Role of 5-hydroxytryptamine in intestinal water and electrolyte movement during gut anaphylaxis

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Abstract
Exposure of sensitised intestine to specific allergen is known to produce appreciable reduction in water and electrolyte absorption. The mediators participating in this process have not been fully characterised. The effects of the 5-hydroxytryptamine, (5-HT₃) and 5-HT, receptor antagonists, ketanserin and granisetron, respectively, on water movement during intestinal anaphylaxis were studied. Hooded Lister rats (120-150 g) were sensitised to ovalbumen and 14 days later, intestinal water and electrolyte movement was assessed at 10 minute intervals by in situ jejunal perfusion with a plasma electrolyte solution (PES) or PES containing 20 mg/l ovalbumen. Within 20 minutes of exposure to PES+ovalbumen, net water secretion that could be completely prevented by the mast cell stabilising agent doxantrazole occurred compared with absorption with PES alone (median -20 µl/min/g (interquartile range -43 to -5), n=11), v (107 (86 to 113), n=10; p<0.01). Pre-treatment with subcutaneous ketanserin 200 µg/kg (n=7) or granisetron 300 µg/kg (n=5) partially inhibited the secretory response to PES+ovalbumen (18 (11 to 48) and 13 (6 to 32) respectively; both p<0.01 compared with PES+ovalbumen control). After 40 minutes perfusion with PES+ovalbumen, the changes in water movement were less pronounced 24 (--3 to 43) and neither ketanserin or granisetron had any effect (ketanserin: 48 (28 to 87), granisetron: 41 (32 to 83); NS). In all experiments, sodium and chloride movement paralleled that of water. Thus, the profound water secretion that occurs in the early stages of intestinal anaphylaxis is partly 5-HT dependent because it can be reversed by 5-HT₂ and 5-HT₃ receptor antagonists. Other mediators must also be involved, especially in the late phase of anaphylaxis.

(Gut 1995; 36: 553–557)

Keywords: 5-hydroxytryptamine, gut anaphylaxis, intestinal perfusion, food allergy.

IgE mediated food allergy is estimated to affect 2–5% of the general population and up to 27% of children, most of whom report gastrointestinal symptoms including diarrhoea, abdominal pain, nausea, and vomiting. Studies in animal models of food allergy have shown that exposure of a previously sensitised intestinal mucosa to a specific allergen leads to mast cell degranulation and a considerable decrease in water and electrolyte absorption, so called intestinal anaphylaxis. The changes in water and electrolyte movement probably contribute to the symptoms of IgE mediated food allergy in humans. The mediators participating in this process, however, have not been fully characterised. 5-Hydroxytryptamine (5-HT) may be an important mediator in intestinal anaphylaxis for several reasons. 5-HT is present in mast cells and enteric neurons, and is known to be a potent intestinal secretagogue. In vitro studies using sensitised rat intestinal segments mounted in Ussing chambers have shown that 5-HT₂ receptor antagonists can decrease or completely abolish antigen induced changes in short circuit current. Similarly, Baird et al have shown in vitro the importance of 5-HT₃ receptors and enteric neurons in type 1 hypersensitivity reaction in guinea pig intestinal segments. However, in vivo studies of the effect of 5-HT receptor antagonists on water and electrolyte movement during intestinal anaphylaxis have not been reported.

The aim of our study was to assess in vivo the importance of 5-HT as a mediator of the physiological events occurring during IgE intestinal anaphylaxis by examining whether 5-HT₂ and 5-HT₃ receptor antagonists, ketanserin and granisetron respectively, can modulate the changes in water and electrolyte movement in an animal model of gut anaphylaxis.

Methods

Animal model
Male Hooded Lister rats (120-150 g body weight) were inoculated intraperitoneally with 10 µg ovalbumen with alum adjuvant as previously described. Fourteen days later, serum anti-ovalbumen IgE was measured by passive cutaneous anaphylaxis. Briefly, multiple dilutions (1:2 to 1:16) of serum (0-1 ml) were injected intradermally on the shaved back of male Wistar rats. Forty eight hours later, animals were given an intracardiac injection of 2-5 mg egg albumen in 0-5 ml of 1% Evans blue and skin reactions were read after 30 minutes. Titres were recorded as the greatest dilution of serum producing a colour reaction measuring 5 mm or more in diameter.

