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Increased defaecation caused by 5-HT_4 receptor activation in the mouse

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Abstract

The precursor to 5-hydroxytryptamine (5-HT), 5-hydroxytryptophan, (5-HTP, 5–50 mg · kg^{-1}) administered subcutaneously (s.c.) to conscious, fed mice caused a dose dependent increase in faecal pellet and fluid output. To avoid provoking watery diarrhoea, all experiments were performed using 5-HTP at 10 mg · kg^{-1}. This dose caused maximal increases in the fluid content (471 ± 41%) and number of formed faecal pellets defaecated (328 ± 13% $n = 25$), 10 and 20 min respectively after administration, when compared to saline-treated mice. In both saline- and 5-HTP-treated mice methiothepin, ketanserin, mianserin and granisetron reduced defaecation at high s.c. doses (100 μg · kg^{-1} or 1000 μg · kg^{-1}). The 5-HT_4 receptor antagonists, DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylate hydrochloride), SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester) and SB 204070 ([1-butyl-4-piperidinylmethyl]-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate), had no effects when administered s.c. to saline-treated mice, but dose-dependently inhibited the 5-HTP-evoked responses. Only SB 204070 at 1000 μg · kg^{-1} completely inhibited the responses to 5-HTP returning them to normal levels. We conclude that SB 204070 is a potent antagonist for the investigation of 5-HT_4 receptor function in both normal and disturbed gastrointestinal activity.

Keywords: 5-HTP (5-hydroxytryptophan); 5-HT (5-hydroxytryptamine, serotonin); 5-HT receptor antagonist; 5-HT_4 receptor; SB 204070; Defecation; (Mouse)

1. Introduction

The gastrointestinal tract is by far the largest single store of 5-hydroxytryptamine (5-HT) within the mammalian body (Thompson, 1971). Among the many proposed actions of this store of 5-HT is a pathological involvement in the mechanisms of at least some types of watery diarrhoea and disturbed defaecation. These effects are evoked by increased propulsive activity of the intestine and/or by increases in the volume of fluid that is accumulated within the lumen, depending on the severity and type of the disturbance (see Jaffe, 1979 for references). However, in contrast to this extensive literature, very little is known about the nature of the 5-HT receptors that mediate these actions of 5-HT and hence, the true pathophysiological roles of 5-HT cannot be fully elucidated.

Selective 5-HT_3 receptor antagonists can cause constipation so it has been argued that the 5-HT_3 receptor must have a role in the physiology of defaecation (Sanger et al., 1994). However, the involvement of this receptor in human pathological diarrhoea or disturbances in defaecation is less clear, with conflicting efficacy being reported for 5-HT_3 receptor antagonists against stimuli such as cholera toxin (see Eherer et al., 1994). Consequently, if 5-HT is to play a major role in this type of pathology it seems reasonable to suppose that other 5-HT receptors will be at least partly involved.

Among the 5-HT receptors which could be involved in the pathophysiology of defaecation, a likely candidate would seem to be the 5-HT_4 receptor. Thus, 5-HT_4 receptor agonists, such as cisapride, increase stool frequency and can cause diarrhoea in healthy volunteers (Enck et al., 1989). Furthermore, 5-HT_4 receptor activation in isolated gut preparations increases peristaltic reflex sensitivity (Craig and Clarke, 1991; Buchheit and Buhl, 1991; Costall et al., 1993; Tuladhar et al., 1994) and evokes chloride secretion in various animal and human mucosal tissues (Scott et al., 1992; Burleigh and Borman, 1993; Budhoo and Kellum, 1994; Kellum et al., 1994; Franks et al., 1995). More recently, Hegde et al. (1994) have demonstrated an involvement of the 5-HT_4 receptor in a more watery form of diarrhoea evoked by different doses of the 5-HT precursor, 5-hydroxytryptophan (5-HTP).

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The present study investigated the effects of a number of different 5-HT receptor antagonists on normal defaecation in conscious mice and against an increased rate of defaecation caused by a dose of 5-HTP which did not evoke obvious watery diarrhoea, a model previously described by Brittain and Collier (1958). Our findings suggest that the 5-HT_4 receptor serves little or no function in the mechanisms of normal defaecation, but that this receptor exerts an influence on the mechanisms of disturbed defaecation. Some of these results have previously been communicated to the British Pharmacological Society (Banner et al., 1993a,b).

