Br. J. Pharmac (1987), **92**, 647–655

Neuronal involvement in type 1 hypersensitivity reactions in gut epithelia

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1 Using a number of 5-hydroxytryptamine (5-HT)-antagonists we have compared their activity against the chloride-secretory response in guinea-pig ileal and colonic epithelia when challenged with 5-HT or antigen. Guinea-pigs sensitized to β-lactoglobulin (βLG) were used throughout; these were obtained by providing animals with cows' milk for drinking.

2 Of methysergide, ketanserin, cyproheptadine and ICS 205-930, only the latter inhibited both the response to 5-HT and to antigen challenge. Methysergide caused a minor, significant effect on 5-HT but not on βLG responses. Ketanserin had no effect on the responses to 5-HT, but both ketanserin and cyproheptadine inhibited the challenge to βLG.

3 The data are considered in relation to the current views of receptor subtypes for 5-HT. Some of the reported inhibitors may be non-specific, while we consider there is evidence to support the view that 5-HT_3-receptors (neuronal receptors) are involved both in the responses to 5-HT and to antigen challenge.

4 Tetrodotoxin mimicked the effect of ICS 205-930 on both the response to 5-HT and to antigen challenge in sensitized tissues, confirming a neuronal involvement for both types of stimuli.

Introduction

5-Hydroxytryptamine (5-HT) occurs throughout the gut in enterochromaffin cells (Erspamer, 1954), mast cells (Lewis, 1958) and neurones (Furness & Costa, 1982; Costa *et al.*, 1982). Intestinal ion transport can be influenced by exogenously applied 5-HT in a number of species (Donowitz *et al.*, 1980; Hardcastle *et al.*, 1981; Zimmerman & Binder, 1984). In guinea-pig ileal epithelium 5-HT produces chloride secretion by an action on enteric neurones (Cooke & Carey, 1985).

In a number of ealier papers (Cuthbert *et al.*, 1983; Baird *et al.*, 1984; 1985) we have shown that gut epithelia sensitized to various antigens, demonstrate type 1 hypersensitivity reactions upon challenge. In particular there is a large, inappropriate electrogenic secretion of chloride ions and an inhibition of electroneutral sodium chloride absorption, associated with fluid secretion towards the luminal side of the epithelium. We have also presented data (Baird *et al.*, 1987) to show that tetrodotoxin (TTX) can inhibit hypersensitivity reactions of this type.

In this paper we examine the role of neuronal elements in the lamina propria in relation to hypersensitivity reactions. Specifically, we examine whether 5-HT is involved in the response to antigen challenge in the ileal epithelium of guinea-pigs sensitized to β-lactoglobulin. To do this we have made use of a number of antagonists of 5-HT including methysergide, ketanserin, cyproheptadine and a new antagonist, ICS 205-930, which antagonizes the actions of 5-HT at M (Neuronal, 5-HT_3)-receptors (Richardson *et al.*, 1985). Furthermore TTX has been used to establish neuronal involvement in both the responses to 5-HT and to antigen.

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Methods

All experiments were carried out upon epithelial tissues taken from guinea-pigs that had been fed cows' milk. Animals, of the Dunkin-Hartley strain, were fed a normal diet but were given cows' milk to drink for a period of three weeks. After this time they were returned to water for drinking for at least 3 days before being used.

Epithelial preparations were dissected from terminal ileum and the colon. These were mounted in Ussing chambers (window area 0.6 cm^2) and prepared

for short circuit (SCC) recording by methods described in full detail elsewhere (Cuthbert *et al.*, 1983; Baird *et al.*, 1984).

To determine what effect various antagonists have on the responses to 5-HT or to challenge with β-lactoglobulin (βLG) we have used paired preparations, i.e. responses in the control preparations were compared to those in the presence of antagonist. In some instances, when more than one concentration of antagonist was investigated, we took as many as four preparations from each animal, one to be used as control plus three test preparations, each exposed to a different antagonist concentration. Antagonists were added to the bathing solution on both sides of the preparation and allowed to equilibrate before the tissues were challenged with either 5-HT or βLG. These two agonists were added only to the basolateral bathing fluid unless otherwise stated.

