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INHIBITION OF GASTRIC ACID SECRETION BY *BUNODOSOMA CAVERNATA* EXTRACT

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ABSTRACT

The saline extract of Bunodosoma cavernata *(6.0 µg protein/100 g body wt i.v.) did not affect basal acid secretion in rats. The extract, however, completely blocked the induced-release of gastric acid by histamine (0.2 mg/100 g body wt i.v.) and pentagastrin (0.6 µg/kg body wt i.v.). These effects were produced when the extract was administered 50 min before the drugs. Also, the extract abolished the stimulant action of both histamine and pentagastrin when it was administered 50 min before application of the drugs. However, the extract failed to influence the basal acid output.*

INTRODUCTION

Presently available information suggests that coelenterate toxins resemble one another both toxicologically and biochemically (Toom and Chan, 1972; Beress, 1982). The sea anemones, in common with other members of the phylum Cnidaria (Coelenterata), contain toxins which have a number of interesting biologically active substances (Beress, 1988; Gould et al., 1990; Kem et al., 1990). Haemolysis (Elliott et al., 1986; Kem, 1988), neurotoxicity (Fogh et al., 1990; Pennington et al., 1990) and cardiotoxicity (Simpson et al., 1990), are common features of these toxins. A recent report also showed that sea anemone toxins also contain a compound which antagonizes the contractile action of histamine on the guinea pig ileum (Aldeen et al., 1981).

In the current study, we have investigated the histaminolytic action of a crude extract of *Bunodosoma cavernata* (a sea anemone), using gastric acid secretion studies. Gastric acid secretion mediated by histamine receptors of the H_2-subtype.

Keywords: Bunodosoma cavernata, extract, gastric acid, inhibition, secretion.

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MATERIALS AND METHODS

Location and Collection of Specimens

Bunodosoma cavernata is a small slimy animal, weighing 5–10 g when fresh. It is found in abundance throughout the year along the shores of Bonny Sea (near Port Harcourt in S. Nigeria). The exact location is "Opudakiri" fishing port (7°00′E:4°20′N), which is about 5 km from the Bonny estuary with the Atlantic ocean. The animals are found buried in the mud with only the tentacles suspended aerially. The substratum are attached to pieces of rotten wood, mollusc shells and other solids buried in the mud. They were collected with their attachments, detached and then dropped into plastic troughs containing sea water. The animals were washed singly with filtered sea water to remove all accompanying debris and mud. They were finally freeze-dried and stored in sealed containers at −20°C.

Preparation of the Crude Extract

The extract was prepared according to the method of Walker (1977). In a typical experiment, 100 g of freeze-dried specimens of *B. cavernata* was homogenized in an all glass electrical driven tissue grinder for 5 min and then dissolved in 100 ml saline (0.9% NaCl). The

homogenate was then centrifuged (10,000 *g*) for 10 min and the supernatant collected into small tubes. The protein content of the crude extract was estimated by the method of Lowry et al., (1951). The stock solution contained 5.2 mg protein/ml and had an LD_{50} of 40 µg protein/kg mouse (i.p.). The desired concentrations of the extract were then prepared from this stock by serial dilution with saline.

Animal Preparation

Fifty-six white Wister rats weighing 230–280 g were prepared for the measurement of gastric acid secretion by the method of Ghosh and Schild (1958). The animals were fasted for 24 h to ensure that the stomach was empty for perfusion. The trachea was cannulated. An esophageal canula was passed into the esophagus via the mouth and tied firmly in place with a ligature around the esophagus in the neck region. The right femoral vein was also cannulated for the injection of drugs. An abdominal incision was made in the middle to expose part of the liver and small intestine. The pyloric end of the stomach was cannulated at its junction with the duodenum. A 0.9% NaCl solution w/v (saline) was flushed slowly via the esophageal canula to wash out the stomach contents. When the stomach was cleared of food particles and the perfusate was flowing freely, the abdominal incision was covered with moist cotton wool.

Experimental Procedure

The stomach was constantly perfused with saline (pH 7.0) at the rate of 1 ml/min and the perfusate samples collected at 10 min intervals. The perfusion rate was maintained by means of a roller pump (Watson and Marlow 5025). The volumes of the collected samples were then measured and then titrated against 0.01 N NaOH, using phenolphthalein as indicator, to determine total acidity.