2. Materials and methods

2.1. Experimental protocol

Fed male CD1 mice (26–36 g, Charles River) were used. Free access to food and water was allowed up to the beginning of the procedure. The mice were then weighed and placed individually in mesh bottom clear Perspex boxes measuring 190 × 100 × 120 mm raised 50 mm above the bench. A period of 20 min was allowed for acclimatization of the mice to this new environment prior to the start of the experiment.

Mice were used in test groups of five individuals and four groups were used at a time. Non-absorbent paper was placed under each group of mice at the beginning of the experiment and at each recording time-point this was replaced with a clean sheet. Pellets were counted and collected every 10 min for 60 min and again at 75 min. Individual pellet numbers were counted and cumulative faecal pellet calculated for each mouse from the time after dosing 5-HTP or saline control. The collected pellets from each test group were placed into single glass vials, weighed and then dried in an oven overnight at 50°C and weighed again the following day. The fluid content of the pellets was calculated as:

(wet weight − vial weight) − (dry weight − vial weight)

5-HTP or saline control were administered subcutaneously (s.c.) after two 10 min faecal pellet collections. The 5-HT receptor antagonists or vehicle control were dosed s.c. 5 min prior to a standard dose of 5-HTP (10 $mg \cdot kg^{-1}$) or saline. SB 204070 was also tested orally (p.o.) by administration 15, 30 and 60 min prior to dosing of 5-HTP or saline.

2.2. Drugs

5-HTP and DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylate hydrochloride) were obtained from Sigma Chemical Co. (Poole, UK) and Boehringer respectively. Methiothepin, ketanserin and mianserin were obtained from Research Biochemicals. Granisetron, SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester); Buchheit et al., 1991) and SB 204070 [(1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate] were all synthesized by SmithKline Beecham Pharmaceuticals. All drugs were dissolved in 0.9% saline and were administered s.c. or p.o. with saline as vehicle in both 5-HTP- and saline-treated mice.

2.3. Analysis and statistics

The mean faecal pellet output of the individuals within each test group was expressed as a percentage of the mean faecal pellet output of the saline control group at the corresponding time point (± standard error of mean, S.E.M.). Pellet counts at 20 min were compared between days in control groups using the non-parametric Kruskall-Wallis test to determine the acceptability of combining data over the time course of the experiments for any given drug tested. There was no significant difference between control data within the saline-treated and within the 5-HTP-treated groups of mice in the absence of an antagonist, throughout tests performed during the course of this study ($P > 0.05$).

For saline-treated mice the number of pellets defaecated in any group at the 20 min time point are expressed as a percentage of the saline control, taken as 100%. For 5-HTP-treated mice a percentage inhibition of the response to 5-HTP alone is given.

The Mann Whitney *U*-test for statistical significance was used to compare treatment groups against controls. Changes were regarded as not statistically significant if P value for Z statistic > 0.05. For the pellet fluid content data values at the 10 min collection point were compared by one-way analysis of variance, changes were regarded as not statistically significant in the Dunnett's test at > 0.05.

3. Results

3.1. The effects of 5-HTP

In vehicle-treated mice 5-HTP at 5–50 $mg \cdot kg^{-1}$ s.c. dose-dependently increased the number of pellets defaecated within the duration of the experiment, when compared with the values obtained from saline-treated mice. In these initial, extended studies, a maximum difference in faecal pellet output between the saline and 5-HTP-treated mice was generally seen at 20 min post dose, except for the initially very high rate of defaecation caused by 50 $mg \cdot kg^{-1}$ 5-HTP. Thus, 20 min after administration of 5-HTP, at a dose of 10 $mg \cdot kg^{-1}$, cumulative faecal pellet output was 328 ± 13% higher than in saline-treated mice. Fig. 1 shows that at all doses the increase in faecal pellet output evoked by 5-HTP was not maintained beyond 20–30 min; thereafter, the number of pellets defaecated by an