Responses of epithelia to 5-HT were maintained and were measured as SCC in μA. Responses to βLG were slower in onset, sometimes phasic and decayed, presumably as the released autocoids were dispersed or metabolised. By integrating the area under the response curve (with a planimeter) responses were measured in units of current × time, i.e. charge. They were converted to μEq using the Faraday relationship.

Analysis of the data was made, in the first instance, using an unpaired Student's *t* test. If significance was not achieved, less rigorous tests such as a paired *t* test or rank order methods were used. Unless otherwise stated, *P* values refer to those from unpaired *t* tests. Throughout tissues were bathed in Krebs-Henseleit solution (KHS) which had a pH of 7.4 when gassed with 95% O_2 and 5% CO_2 at 37°C. Its composition was (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4, 1.2, $NaHCO_3$ 24.8 and glucose 11.1.

All drugs were purchased from conventional sources except ICS 205-930 ([3α-tropanyl]-1H-indole-3-carboxylic acid ester) which was a generous gift from Dr B. Richardson of Sandoz AG, Basle.

Results

5-HT-induced increase in SCC in ileal epithelium: effects of antagonists

Isolated ileal epithelium has a high basal SCC, which in our present experiments was 101.3 ± 3.2 μA 0.6 cm^{-2} ($n = 46$). 5-HT increased the SCC further, but only when added to the basolateral side of the tissue. At low concentrations the SCC response was maintained, making it practical to obtain concentration-response relationships in a cumulative manner. At very high concentrations the SCC was not maintained and this may have been due to receptor desensitization. At intermediate concentrations there was commonly an initial SCC spike followed by a maintained plateau. These features are illustrated in Figure 1.

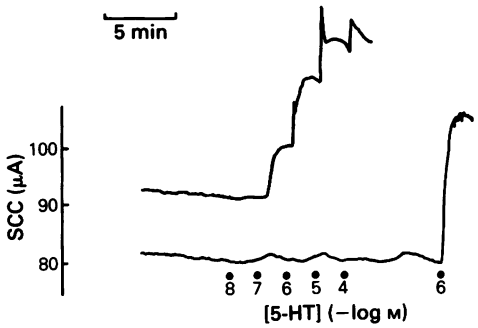

Figure 1 Typical responses to 5-hydroxytryptamine (5-HT) in ileal preparations. In the experiment represented by the upper trace, 5-HT was added to the basolateral side of the preparation. The lower trace represents a preparation in which 5-HT was added to the apical side on all occasions except the last.

We have examined the effects of ketanserin, methysergide and ICS 205-930 on the concentration-response relationship to 5-HT, measured as outlined in the methods. All antagonists were added 30 min before testing with 5-HT in order that their effects, if any, on ileal SCC could be monitored.

Methysergide (10^{-8}–10^{-7}M) treatment did not affect basal SCC. When compared by two way analysis of variance the curve obtained in the presence of 10^{-8}M methysergide was significantly different from the control curve ($P < 0.05$; $F = 9.164$). Increasing the concentration of the antagonist did not produce greater antagonism. Notice that, unlike ketanserin, methysergide caused a bigger rightward shift at the lower concentrations of 5-HT (Figure 2a).

Ketanserin (10^{-6}–10^{-8}M) had no effect on basal SCC and caused only a minor shift of the concentration-response curve to 5-HT to the right with depression of the maximal responses. However, by use of two-way analysis of variance the curves obtained in the presence of the antagonist did not differ significantly from the control curve (Figure 2b). The effects of 5-HT at the highest agonist concentrations used were not significantly reduced by ketanserin.

ICS 205-930, 10^{-8}–10^{-7}M, had no effect on the basal SCC in the ileum, while at 10^{-6}M it caused a transient increase of SCC (38.0 ± 4.1 μA 0.6 cm^{-2}, $n = 4$) which had disappeared by the time the effects of 5-HT were examined. Two way analysis of variance indicated that ICS 205-905 (10^{-6}M) caused a significant change in the concentration-response curve compared to control ($P < 0.01$, $F = 380.14$). The antagonist caused a rightward, non-parallel shift in the response curve at 10^{-7}M, but at 10^{-8}M control and test curves were superimposable (Figure 2c).