Intestinal perfusion
On day 14 and after 18 hours fast with free access to water, sensitised rats were anaesthetised with intraperitoneal injection of sodium pentobarbitone (60 mg/kg) and maintained throughout the experiment by...
interval intraperitoneal injections (15–30 mg/kg) as necessary. The abdomen was opened through a midline incision and a 15 cm segment of jejunum, starting 5 cm distal to the ligament of Treitz, was cannulated at proximal and distal ends. The isolated intestinal segment was gently flushed with warm isotonic saline and the intestine returned to the abdominal cavity and the abdomen closed. The intestinal segment was then perfused at a rate of 0.25 ml/min with either plasma electrolyte solution (PES) containing Na 140, K 4 Cl 104, HCO_3 40 mmol/l, and 4 μCi/l of [14C]-PEG 4000 (non-challenged group), or else with the same solution to which 20 mg/l of ovalbumen was added (challenged group). Twenty minutes were allowed to elapse to reach steady state before five consecutive 10 minutes collections of the effluent were obtained from the distal cannula. Animals were maintained at 37°C using a heat pad and an overhead lamp. At the end of the experiments the rats were killed by an overdose of pentobarbitone and the perfused intestinal segment was removed, rinsed, blotted, and desiccated in an oven at 100°C to obtain dry weight. The samples of effluent were analysed immediately or kept frozen at −20°C and analysed within two weeks.

### Drug administration
At the time of anaesthetic administration, 90 minutes before intestinal perfusion, the animals were injected subcutaneously with either (a) ketanserin (200 μg/kg), (b) granisetron (300 μg/kg), (c) the same doses of ketanserin and granisetron in combination or (d) saline as control. In a further group of sensitised animals the mucosal mast cell stabiliser, doxantrazole in a dose of 30 mg/kg subcutaneously was given 30 minutes before perfusion.

### Analytical methods
[14C]-PEG concentrations in the effluent were measured in triplicate by liquid scintillation spectroscopy in KB Wallac Ultra-beta 1210 scintillation counter. Sodium and potassium concentrations were determined using flame photometer (Instrument Laboratories 943), and chloride concentrations by Chemlab (CCMI chloridimeter). The net water and solute movement was calculated and expressed respectively in μg/min and μmol/min/g of dry intestinal weight. Positive values denote net absorption and negative values net secretion.

### Materials
Chicken egg albumen (grade V) was obtained from Sigma Chemical Company. The 5-HT_2 antagonist ketanserin was supplied by Janssen, Belgium; and the 5-HT_3 antagonist granisetron from SmithKline Beecham, UK. Doxantrazole was obtained from Wellcome, UK. Radio-labelled polyethylene glycol ([14C]-PEG 4000) was obtained from Amerham International and all other chemicals were supplied by British Drug House (BDH Chemicals).

### Statistics
Results are expressed as median and interquartile range and Wilcoxon rank sum test was used for statistical analysis.

### Results
All rats developed a specific anti-ovalbumen IgE titre of >1:8 14 days after initial inoculation of egg albumen and adjuvant.
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Intestinal segments perfused with PES alone showed constant water absorption during the entire experimental period (Fig 1A) with less than 10% variation between the consecutive 10 minute intervals. Continuous exposure of the intestine to ovalbumen resulted in profound water secretion after 20–30 minutes (median 20 μl/min/g, interquartile range 43 to −5), n=11) compared with control in which there was net water absorption (107 (86 to 113), n=10; p<0.01) (Fig 1A). Thirty minutes after the ovalbumen exposure, the secretory state ceased, but there was still a noticeable decrease in water absorption compared with controls perfused with PES alone. This reduction in water absorption persisted throughout the 70 minutes experimental period. Sodium and chloride movement paralleled that of water, with net sodium and chloride secretion occurring after 20 minutes of ovalbumen challenge (Figs 1B and 1C) with progressive recovery during 70 minutes of experimental period.

These changes in water and electrolyte movement were shown to be dependent on mast cell degranulation as they were completely abolished by the mast cell stabiliser, doxantrazole (Fig 1).