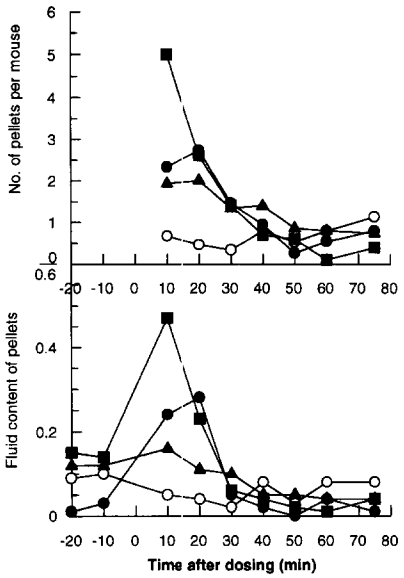

Fig. 1. The effect of subcutaneous administration of 5-HTP 5 mg·kg^{-1} (●), 10 mg·kg^{-1} (▲), 50 mg·kg^{-1} (■) and saline (○) on faecal pellet output (upper graph) and pellet fluid content (lower graph) in mice. Points in the upper graph represent the mean of 10–25 mice, the S.E.M. is omitted for clarity. In the lower graph points are a single, representative, experiment with pellets from five mice aggregated for each test group.

individual were similar to those of saline control animals.

The faeces produced by the 5-HTP-treated mice (5–50 mg·kg^{-1}) were in pellet form but contained a higher water content than the pellets excreted by saline-treated mice, an example is shown in Fig. 1. Again, as with the increased faecal pellet output, the peak increase in fluid content of the pellets obtained from each group of mice occurred very rapidly. Thus, after 10 min 5-HTP (10 mg·kg^{-1}) raised the fluid content from a mean of 0.056 ± 0.008 g ($n = 5$) in saline-treated mice to 0.264 ± 0.023 g ($n = 5$) in the 5-HTP-treated mice, an increase of 471 ± 41%.

To determine the effects of 5-HT receptor antagonists against 5-HTP-evoked defaecation, compounds were subsequently tested against the submaximally effective dose of 10 mg·kg^{-1} 5-HTP. Changes in faecal pellet output were determined 20 min after 5-HTP whilst changes in faecal pellet fluid content were determined 10 min after 5-HTP.

3.2. The effect of 5-HT receptor antagonists on faecal pellet output in saline-treated mice

In saline-treated animals methiothepin, ketanserin and mianserin, each at 10 μg·kg^{-1} and granisetron at 10 and 100 μg·kg^{-1} had no statistically significant inhibitory effect on faecal pellet output. However, at the higher dose of 100 μg·kg^{-1} methiothepin reduced faecal pellet output by 83 ± 13% ($P < 0.01$, $n = 10$) vs. saline controls. Statistically significant ($P < 0.05$, $n = 10$) reductions of 59 ± 20% and 60 ± 24% were also seen with 30 and 100 μg·kg^{-1} mianserin respectively. Granisetron at 1000 μg·kg^{-1} (33 ± 29%; $P < 0.05$, $n = 10$) also inhibited defaecation. By comparison, the 5-HT$_4$ receptor antagonists SDZ 205-557 (1–5000 μg·kg^{-1}; $n = 10$–20 each dose), DAU 6285 (1–1000 μg·kg^{-1}; $n = 10$) and SB 204070 (0.01–1000 μg·kg^{-1}; $n = 5$–10) did not consistently affect faecal pellet output in saline-treated mice.

3.3. The effect of 5-HT receptor antagonists on faecal pellet output in 5-HTP-treated mice

In the dose range 10–30 μg·kg^{-1}, the 5-HT$_1$ and 5-HT$_2$ receptor antagonists, methiothepin and mianserin, had no statistically significant effect on faecal pellet output in mice treated with 5-HTP. However, at the higher dose of 100 μg·kg^{-1}, both methiothepin (56 ± 24%; $P < 0.01$; $n = 10$) and mianserin (84 ± 14%; $P < 0.001$, $n = 15$) inhibited defaecation compared to the 5-HTP-treated control mice. Ketanserin at 30 μg·kg^{-1} (49 ± 20%; $P < 0.05$, $n = 10$) and 100 μg·kg^{-1} (66 ± 22%; $P < 0.01$, $n = 10$) also inhibited 5-HTP-induced faecal pellet output as did the high dose of 1000 μg·kg^{-1} granisetron (50 ± 21%; $P < 0.01$, $n = 10$); lower doses of all drugs being without effect.