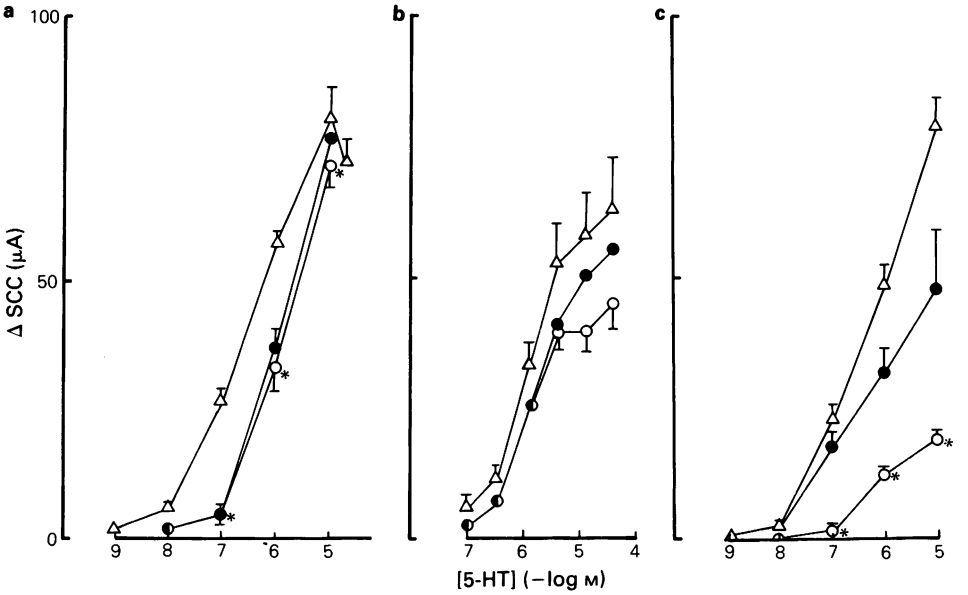

Figure 2 Concentration-response curves to 5-hydroxytryptamine (5-HT) and the effects of antagonists. Antagonists used were in (a) methysergide 10 nM (○) and 100 nM (●), in (b) ketanserin 100 nM (○) and 1 μM (●) and in (c) ICS-205-930 100 nM (●) and 1 μM (○). Means are shown for $n = 6$ (a and b) or $n = 4$ (c); s.e. shown by vertical lines.

Interaction of 5-HT with TTX

Experiments were carried out with TTX using the same protocols as were used with the 5-HT antagonists. In this series the basal SCC, 30 min before addition of 5-HT was 113.0 ± 5.0 μA 0.6 cm^{-2} ($n = 21$). TTX added to the preparations caused a rapid fall in SCC of 29.0 ± 5.0 μA 0.6 cm^{-2} (10^{-8} M TTX, $n = 6$) and 29.0 ± 7.0 μA 0.6 cm^{-2} (10^{-7} M TTX, $n = 6$) compared to a fall of only 6.0 ± 1.0 μA 0.6 cm^{-2} over the same period in the control group ($n = 9$).

TTX (10^{-7} M) significantly attenuated the 5-HT-induced increases in SCC, the concentration-response curve being significantly different from the control by two-way analysis of variance ($P < 0.01$; $F = 52.3$) (Figure 3).

Inhibition of type 1 hypersensitivity reactions to βLG by ketanserin, methysergide and cyproheptadine

In this series of experiments, measurements in control preparations were compared to those in paired tissues exposed to high antagonist concentrations for 5 min. This allowed a comparison of the basal SCC, the effect of the antagonist on the basal current, the attenuation or otherwise of the response to challenge with βLG and finally, after the βLG response had subsided, the effect upon exogenous 5-HT addition.

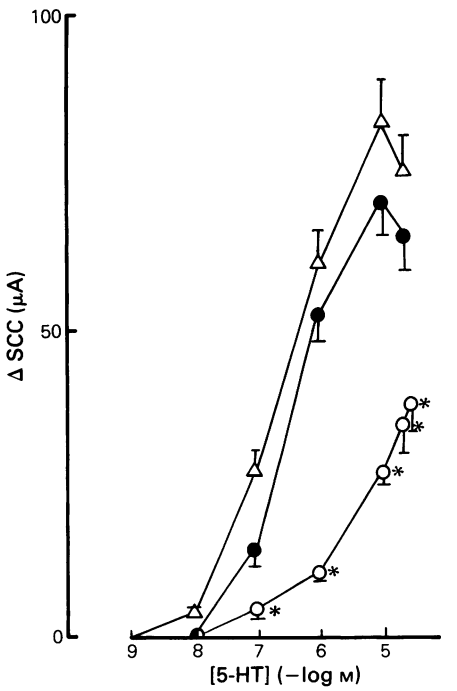

Figure 3 Effect of tetrodotoxin (TTX) on concentration response curves for 5-hydroxytryptamine (5-HT): control values (Δ); TTX, 10 nM (●) and 100 nM (○). Means for 6 values are given; s.e. shown by vertical lines. Significant difference indicated by asterisks.