Various doses of the crude extract (1–20 µg protein/100 g body wt, i.v.) were injected to determine the relationship between extract concentration and pentagastrin (0.6 mg/kg body wt, i.v.) stimulated secretion of gastric acid. From the results, the ED_{50} was estimated, and a test dose of 6 µg protein/100 g body wt was selected.

Histamine (0.2 mg/100 g body wt, i.v.) or pentagastrin (0.6 mg/kg body wt, i.v.) (both from Sigma, U.K.) was administered 50 min before and 50 min after injection of the crude extract.

In one group of animals, the effect of the drugs (histamine and pentagastrin) was studied after 8 h pretreatment with the extract (6 µg protein/100 g body wt, i.v.). The animals received the extract via the femoral vein under ether anaesthesia. The incisions were sutured and covered with flexible colloidion and the animals were left to recover. They were later prepared for gastric acid studies and the drug (histamine or pentagastrin) was injected 8 h after the administration of the extract. Drugs were injected in a volume of 0.2 ml followed by a washing injection of the same volume of saline.

Statistical Analysis

All data were expressed as mean ± SEM, and analysed by the Student's *t*-test. Regression lines were constructed from the linear portion of log concentration response curves. ED_{50} values of the extract were extrapolated from this curve.

RESULTS

Resting Gastric Acid Secretion

The pH of the saline perfusate (effluent) from the stomach, taken as the control value, was 2.6. Results obtained by titration showed that the mean basal gastric acid secretion was 3.92 ± 0.45 (mean ± standard error of mean) µ-equivalents [= wt (g) × 10^{-6} that reacts with 1 mole H^+] per 10 min. This value represented the total acidity.

Effect of Extract Concentration on Pentagastrin-induced Secretion of Gastric Acid

The crude extract (1–20 µg protein/100 g body wt, i.v.) dose-dependently decreased the peak levels of pentagastrin-induced secretion of gastric acid. The concentration-response relationship was apparently sigmoidal, and from this, the ED_{50} of 6.31 µg protein/100 g body wt was estimated (Fig. 1). The extract (1–20 µg protein/100 g body wt) produced about 9.6 ± 3.2 – 78.4 ± 2.4% (SEM, $n = 8$) inhibition of pentagastrin-induced secretion of gastric acid.

Effect of Histamine and Pentagastrin on Gastric Acid Secretion

After steady levels of gastric acid secretion were obtained for 50 min in the first group of 10 rats, histamine (0.2 mg/100 g body wt, i.v.) increased acid secretion from a mean control level of 3.92 ± 0.45 µ-equivalents per 10 min to a peak value of 9.70 ± 0.18 µ-equivalents per 10 min ($p < 0.001$), after 50 min perfusion (Fig. 2). However, after reaching this

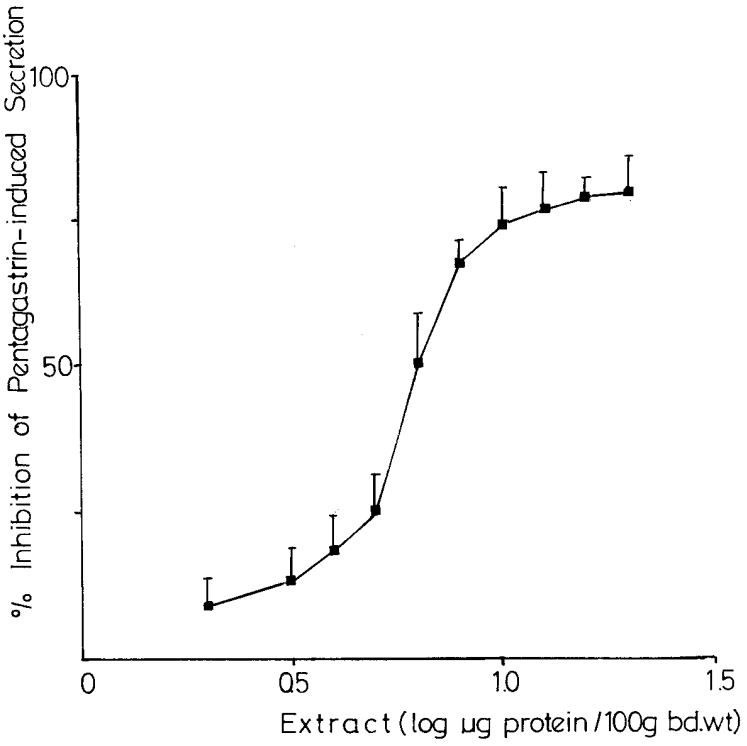

Fig. 1. Percentage inhibition of pentagastrin-induced secretion of gastric acid produced by the crude extract (1–20 µg protein/100 g body wt, i.v.). Each point represents the mean value ± SEM. $n = 10$.

