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

F H Mourad, L J D O'Donnell, E Ogutu, J A Dias, M J G Farthing

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-HT₂) and 5-HT₃ 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=8) 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,¹ most of whom report gastrointestinal symptoms including diarrhoea, abdominal pain, nausea, and vomiting.^{2,3} 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 degranulation⁴ and a considerable

decrease in water and electrolyte absorption,⁵ 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.⁶ In vitro studies using sensitised rat intestinal segments mounted in Ussing chambers have shown that 5-HT₂ receptor antagonists can decrease⁷ or completely abolish⁸ antigen induced changes in short circuit current. Similarly, Baird *et al*⁹ 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.¹⁰ Fourteen days later, serum anti-ovalbumen IgE was measured by passive cutaneous anaphylaxis.⁵ 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

Department of
Gastroenterology,
St Bartholomew's
Hospital, London
F H Mourad
L J D O'Donnell
E Ogutu
J A Dias
M J G Farthing

Correspondence to:
Professor M Farthing,
Department of
Gastroenterology,
St Bartholomew's Hospital,
London EC1A 7BE.

Accepted for publication
8 August 1994

Figure 1: Water (A), sodium (B), and chloride (C) movement in control (PES, $n=10$) and in challenged rats during gut anaphylaxis (PES+ovalbumen, $n=11$) and the effect of doxantrazole (PES+OVA+Dox, $n=8$). Time (min): time after the start of perfusion; PES plasma electrolyte solution, OVA: ovalbumen. * $p<0.01$, † $p<0.05$, ‡NS compared with PES. § $p<0.05$ compared with 30–40 minute time period. ‡ median, IQR.

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 $\mu\text{Ci/l}$ of [^{14}C]-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 $\mu\text{g/kg}$), (b) granisetron (300 $\mu\text{g/kg}$), (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

[^{14}C]-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 $\mu\text{g/min/g}$ and $\mu\text{mol/min/g}$ 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-HT₂ antagonist ketanserin was supplied by Janssen, Belgium; and the 5-HT₃ antagonist granisetron from SmithKline Beecham, UK. Doxantrazole was obtained from Wellcome, UK. Radio-labelled polyethylene glycol ([^{14}C]-PEG 4000) was obtained from Amersham 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.

Figure 2: Effect of ketanserin on water (A), sodium (B), and chloride (C) movement in control (PES+Ket, $n=10$) and in challenged rats during gut anaphylaxis (PES+OVA+Ket, $n=7$). Time (min): time after the start of perfusion. All p values compared with PES+OVA.

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 $\mu\text{l}/\text{min}/\text{g}$ (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 $\mu\text{g}/\text{kg}$) and granisetron (300 $\mu\text{g}/\text{kg}$) reversed the water secretion seen at 20–30 minutes to absorption (ketanserin: 18 (11–48) $\mu\text{l}/\text{min}/\text{g}$, $n=7$; 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 antagonists suggesting a role for 5-HT as a mediator during this stage. The decrease in water absorption occurring later in anaphylaxis is unlikely to be mediated by 5-HT₂ or 5-HT₃ receptors, as it is not affected by antagonists to these receptors. These changes in water movement during the entire period of intestinal anaphylaxis are dependent on mediators derived from mast cells as they were completely prevented by pre-treatment with the mast cell stabilising agent, doxantrazole.

Perdue *et al*⁵ 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.^{9–11} These changes in water and

Figure 3: Effect of granisetron on water (A), sodium (B), and chloride (C) movement in control (PES+Gra, $n=9$) and in challenged rats during gut anaphylaxis (PES+OVA+Gra, $n=8$). Time (min): time after the start of perfusion. All p values compared with PES+OVA.

electrolyte movement are thought to represent a host defence mechanism to facilitate expulsion of unwanted antigens and parasites by a process involving release of mast cell mediators.¹² The occurrence of severe water secretion in the present experiments may be due to the use of a higher antigen load in the perfusate compared with that used by Perdue *et al*⁵ or to collection of effluent at more frequent intervals; this permitted us to detect changes occurring over very short periods of time especially as the secretion was short lived and

could have been easily missed by less frequent collections.