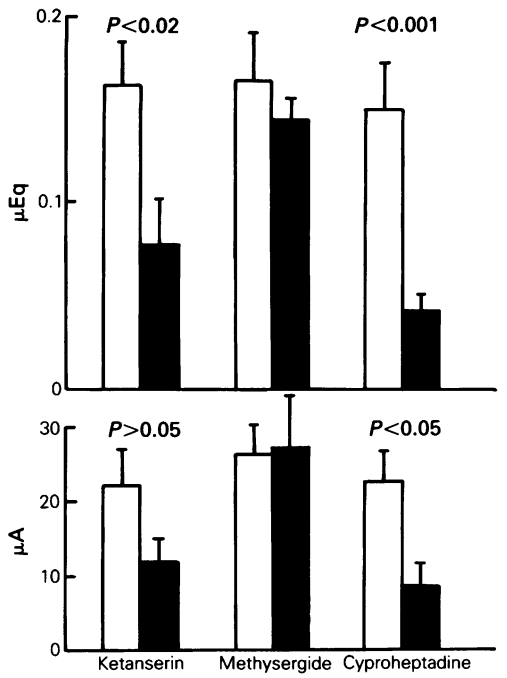

Figure 4 Effect of ketanserin, methyserigde and cyproheptadine (all at 5 μM) on response to challenge with β-lactoglobulin (βLG, 0.55 μM) expressed in μEq (top) and to subsequent challenge with 5-hydroxytryptamine (5-HT) (0.5 μM except with methysergide when 1.25 μM was used) expressed in μA. Open columns show control responses while closed columns show responses in the presence of one antagonist. Mean values are given for *n* values ranging from 4–12; s.e. shown by vertical lines. Significant differences are shown.

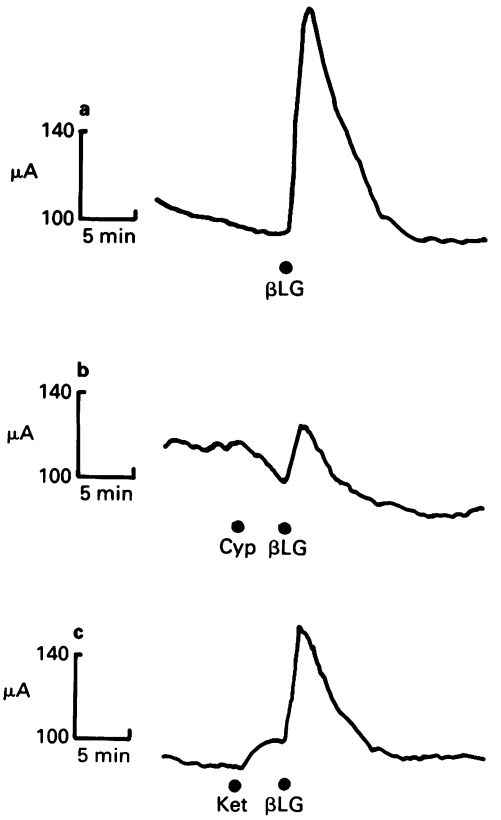

Figure 5 Typical responses of three ileal preparations all from the same animal to β-lactoglobulin (βLG, 0.55 μM). (a) Represents the control, (b) and (c) tracings show results in the presence of cyproheptadine (Cyp) and ketanserin (Ket), respectively, both at 5 μM.