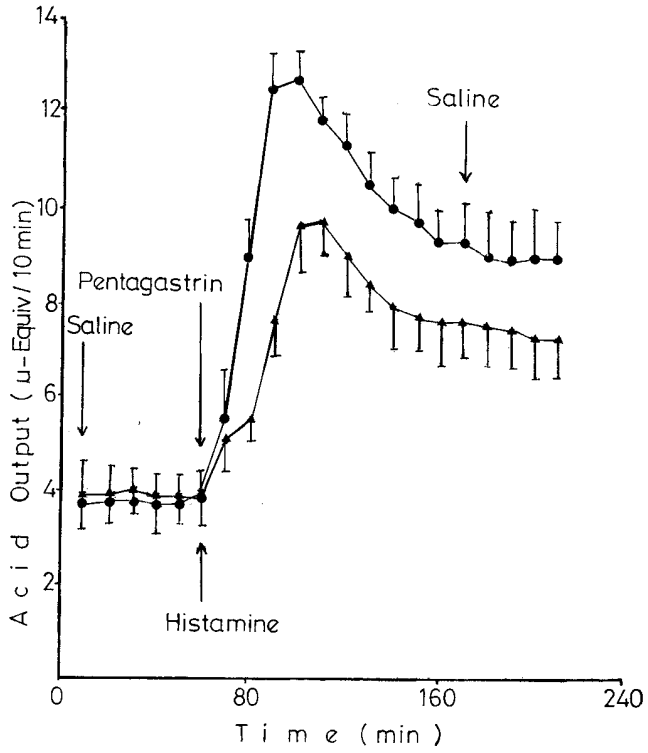

Fig. 2. Effect of histamine (0.2 mg/100 g body wt, i.v.) and pentagastrin (0.6 µg/kg body wt, i.v.) on gastric acid secretion. Values are the means ± SEM. $n = 10$.

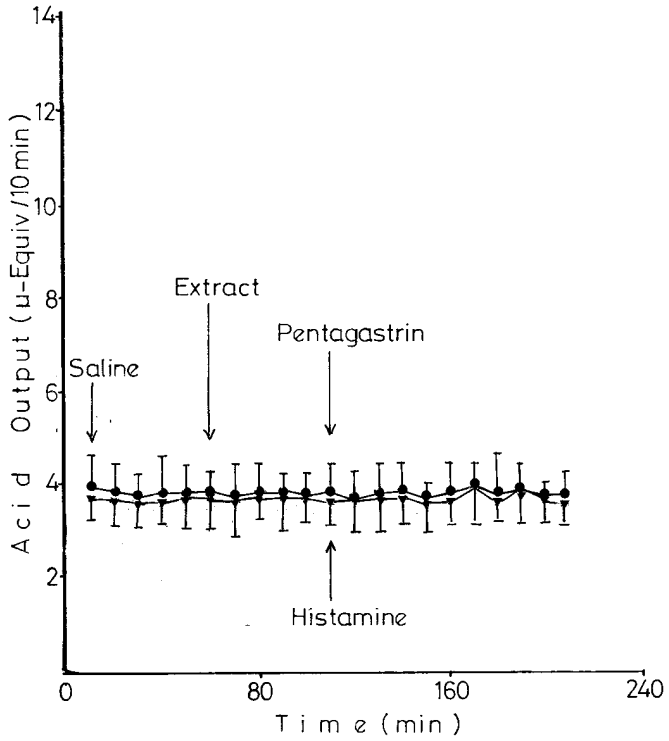

Fig. 3. Effect of administering the extract (6 µg protein/100 g body wt, i.v.) 50 min before histamine- or pentagastrin-stimulated acid secretion. Values are the means ± SEM. $n = 10$.

