, reduced and/or abolished this spasmogenic effect of the extract, suggesting a significant contribution of a cholinergic mechanism in this action, and this explains why the drug (atropine) was added to the reservoir Tyrode solution when carrying out the assay experiments. In other species of sea anemone, such spasmogenic effects were caused by the presence of tetramethylammonium in the extract (Mathias et al., 1960), and it has been suggested that anemone toxins resemble one another both toxicologically and biochemically (Toom et al., 1975).

The histamine blocking action of the extract was slow in onset and could only be partially reversed by repeated washing of the preparation with extract-free Tyrode solution. These data could reflect the low concentration of the active compound present in the extract or the probable large molecular weight compound which may only penetrate slowly to its site of action (Bowman & Rand, 1980). The presence of large molecular weight compounds seems a more likely explanation since many sea anemone extracts are reported to contain large molecular weight compounds (Alsen et al., 1976, Mebs & Gebauer, 1980; Bernheimer & Avigad, 1976). Also, the observation that the antagonist action of the extract was maintained for several hours despite repeated washing of the preparation with the reservoir tyrode solution, was of tremendous interest since the duration of action of a drug is said to be of immense clinical importance (Bowman & Rand, 1980).

The inhibition of histamine-induced contraction of the ileum by the extract was dose-dependent. However, the extract was relatively non-selective as an antagonist since the responses to ACh were similarly inhibited by the extract. Furthermore, the antagonism of histamine action produced

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*Figure 3.* Typical mechanical records showing the effects of the crude extract (10.4 μg protein/ml) on (a) acetylcholine (ACh)-induced contractions, (b) 5-hydroxytryptamine (5-HT)-induced contractions, and (c) potassium chloride (KCl)-induced contractions of the ileum.
by the extract was probably of a non-competitive type since it was not surmounted by doubling the concentration of histamine.

Finally, the inhibition of ileal responses to histamine by the extract could result from a direct action of the active principle(s) on the tissue or an indirect (receptor) action. However, that the extract failed to inhibit 5-HT- and KCl-induced contractions seems to exclude a general toxic or local anaesthetic action on the tissue, and a receptor action is favoured. This is not surprising since neuroactive compounds have been isolated from some species of sea anemone extracts (Beress et al., 1976; Shibata et al., 1976; Norton et al., 1982). In conclusion, there seems to be convincing evidence that the crude extract from B. cavernata contains histaminolytic agents that act non-selectively and non-competitively on histaminergic neurons H1 to reduce and/or abolish smooth muscle contractions. However, it is premature to speculate on the actions of the crude extract on smooth muscles since the active principles are still unknown. Further progress must await refinement in the separation techniques.

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References