In experiments with ketanserin (5 μM) there was no difference in basal SCC values between the control (100 ± 8.0 μA $0.6\,cm^{-2}$, $n = 7$) and test preparations (104 ± 12.0 μA $0.6\,cm^{-2}$, $n = 12$) before drugs were added. The antagonist caused an increase in SCC of 26.6 ± 3.9 μA $0.6\,cm^{-2}$, ($n = 12$) during the pretreatment, compared to a slight fall in the untreated control group (-4.2 ± 1.7 μA $0.6\,cm^{-2}$, $n = 7$). These values were significantly different ($P < 0.001$). There was a significant reduction in the response to βLG while the response to 5-HT, after the βLG response had subsided, was not significantly reduced (Figure 4).

With methysergide (5 μM) there was a small (-5.0 ± 2.6 μA $0.6\,cm^{-2}$) insignificant effect on SCC upon addition of the antagonist. The basal SCC values were 101.2 ± 15.2 μA $0.6\,cm^{-2}$ in controls ($n = 5$) and 99.6 ± 17.4 μA $0.6\,cm^{-2}$ ($n = 5$) in the corresponding test preparations after addition of the methysergide. Neither the response to βLG, or the subsequent responses to 5-HT were affected by methysergide (Figure 4).

In this series the effects of cyproheptadine (5 μM) were also examined. Control and test preparations had similar SCCs before any drugs were added (104 ± 10 μA $0.6\,cm^{-2}$, $n = 14$ versus 111 ± 8 μA $0.6\,cm^{-2}$, $n = 8$). Cyproheptadine caused a fall in SCC of 20.5 ± 2.7 μA $0.6\,cm^{-2}$ ($n = 8$) compared to a reduction of only 2.8 ± 1.9 μA $0.6\,cm^{-2}$ in controls. These values were significantly different ($P < 0.001$).

The response to challenge with βLG was significantly reduced by cyproheptadine (Figure 4) as was the response to exogenously added 5-HT.

Typical responses from three adjacent preparations taken from the same animal are given in Figure 5, in which some of the features described above are illustrated.

Table 1 Short circuit (SCC) values (μA 0.6 cm^{-2}) in sensitized ileum and colon preparations of guinea-pig, before and after addition of ICS 205-930

	Ileum		*Colon*	
[ICS 205.930]	*Basal*	*+ Antagonist*	*Basal*	*+ Antagonist*
0.0	70.6 ± 5.0	4.6 ± 2.2	91.2 ± 21.9	− 0.6 ± 1.4
0.01 μM	93.4 ± 9.0	− 2.8 ± 2.3	85.6 ± 25.3	− 1.4 ± 1.6
0.1 μM	78.0 ± 6.7	− 1.4 ± 2.9	78.2 ± 24.8	1.2 ± 0.4
1.0 μM	72.6 ± 7.3	− 4.6 ± 5.1	76.2 ± 14.0	2.8 ± 0.7

All values for $n = 5$.