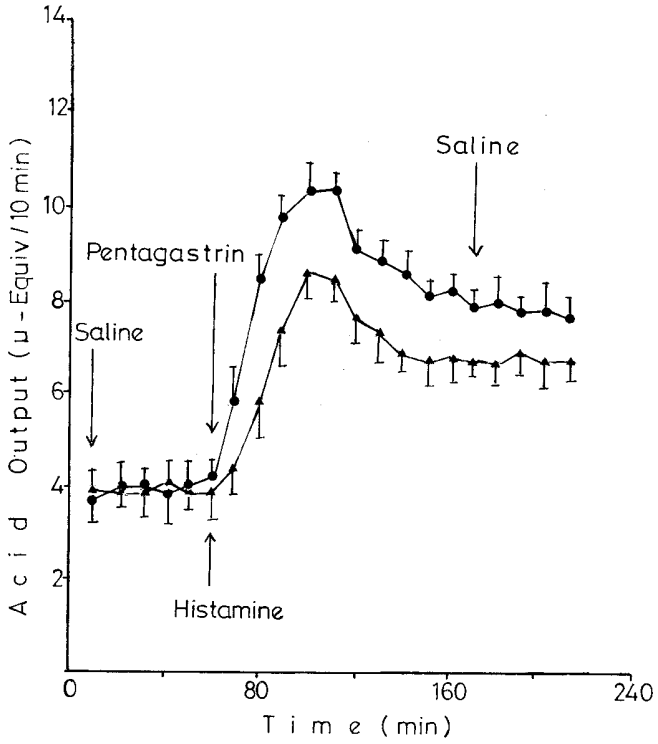

Fig. 4. Effect of administering the extract (6 µg protein/100 g body wt, i.v.) 8 h before histamine- or pentagastrin-stimulated acid secretion. Values are the means ± SEM. $n = 10$.

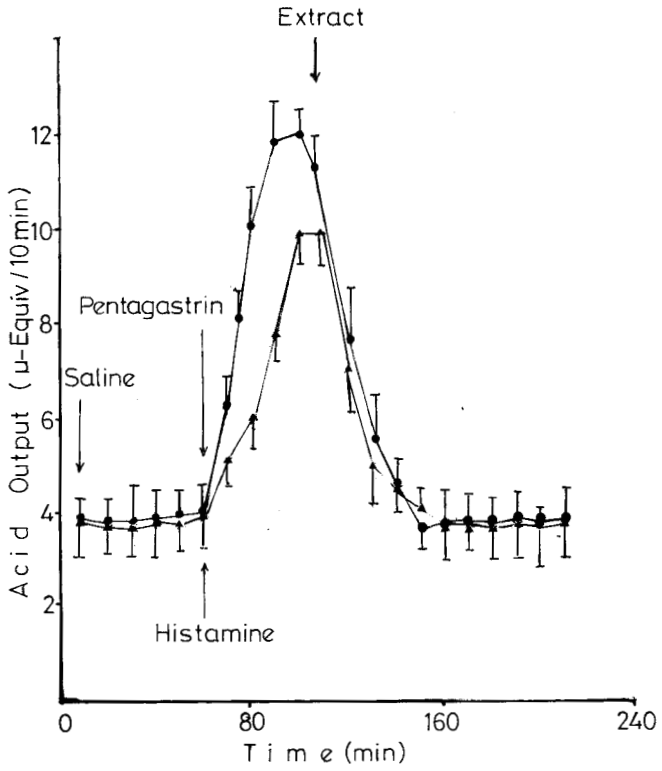

Fig. 5. Effect of administering the extract (6 μg protein/100 g body wt, i.v.) 50 min after histamine- or pentagastrin-stimulated acid secretion. Values are the means ± SEM. $n = 10$.

peak level, acid output progressively declined and reached a steady state level of about 7.51 ± 0.20 μ-equivalents per 10 min, which was still above the control level (Fig. 2).

Pentagastrin (0.6 μg/kg body wt, i.v.) was more potent than histamine in stimulating gastric acid secretion in another group of 10 rats. Pentagastrin (0.6 μg/kg body wt, i.v.) increased acid secretion from a mean control level of 3.84 ± 0.55 μ-equivalents per 10 min to a peak value of 12.8 ± 0.23 μ-equivalents per 10 min ($p < 0.001$), after the first 50 min of drug administration. As seen with histamine, there was a gradual decrease in acid output until a steady level of 9.14 ± 0.19 μ-equivalents per 10 min was reached (Fig. 2).