The 5-HT$_4$ receptor antagonists each reduced the 5-HTP-evoked increase in faecal pellet output. However, the potency and magnitude of effect depended on the compound tested. Thus, in the dose ranges tested SDZ 205-557 and DAU 6285 did not completely prevent the effect of 5-HTP, being maximally active at 5000 μg·kg^{-1} (55 ± 12% inhibition; $n = 10$) and 1000 μg·kg^{-1} (78 ± 11% inhibition; $n = 15$), respectively. In contrast, SB 204070 (Fig. 2) dose-dependently and potently reduced the evoked response to 5-HTP at doses as low as 10 μg·kg^{-1}

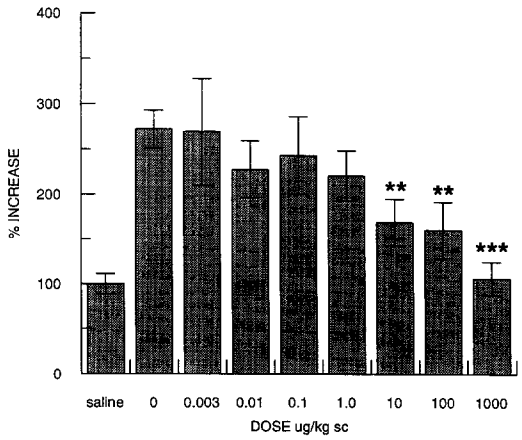

Fig. 2. The effect of SB 204070 on faecal pellet output in 5-HTP treated mice. The first column represents the effects of saline on faecal pellet output, 20 min after administration of saline control for 5-HTP. In the subsequent columns, mice received 5-HTP 10 mg·kg^{-1} s.c., plus different s.c., doses of antagonist, administered 5 min prior to 5-HTP. The effects of these antagonists were compared statistically with the effects of 5-HTP in the absence of antagonist; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Points represent the mean ± S.E.M. of 10–25 mice.

(60 ± 15% inhibition; $n = 15$); SB 204070 1000 $\mu g \cdot kg^{-1}$ fully antagonised the effect of 5-HTP ($n = 20$). An estimated ED_{50} value of 3.9 $\mu g \cdot kg^{-1}$ was obtained. SB 204070 was also tested orally with 15, 30 or 60 min pre-dose times in the range 1–1000 $\mu g \cdot kg^{-1}$ ($n = 10$ each dose), against the 5-HTP-evoked response, but was found to have no statistically significant inhibitory action at any dose tested at these different time points.

3.4. The effect of 5-HT receptor antagonists on faecal water content

Pellets from five individual mice were accumulated for pellet fluid content, so the overall n values are small. Nevertheless, the results obtained for pellet fluid secretion in both treatment groups followed a similar pattern to those obtained for faecal pellet output, although no statistically significant effects of the $5\text{-}HT_1$, $5\text{-}HT_2$ and $5\text{-}HT_3$ receptor antagonists were recorded in either the saline or the 5-HTP-treated groups of mice.

SDZ 205-557, did not have a consistent effect on fluid content in saline-treated mice, significance only being achieved at 10 $\mu g \cdot kg^{-1}$ (27 ± 17% of saline controls; $P < 0.05$, $n = 4$ groups of mice). However, this antagonist dose-dependently returned the 5-HTP-evoked response to control values (Fig. 3) with a maximum inhibitory effect at the highest dose tested (5000 $\mu g \cdot kg^{-1}$; 96 ± 17% inhibition). DAU 6285 (1–1000 $\mu g \cdot kg^{-1}$; $n = 10$ each dose) had no statistically consistent effects on fluid content of pellets in saline- or 5-HTP-treated mice, except that higher doses tended to reduce the 5-HTP-evoked increase in pellet fluid content. SB 204070 again had no effect on saline-treated mice but like SDZ 205-557 dose-dependently inhibited the 5-HTP-evoked increase in the pellet fluid content, with complete reversal at 1000 $\mu g \cdot kg^{-1}$ (Fig. 4).