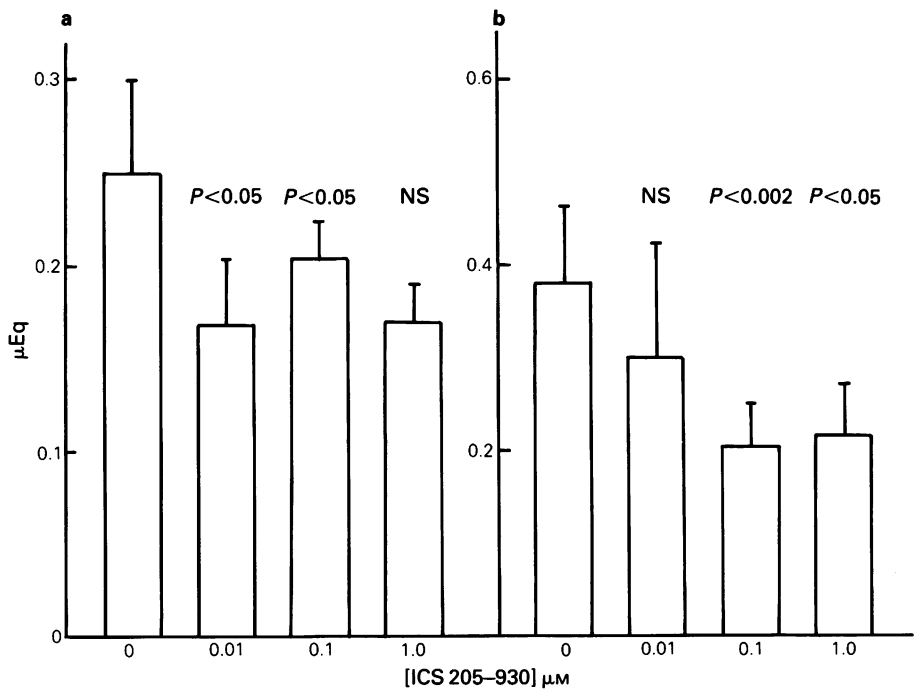

Figure 6 Responses of ileal (a) and colonic (b) preparations to challenge with β-lactoglobulin (βLG, 0.55 μM) in the presence and absence of ICS 205-930 at three different concentrations. Values given are means for five preparations; s.e. shown by vertical lines. With ileal preparations and 0.1 μM ICS 205-930 a sign test was used to show significance, while *t* tests were used for the others. The *t* test was paired in the case of 0.01 μM ICS 205-930 in ileal preparations.

Antagonism of βLG by ICS 205-930 in sensitized preparations

The effects of ICS 205-930 on the responses to βLG in sensitized ileum were examined at three different concentrations because of the reported high potency and specificity of this compound. Twenty preparations, in groups of four, from five animals were used. One from each group was used as a control, while the other three were each exposed to one of three different antagonist concentrations for 5 min. The antagonist was added to both sides of the epithelium prior to challenge with βLG. Table 1 shows the basal SCC of the four groups of ileal preparations and the effects of the antagonist on the SCC. It is clear that the SCC is not significantly altered after transient effects have disappeared.

Figure 6 shows how the responses to βLG (0.55 μM) are modified by ICS 205-930. At 0.01 μM there was a significant reduction in the responses to βLG, although this inhibition was incomplete, reaching a value of only 32%. In fourteen of the fifteen comparative tests the response in the presence of antagonist was smaller than in its absence. This was not so in the

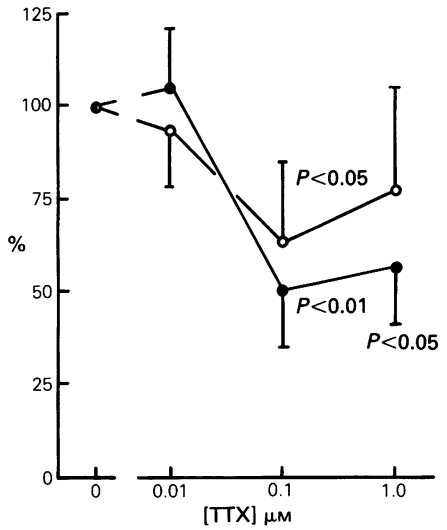

Figure 7 Responses of ileal (●) and colonic (○) preparations to challenge with β-lactoglobulin (βLG, 0.55 μM) in the presence and absence of tetrodotoxin (TTX). Values are given as a percentage of the control responses. Mean percentages are given for $n = 5$; s.e. shown by vertical lines. Significant differences are shown.

fifteenth pair which explains why significance was not achieved at 1.0 μM ICS 205-930.

We used colon preparations from the same animals to examine the effects of ICS 205-930 on the response to βLG. As with the ileum the antagonist had no effect upon basal SCC (Table 1). With the colon all responses in the presence of antagonist were smaller than those in its absence. Significant inhibition of the responses was obtained at 0.1 μM and 1.0 μM ICS 205-930. Maximal achievable inhibition in the colon was 47.0%, somewhat larger than in the ileum.

Effects of TTX on responses to βLG in sensitized epithelia

In an earlier report (Baird *et al.*, 1987) we showed that in ileal preparations sensitised to βLG the response to challenge (or electrical field stimulation) was inhibited by TTX. For the sake of completeness we have also carried out experiments with colon preparations from guinea-pigs that had been fed cows' milk. The results are presented in Figure 7 in which responses are given as a percentage of control. For comparative purposes the earlier ileum data have been presented in the same form.