The mediators responsible for the physiological changes during IgE mediated anaphylaxis still remain to be fully characterised. Histamine, being abundant in mast cells was thought to be an important mediator. In vitro studies using histamine antagonists have produced conflicting results, however, and failed to provide clear evidence that histamine acts as a secretagogue during gut anaphylaxis.^{7 8 11 13} 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^{7 9} or abolish⁸ 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.⁹ 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-HT₂ or 5-HT₃ receptor antagonists.¹⁴ Beubler *et al*¹⁵ 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 preformed 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.¹⁶⁻¹⁹ This notion is supported by the in vitro finding of a biphasic rise and fall (phase I and II) in short circuit current during exposure of a sensitised intestine to antigen.^{8 11} Castro *et al*¹¹ 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*⁸ 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 antagonism; 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,

Figure 4: Effect of a combination of ketanserin and granisetron on water movement during gut anaphylaxis compared with either ketanserin ($n=7$) or granisetron ($n=8$) alone. Time (min): time after the start of perfusion. * NS compared with either ketanserin or granisetron alone. $\bar{x}$ median, IQR.

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-HT₂ and 5-HT₃ antagonists, but it is highly likely that other mediators play a part in both the early and late phases of the anaphylactic reaction.

- 1 Kajosaari M. Food allergy in Finnish children aged 1 to 6 years. *Acta Paediatr Scand* 1982; 71: 815-9.
- 2 Lessof MH, Wraith DG, Merret TG, Merret J, Buisseret PD. Food allergy and intolerance in 100 patients - local systemic effects. *Q J Med* 1980; 195: 259-71.
- 3 Allen DH, van Nunen S, Loblay R, Clarke L, Swain A. Adverse reactions to foods. *Med J Aust* 1984; 141 (suppl 5): S37-42.
- 4 Patrick MK, Dunn IJ, Buret A, Miller HRP, Huntley JF, Gibson S, et al. Mast cell protease release and mucosal

- ultrastructure during intestinal anaphylaxis in the rat. *Gastroenterology* 1988; 94: 1-9.
- 5 Perdue MH, Chung M, Gall DG. Effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984; 86: 391-7.
- 6 Jaffe BM, Ferrara A, Sherlock DJ. Comparative effects of ketanserin, atropine and methysergide on the gastrointestinal effects of hyperserotoninemia in the awake dog. *J Pharmacol Exp Ther* 1986; 238: 536-41.
- 7 Crowe SC, Sestini P, Perdue MH. Allergic reaction of rat jejunal mucosa. Ion transport responses to luminal antigen and inflammatory mediators. *Gastroenterology* 1990; 99: 74-82.
- 8 Catto-Smith AG, Patrick MK, Hardin JA, Gall DG. Intestinal anaphylaxis in the rat; mediators responsible for the ion transport abnormalities. *Agents and Actions* 1988; 28: 186-91.
- 9 Baird AW, Cuthbert AW. Neuronal involvement in type 1 hypersensitivity reaction in gut epithelia. *Br J Pharmacol* 1987; 92: 647-55.
- 10 Perdue MH, Gall DG. Intestinal anaphylaxis in the rat, jejunal response to in vitro challenge. *Am J Physiol* 1986; 250: G427-31.
- 11 Castro GA, Harari Y, Russell D. Mediators of anaphylaxis-induced ion transport changes in small intestine. *Am J Physiol* 1987; 253: G540-8.
- 12 Miller HRF, Woodbury RG, Huntley JK, Newlands G. Systemic release of mucosal mast cell protease in primed rats challenged with *Nippostrongylus brasiliensis*. *Immunology* 1983; 49: 471-9.
- 13 Perdue MH, Gall DG. Rat jejunal mucosa responses to histamine and antihistamines in vitro. Comparison with antigen-induced changes during intestinal anaphylaxis. *Agents Actions* 1986; 19: 5-9.
- 14 Beubler E, Horina G. 5-HT₂ and 5-HT₃ receptor subtypes mediate cholera toxin-induced intestinal fluid secretion in the rat. *Gastroenterology* 1990; 99: 83-9.
- 15 Beubler E, Shirgi-Degen A, Gamse R. Inhibition of 5-hydroxytryptamine and enterotoxin-induced fluid secretion by 5-HT receptor antagonists in the rat jejunum. *Eur J Pharmacol* 1993; 248: 157-62.
- 16 Serafin WE, Austen KF. Mediators of immediate hypersensitivity reactions. *N Engl J Med* 1987; 317: 30-4.
- 17 Barret KE, Metcalfe DD. Mucosal mast cells and IgE. In: Heyworth MF, Jones AL, ed. *Immunology of the gastrointestinal tract and liver*. New York: Raven, 1988; 65-91.
- 18 Donowitz M, Welsh MJ. Regulation of mammalian small intestinal electrolyte secretion. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. New York: Raven, 1987: 1351-88.
- 19 Hanglow AC, Bienenstock J, Perdue MJ. Effect of platelet-activating factor on ion transport in isolated rat jejunum. *Am J Physiol* 1989; 257: G845-50.