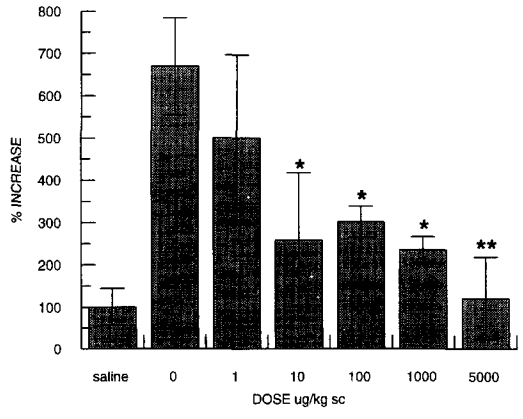

Fig. 3. The effect of SDZ 205-557 on pellet fluid content in 5-HTP treated mice. The first column represents the effects of saline on fluid content of the pellets, 10 min after administration of saline control for 5-HTP. In the subsequent columns, mice received 5-HTP 10 $mg \cdot kg^{-1}$ s.c., plus different s.c. doses of antagonist, administered 5 min prior to 5-HTP. The effects of SDZ 205-557 were compared statistically with the effects of 5-HTP in the absence of antagonist; * $P < 0.05$, ** $P < 0.01$. Points represent the mean ± S.E.M. of 2–5 groups of mice.

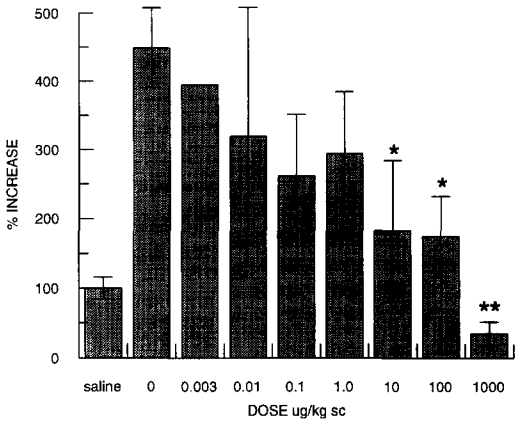

Fig. 4. The effect of SB 204070 on pellet fluid content in 5-HTP treated mice. The graph is configured as for Fig. 3. The effects of SB 204070 were compared statistically with the effects of 5-HTP in the absence of antagonist; * $P < 0.05$, ** $P < 0.01$. Points represent the mean ± S.E.M. of 2–5 groups of mice.

4. Discussion

5-HT has long been known to facilitate defaecation and cause diarrhoea (see Introduction). The receptors involved in these responses and the true pathological significance of this observation has never been fully characterised, primarily because of the lack of specific pharmacological tools. In this study we have now investigated a range of different 5-HT receptor antagonists for their ability to modulate the rate of defaecation in normal mice and for their ability to reduce the increased rate of defaecation caused by 5-HTP. The method for the latter is taken from a much earlier study by Brittain and Collier (1958), with which we now show clearly that even low doses 5-HTP can markedly increase the defaecatory habits of fed, conscious mice. These effects of 5-HTP are likely to be prolonged when compared with a single dose of 5-HT (Sanger and McClelland, 1986). Nevertheless, after the initial response to 5-HTP has been observed, the rate of faecal pellet output tends to decline to that of saline control levels. This is probably a reflection of an initial emptying of the colorectum. In particular, the initial large 'insult', achieved by 50 $mg \cdot kg^{-1}$ 5-HTP, propels most of the contents of the colon out of the body in a watery state.

For pharmacological analyses the dose of 10 $mg \cdot kg^{-1}$ 5-HTP s.c. was chosen as it consistently produced a submaximal increase in the defaecation of formed faecal pellets, with an associated increase in fluid content, rather than the watery diarrhoea which was observed at higher doses. The chosen dose thereby seems to mimic the disturbed motility and secretory effects observed with 5-HTP in healthy volunteers (Davidson et al., 1957).

5-HT_1 and 5-HT_2 receptors have been implicated in colonic motility (Gué et al., 1993), but the results presented here indicate varied and weak defaecatory responses to these antagonists. Methiothepin had little or no consistent effect up to 30 $\mu g \cdot kg^{-1}$ on faecal pellet output and fluid content in both saline-treated and 5-HTP-treated mice, but at the higher dose of 100 $\mu g \cdot kg^{-1}$, methiothepin caused inhibition of responses in both of these test groups. However, pharmacological activity of this dose can be regarded as non-specific (Connor et al., 1986). Ketanserin and mianserin also inhibited defaecation in both saline- and 5-HTP-treated mice, again at the higher doses. Thus, the inconsistent and perhaps non-selective effects of the high doses of these 5-HT_1 and 5-HT_2 receptor antagonists, suggest that these receptors may at best have only a minor role in the defaecatory processes. However, it cannot be dismissed that some of these antagonists may be have very specific roles in gastrointestinal motility or secretion which are not clearly apparent under the present experimental conditions.