Discussion

There is already substantial evidence that 5-HT affects transepithelial ion transport in the gut of a number of species including the rat (Hardcastle *et al.*, 1981; Zimmerman & Binder, 1984), rabbit (Donowitz *et al.*, 1977) and guinea-pig (Cooke & Carey, 1985). Some have considered that 5-HT acts directly on the enterocytes (Zimmerman & Binder, 1984) yet radioligand binding studies failed to show binding sites on the epithelial cells although they were present in the lamina propria (Gaginella *et al.*, 1983; Gershon *et al.*, 1983). Cooke & Carey (1985) using the guinea-pig ileal epithelium showed that 5-HT caused electrogenic chloride secretion which was inhibited by TTX. Our data with TTX, although less extensive, essentially confirm their view, except in one detail. We found that TTX inhibited basal SCC in the ileum, indicating tonic neural activity, apparently affecting chloride secretion. Our finding agrees with results reported by Keast (1987) for the guinea-pig and Hubel (1978) for the rabbit ileal epithelium. In contrast to this Cooke *et al.* (1983) and Cooke (1986) did not find this effect of TTX, except in the absence of glucose.

From the foregoing it is clear that a major part of the effect of 5-HT on chloride secretion is mediated indirectly via an action upon neural elements in the tissue. Independent evidence that TTX can block the effects of direct electrical stimulation of the submucosal plexus has been given previously (Baird *et al.*, 1987).

Major questions are, therefore, upon what kind of receptors and where does 5-HT act to induce chloride secretion in the mucosa? Also, is 5-HT a primary mediator of the response to antigen challenge, whatever the final effect or process may be? Our approach here has been to use a series of 5-HT antagonists and to examine their relative effectiveness against both 5-HT and antigen challenge. We do so against a background of uncertainty about 5-HT receptor subtypes, antagonist specificity and the possibility that our tissue contains a mixture of receptor subtypes. We have made considerable use of the findings from a recent symposium (Bradley *et al.*, 1986) on the classification of 5-HT receptors. Even though the classification incorporates data from over 100 studies, none of these refer to epithelial actions of 5-HT. Methysergide is a weak antagonist at 5-HT_1-like receptors but also antagonizes 5-HT at 5-HT_2-receptors. This agent produced a shift in the concentration-response curve at 10 nM which was evident only at low agonist concentrations, but no effect on the response to antigen-challenge, even at 5 μM. One might conclude, therefore, that no 5-HT_1-like or 5-HT_2 receptors are involved in the response to antigen challenge. Nevertheless the preparation might contain 5-HT_1 receptors in sites where 5-HT, liberated by antigen challenge, cannot reach an appreciable concentration. Ketanserin, with negligible affinity for 5-HT_1 binding sites (Van Neuten & Vanhoutte, 1981) is a potent antagonist at 5-HT_2 receptors (Leysen *et al.*,

1981) yet this compound has no effect on the response curves to 5-HT. There was an apparent depression of the maximal response in the presence of ketanserin, but this effect was not significant. More confusingly, ketanserin significantly reduced the response to βLG challenge. One is forced to conclude that the effects of ketanserin on the hypersensitivity reaction are not specific, in the sense that inhibition is unlikely to be due to antagonism of endogenously release 5-HT. Cyproheptadine, another 5-HT_2 antagonist also inhibited both the response to antigen challenge and exogenously applied 5-HT. However, this agent is a potent calcium antagonist (Lowe *et al.*, 1981; Winquist *et al.*, 1984) and as raised intracellular calcium causes chloride secretion in many epithelia (see for example, Cuthbert, 1985) no useful conclusions can be made from the actions of cyproheptadine. This agent would have been useful in a confirmatory role if both ketanserin and methysergide had produced evidence for the involvement of 5-HT_2 receptors. From the results we have obtained with ketanserin, methysergide and cyproheptadine, we cannot conclude 5-HT is involved in the hypersensitivity reaction. The inhibition of 5-HT action on chloride secretion by methysergide but not by ketanserin might indicate the involvement of 5-HT_1-like receptors in the response to 5-HT. However, a very contrived explanation would be required in terms of known functional responses mediated by 5-HT_1-like receptors to explain the minor inhibition we have recorded.