Neither ketanserin nor granisetron had any effect on water and electrolyte absorption in normal, non-challenged rats (Figs 1, 2, 3). In the challenged group, however, both ketanserin (200 μg/kg) and granisetron (300 μg/kg) reversed the water secretion seen at 20–30 minutes to absorption (ketanserin: 18 (11–48) μl/min/g, n=7; and granisetron: 13 (6–32); n=8; both p<0.01 compared with challenged animals) and significantly improved water absorption during the 30–40 minute period after challenge (Figs 2A and 3A). During these two periods (20–30 and 30–40 minutes), water absorption was still significantly less than that in normal, non-challenged controls. After 40 minutes of challenge, 5-HT2 and 5-HT3 antagonism had no effect on water absorption, which still remained less than in normal controls. Sodium and chloride movement was similarly improved by prior treatment with ketanserin (Figs 2B and 2C) and granisetron (Figs 3B and 3C). Giving a combination of ketanserin and granisetron did not show any additive effect over each one alone in reversing water (Fig 4), sodium or chloride (data not shown) secretion.

**Discussion**

Our results show that a severe cholera like water secretion occurs within 20 minutes of exposure of sensitised intestine to allergen. This secretory phase is followed by a period of appreciable decrease in water absorption lasting for at least 30 minutes. The secretion during the early stage can be partially inhibited by 5-HT2 and 5-HT3 antagonism tool no effect on water absorption, which still remained less than in normal controls. Sodium and chloride movement was similarly improved by prior treatment with ketanserin (Figs 2B and 2C) and granisetron (Figs 3B and 3C). Giving a combination of ketanserin and granisetron did not show any additive effect over each one alone in reversing water (Fig 4), sodium or chloride (data not shown) secretion.

Perdue et al. have shown in vivo that a decrease in water and electrolyte absorption but not net secretion occurs during exposure of presensitised intestine to ovalbumen and these findings have been confirmed in in vitro studies. These changes in water and
The effect of 5-HT and 5-HT antagonists have also been studied in vitro. Ketanserin and cinanserin (5-HT2 antagonists) were found respectively to decrease or abolish the chloride response to antigen in sensitised gut. The 5-HT3 antagonist, ICS 205–930 also decreased chloride response to antigen in sensitised intestine to β-lactoglobulin. These experiments are the first to evaluate the effects of 5-HT receptor antagonists during anaphylaxis in vivo. It has previously been shown that 5-HT induces water secretion in rat jejunum when given intravenously, a process that can be attenuated or reversed by 5-HT3 or 5-HT3 receptor antagonists. Beubler et al showed a complete inhibition of 5-HT induced intestinal secretion by subcutaneous injection of ketanserin or granisetron in doses similar to those used in our study. Our data suggest that 5-HT plays a part early in the anaphylactic reaction but not in the later stages. A possible explanation would be that mast cell degranulation leads to release of performed mediators such as 5-HT stored in the secretory granules, which act early to promote the secretory process. In addition, mast cell degranulation leads to de novo generation of lipid metabolites from cell membranes, which results in synthesis of prostaglandins, leukotrienes, and platelet activating factor, which could also act as secretagogues in the late phase. This notion is supported by in vitro findings of a biphasic rise and fall (phase I and II) in short circuit current during exposure of a sensitised intestine to antigen. Castro et al have found that the phase I is mimicked by exogenous 5-HT and blocked by 5-HT receptor antagonists; and phase II is mimicked by exogenous prostaglandin E2 and blocked by an inhibitor of prostaglandin synthesis. In addition, Catto-Smith et al have found that indomethacin inhibited the phase I response by 56% and completely abolished phase II, implying that prostaglandins play a part in phase I but are more important in phase II. Our results are in accordance with their findings and the water secretion seen during the early phase of anaphylaxis could be mediated by a combination of 5-HT and other mediators like prostaglandins leading to changes in the activity of multiple ion transporters, only some of which are blocked by 5-HT receptor antagonists; whereas the late changes are not 5-HT dependent.

We have found that both ketanserin and granisetron have the same effect on water movement implying that 5-HT acts through both type 2 and type 3 receptors to produce its secretory action. The present experiments,
however, do not provide an explanation for the fact that a combination of ketanserin and granisetron at the doses used did not provide any additional benefit over each one alone.

In conclusion, our findings show that 5-HT plays a part in water movement changes during early phase of intestinal anaphylaxis in vivo as it is partially blocked by 5-HT\textsubscript{3} and 5-HT\textsubscript{3} antagonists, but it is highly likely that other mediators play a part in both the early and late phases of the anaphylactic reaction.

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Gut 1995 36: 553-557
doi: 10.1136/gut.36.4.553

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