Granisetron had a significant effect on defaecation in the saline-treated mice at 1000 $\mu g \cdot kg^{-1}$. This would tend to agree with the constipatory effects seen in healthy volunteers and may support a possible role for 5-HT_3 receptors in the normal mechanisms of human defaecation (see Sanger et al., 1994). Granisetron also significantly reduced faecal pellet output in 5-HTP-treated mice at the highest dose tested and this agrees with its reported ability to decrease the effects of cholera toxin (Eherer et al., 1994).

The time of s.c. administration of the antagonists administered prior to 5-HTP may not allow the full activity of these compounds to be realised; further experiments are required to optimise the pharmacological conditions for each compound. Nevertheless, the choice of the 5-HT precursor as the stimulus for altered defaecation in the mouse allows the compounds a window of activity that is created by the duration of increased synthesis of 5-HT within the colon. Consequently, it is suggested that whilst 5-HT_1, 5-HT_2 and 5-HT_3 receptors may contribute to both normal and/or disturbed defaecatory functions they do not play a major role in normal defaecation or in the mechanisms by which 5-HTP induces disturbed bowel conditions in the conscious mouse.

The identification of selective 5-HT_4 receptor antagonists has enhanced the study of 5-HT mechanisms in disturbed colonic activity. The data presented here demonstrates an effect with each of the 5-HT_4 receptor antagonists, but whilst the metabolically unstable (Ku et al., 1992), SDZ 205-557 required high concentrations to have an effect on the 5-HTP-evoked faecal pellet output response it was more effective at inhibiting the evoked increase in fluid content, returning fluid content to saline control levels. In contrast DAU 6285 was a better blocker of faecal pellet output in the presence of 5-HTP but the highly potent antagonist, SB 204070 (Wardle et al., 1994), completely inhibited the effects of 5-HTP on faecal pellet output and fluid content. This is in contrast to the maximum effects reported by Hegde et al. (1994). They found that DAU 6285 and SB 204070 had 63% and 36% of maximal inhibition of the 5-HTP-evoked response when administered intraperitoneally and analysed by subjective scoring. The reason for this difference is not clear, but may be related to the development of a more watery (and hence, severe) type of diarrhoea in the studies of Hegde et al. (1994). Notably each of the 5-HT_4 receptor antagonists used in the present study did not have any consistent effect on colonic function in saline-treated mice, suggesting that the 5-HT_4 receptor is not greatly involved in normal bowel function but appears only to be recruited in disturbed conditions. Furthermore, the lack of effect seen with SB 204070 orally may be explained by one or more factors. Thus, it is possible that the time of administration prior to 5-HTP was inappropriate, or more likely that SB 204070 exhibits metabolic instability because of the ester linkage (Gaster et al., 1993). Hegde et al. (1995) have since reported that the 5-HT_4 receptor antagonist RS 39604 when administered orally at 30 $mg \cdot kg^{-1}$ inhibited the diarrhoea score in response to 5-HTP.

These results show an involvement of 5-HT_4 receptors in altered defaecatory processes, but do not describe a mechanism of action. However, as formed pellets were being measured it is likely that a facilitation of the propulsive reflex is involved, since in the ileum of various species, others have shown a facilitation of the peristaltic reflex in vitro (see Introduction for references). In addition to this effect, the fluid content of the pellets was also increased. There exist two possible explanations for this effect, firstly, there may be less time in which water reabsorption can occur and/or there may be an active secretion of fluid (Burleigh and Borman, 1993; Budhoo and Kellum, 1994). In either case it has been possible to demonstrate the return towards normalization of these secretory, as well as motility, events manifested as altered defaecation by use of the 5-HT_4 receptor antagonist, SB 204070. However, precisely which gastrointestinal processes the 5-HT_4 receptor is involved in remains to be elucidated. The importance of understanding the mechanism of action of the 5-HT_4 receptor is now implicated in its pathophysiological role for 5-HT in disturbed defaecation.

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