Effect of the Crude Extract from *Bunodosoma cavernata* on Histamine and Pentagastrin Stimulation of Gastric Acid Secretion

The effect of the extract on acid secretion 50 min before and after administration of either histamine or pentagastrin was tested. The animals were divided into three batches, consisting of two groups per batch. In the first batch, one group ($n = 8$ rats) received the extract (6 μg protein/100 g body wt, i.v.) 50 min before histamine (0.2 mg/100 g body wt, i.v.) and the other group ($n = 8$ rats) received the extract 50 min before pentagastrin (0.6 μg/kg body wt, i.v.). The mean basal acid outputs for the histamine and pentagastrin groups were 3.68 ± 0.45 and 3.80 ± 0.71 μ-equivalents per 10 min, respectively. From the results (Fig. 3), both histamine and pentagastrin failed to stimulate gastric acid secretion above the basal level following the administration of the extract 50 min earlier.

The same procedure described above was repeated for the groups in this second batch. The only difference was that animals in the batch received the extract 8 h before histamine or pentagastrin, and not 50 min before, as previously described. Results show that the mean basal acid output was about 3.87 ± 3.1 ($n = 10$), and pH = 3.4. Following the administration of histamine or pentagastrin, gastric acid secretion increased from the mean basal level to peak levels of 8.84 ± 1.8 and 10.64 ± 2.2 μ-equivalents per 10 min for the histamine and pentagastrin groups, respectively (Fig. 4). Comparing Figures 2 (considered as controls) and 4, administration of the crude extract 8 h before histamine

or pentagastrin produced only about 8.86 and 16.95% inhibition of gastric acid secretion stimulated by histamine and pentagastrin, respectively.

Figure 5 shows the results of administering the extract 50 min after the injection of histamine or pentagastrin. This third batch consisted of two groups of 10 rats per group. From the results, both histamine and pentagastrin progressively increased the acid output to reach peak values of 9.90 ± 0.21 and 11.99 ± 0.28 µ-equivalents per 10 min, respectively. Following the administration of the extract, there was a rapid drop towards basal levels in both histamine- and pentagastrin-induced secretion of gastric acid.

Also, it was evident from the results (Fig. 3) that the crude extract, at the given concentration, could not alter the basal acid output level, at least within the first 50 min of administration.

DISCUSSION

It is clear from these results that the crude *B. cavernata* extract dose-dependently inhibited stimulated release of gastric acid but failed to influence the basal acid output. That the extract had no action on the basal acid secretion could be either due to the relative insensitivity of the technique in terms of measuring low acid levels or to the basal secretory mechanism being different from that of stimulated secretion. The inhibition of both histamine- and pentagastrin-stimulated release of gastric acid probably indicates a receptor action of the extract.

A previous report that an extract from a sea anemone (*Tealia felina*) inhibited histamine-induced contractions of the guinea pig ileum but failed to prevent 5-HT- or KCl-evoked contractions (Aldeen et al., 1981) tends to suggest a receptor action of the animal extract. Substances given by intravenous administration which influence gastric secretion, either act on the atropine-sensitive muscarinic cholinoceptors or directly on the histamine H_2-receptors, and blockade of pentagastrin-stimulated release of gastric acid is thought to be due specifically to an action on the H_2-receptor subtype (Bowman & Rand, 1980).

In this study, although histopathological studies were not performed, generalized tissue toxicity could be ruled out, since basal flow was still maintained for at least 50 min after i.v. injection of the extract. Furthermore, although the crude extract, injected 50 min before histamine or pentagastrin administration (Fig. 3), abolished the actions of these drugs, it failed to be active when injected 8 h before the administration of the drugs (Fig. 4). A possible explanation for these effects could be that after 8 h, there was partial recovery from the effect of the extract. Secondly, the basal output pH of 3.4, recorded 8 h after the injection of the extract, probably indicates there was still spontaneous and continuous secretion of gastric acid by the parietal cells.

Therefore, it is unlikely that the crude extract from *B. cavernata* at the given concentration, produced an irreversible action in rats. This suggestion is also based on the results of our earlier findings that the extract, at a dose range of 1–8 µg protein/100 g body wt, i.v., had no effect on blood pressure, electrocardiogram and respiration (Eno, 1995). However, it is premature to speculate on the precise action of the extract in its crude form because it may contain more than one active compound. Nonetheless, we strongly suspect a receptor-based action of the active agent or agents contained in the extract.

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