Two new compounds which inhibit 5-HT responses at 5-HT_3 receptors (M-receptors) were described recently, MDL 72222 (Fozard, 1984) and ICS 205-930 (Richardson *et al.*, 1985). The former is apparently a weak, non-specific antagonist on enteric neurones in the guinea-pig (Fozard, 1984) so we have favoured ICS 205-930. This compound blocks depolarization and transmitter release from a number of different peripheral neurones, but its actions are less potent on the guinea-pig ileum neurones than on the heart or vagus. Recently Mawe *et al.* (1986) have shown ICS 205-930 blocks the fast e.p.s.p. caused by 5-HT in guinea-pig myenteric neurones. We have found significant inhibition with ICS 205-930 on the chloride secretory response to 5-HT and to antigen challenge in sensitized preparations at sub-micromolar concentrations. Shifts in the concentration-response relationship were not parallel, but we know the effects of 5-HT are indirect and may involve various amplification stages. Furthermore, there is desensitization at high 5-HT concentrations so that inhibition by ICS 205-930 may not be surmountable. In addition, the form of the inhibition with ICS 205-930 is not unlike that with TTX. If there is a crucial involvement of 5-HT in a neural pathway in the lamina propria then block of conduction, with TTX, or at a synapse, with ICS 205-930 would have the same final effect on secretion. In both ileum and colon preparations inhibition by ICS 205-930 was similar and incomplete, suggesting that only part of the hypersensitivity reaction is neuronally mediated. In the guinea-pig, separate populations of cholinergic and non-cholinergic nerves exist in the submucosal layer (Furness *et al.*, 1984). Low concentrations of 5-HT apparently stimulate the non-cholinergic population, as the effects on secretion are blocked by TTX but not hyoscine, while high concentrations of 5-HT give a secretory response that is partially hyoscine-sensitive (Keast *et al.*, 1985). We have shown previously (Baird *et al.*, 1984) that atropine does not affect the secretory response to βLG challenge in the ileum, suggesting that if 5-HT is involved it can affect only neurotransmitter release from non-cholinergic nerves, such as those releasing vasoactive intestinal peptide (VIP) or substance P. Cook & Carey (1984) reported that the 5-HT antagonist, cisapride inhibited the effects of 5-HT on SCC in guinea-pig ileal mucosa. In another study, interactions between 5-HT and cisapride were studied on myenteric neurones in the guinea-pig ileum (Nemeth *et al.*, 1985). Antagonist effects of cisapride were described which could be mimicked by methysergide, an antagonist we found to be ineffective against antigen challenge. In another study (Hardcastle *et al.*, 1984), cisapride was shown to cause acetylcholine release and so affect chloride secretion. It seems unlikely that cisapride offers real prospects for clarifying the hypersensitivity reaction we have described.

In the guinea-pig colonic mucosa, sensitized to βLG, we found that indomethacin completely blocked the response to antigen challenge, while mepyramine was able to cause partial inhibition (Cuthbert *et al.*, 1983). In the sensitized ileum these antagonists had no effect, neither did atropine nor sodium cromoglycate (Baird *et al.*, 1984). We concluded that antigen challenge liberated a whole cascade of mediators, of which some exerted a permissive effect on the action of others. This appeared to be so for eicosanoids and the colon. Here we have produced data to suggest that 5-HT is involved in both the ileum and colon, although our conclusion depends entirely on the specificity of ICS 205-930. Also if there is a cascade of mediators some, such as histamine, eicosanoids and kinins, have a direct action on the enterocyte, thus explaining the failure to antagonize completely the response to antigen challenge.

Finally, the origin and site of action of 5-HT needs to be considered. 5-HT-containing neurones do occur in the guine-pig ileum but they appear to terminate in submucous ganglia (Furness & Costa, 1982). It is possible that mediators released upon antigen challenge act upon these tryptaminergic terminals to cause transmitter release and activation of non-cholinergic postganglionic fibres. In some species, for example the rat, mast cells release 5-HT along with other

substances which may then affect postganglionic neurones. However, this is not so for the guinea-pig. Alternatively mast cell mediators might liberate 5-HT from enterochromaffin cells (Burks & Lang, 1966), the major source of 5-HT in the gut, which then act upon 5-HT_3 receptors. It would seem from our data that a single antagonist is unlikely to block completely type 1 hypersensitivity reactions in the gut. As these reactions are thought to be involved in some food allergic states, antagonists at 5-HT_3 receptors may be useful adjuncts for treatment. The first reports are now appearing (Coupe *et al.*, 1986) that 5-HT_3 antagonists prevent the secretory diarrhoea associated with carcinoid syndrome, indicating that mechanisms in common with those that we are proposing may be involved.

This work was supported by Fisons p.l.c.

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(Received May 12, 1987.
Revised July 1, 1987.
Accepted July 17, 1987.)