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Increased defecation during stress or after 5-hydroxytryptophan: selective inhibition by the 5-HT_4 receptor antagonist, SB-207266

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1 5-HT_4 receptor antagonism pr2events the ability of exogenous 5-HT or 5-HTP to sensitize the intestinal peristaltic reflex and increase the rate of defecation, generally without affecting non-stimulated intestinal function. In this study we confirmed the ability of the selective 5-HT_4 receptor antagonist SB-207266 1–1000 μg kg^{-1} p.o., to prevent the increase in defecation evoked over a 60 min period by 5-HTP 10 mg kg^{-1} s.c. in conscious mice, in the absence of an apparent constipating action.

2 The role of endogenous 5-HT in the mechanisms of increased defecation and/or diarrhoea was then investigated in conscious, fed rats. This was evoked by 180 min exposure to restraint stress, which increased both the number and mean weight of formed, faecal pellets excreted over the entire time period.

3 SB-207266 1–1000 μg kg^{-1} p.o. (dosed 30 min before restraint) did not affect the increase in defecation evoked during the first 60 min of restraint stress, but significantly and dose-dependently reduced or prevented the increased defecation during the remaining 120 min of the experiment; this action occurred in the absence of an apparent constipating action of SB-207266.

4 In fasted rats exposed to restraint stress, watery diarrhoea developed and although there was a tendency for SB-207266 1–1000 μg kg^{-1} p.o. (dosed 30 min before restraint) to reduce the incidence of diarrhoea, this inhibition was not complete.

5 We conclude that selective 5-HT_4 receptor antagonism prevents disruptions in defecation behaviours caused by exogenous or endogenous enteric 5-HT and that this activity is not accompanied by a concomitant suppression of activity (constipation-like) within the intestine itself. *British Journal of Pharmacology* (2000) **130,** 706–712

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Abbreviations: 5-HT, (5-hydroxytryptamine); 5-HTP, (5-hydroxytryptophan); IBS, irritable bowel syndrome; SB-207266, (*N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2*H*-[1,3]-oxazino [3,2-*a*]indole-10-carboxamide)

Introduction

Selective antagonists at the 5-HT_4 receptor do not generally affect normal, healthy gut function. Thus, this class of compound did not modify faecal pellet output by conscious mice (Banner *et al*., 1996; Sanger *et al*., 1998), affect baseline potential differences across different intestinal regions of anaesthetized rats (Franks *et al*., 1994), change intestinal motility in conscious rats (Clayton & Gale, 1996), sheep (Plaza *et al*., 1997) and dogs (Nagakura *et al*., 1996) or affect Heidenhain gastric pouch motility in conscious dogs (Wardle *et al*., 1996). Similarly, 5-HT_4 receptor antagonism did not affect the peristaltic reflex caused by intraluminal distension in various isolated but otherwise-untreated intestinal preparations (Craig & Clarke, 1991; Costall *et al*., 1993; McLean & Coupar, 1996; Sanger *et al*., 1998), or the ascending and descending components of the reflex (Yuan *et al*., 1994). An exception to this general inactivity is reported by Lordal & Hellstrom (1999) who suggest that in rodents, both 5-HT_4 and 5-HT_3 receptor antagonism can disrupt migrating motor complex activity. Further, one other laboratory (Foxx-Orenstein *et al*., 1996; Grider *et al*., 1996) found that in flat preparations of rat colon, human jejunum or guinea-pig colon, brush stroking the mucosa could evoke ascending and descending nerve pathways, possibly *via* the release of 5-HT and activation of 5-HT_4 receptors, either alone or in combination with the $5HT_3$ receptor or the putative 5-HT_{1P} receptor.

In contrast to the absence of a clear effect on normal gut function, selective 5-HT_4 receptor antagonists prevent the ability of exogenously-applied 5-HT to sensitize the peristaltic reflex in intact, isolated preparations of small (Craig & Clarke, 1991; Costall *et al*., 1993; McLean & Coupar, 1996; Tuladhar *et al*., 1996; Sanger *et al*., 1998) and large (Jin *et al*., 1997) intestine. 5-HT_4 receptor antagonism also prevents the ability of 5-HT or 5-HTP to increase the excretion of faecal pellets in conscious mice (Hegde *et al*., 1995; Banner *et al*., 1996; Sanger *et al*., 1998), to evoke chloride (Borman & Burleigh, 1993; Kellum *et al*., 1994) and mucus (Moore *et al*., 1996) secretion from the mucosa and to induce watery diarrhoea (Hegde *et al*., 1994).

If 5-HT_4 receptor antagonists do not greatly affect normal gut function, but instead, prevent the disturbances caused by inappropriate levels of 5-HT within the gut, such compounds might find clinical utility in the treatment of functional bowel disorders, in which a role for 5-HT has been suggested (Sanger, 1996). This profile of activity would contrast with current approaches for the treatment of, for example, Irritable Bowel

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Syndrome (IBS). Thus, for IBS, drugs deliberately evoke substantial changes in normal gut physiology in order to oppose this condition, risking the replacement of one symptom with another, drug-related symptom (Sanger, 1999). Given the potential for 5-HT_4 receptor antagonism to desensitize the bowel in the absence of marked changes in gut function we have, therefore, investigated the effects of the selective 5-HT_4 receptor antagonist SB-207266 (Wardle *et al*., 1996) on the increased rate of defecation by mice and rats induced firstly by 5-HTP and then by restraint stress. The effects of the latter procedure on intestinal function is thought to be accompanied by the release of large amounts of 5-HT within the gut (Kundrotas & Gregg, 1977). This study was, therefore, designed to study the role of 5-HT_4 receptors in the mechanisms by which endogenous 5-HT might disrupt gastrointestinal function.

Methods

Animals

Male rats (Crj: Donryu, 5 weeks, 115–162 g) or mice (Crj: CD-1 (ICR), 5 weeks; 24–32 g) were obtained from Charles River Japan Inc. They were randomized into groups according to body weight, so that the group mean body weights were approximately equal and were initially housed up to five (rats) or eight (mice) in a group and allowed free access to food (solid diet; CRF-1, Oriental Yeast Industry Ltd) and tap water. One week after grouping, they were housed individually and the experiment conducted.

5-HTP-induced defecation in fed mice

For each dose of antagonist tested, a treatment group of eight mice with a weight range of 29–34 g were used. In each group, the mice were given the vehicle or SB-207266 orally and 30 min later, 10 mg kg^{-1} of 5-HTP or vehicle was injected subcutaneously. For both groups, the mice were placed individually in plastic cages (W: 170 mm × D: 270 mm × H: 140 mm), and the number and weight of faecal pellets were determined during 0–60, 61–120, and 121–180 min periods.

Restraint stress-induced defecation in fed rats

For each dose of antagonist tested, a treatment group of eight rats with a weight range of 173–202 g was used. Control rats, which were not restrained, were placed individually in plastic cages (W: 400 mm × D: 250 mm × H: 200 mm) 30 min after oral administration of the drug vehicle. In the restraint-stress groups, the rats were placed individually into stainless steel restraint cages (W: 45 mm × D: 180 × H: 50 mm) arranged in parallel and in the same direction, 30 min after oral administration of the vehicle or SB-207266; faecal pellets were collected using trays located just below each rat. For each group, the number and weight of faecal pellets was determined during 0–60, 61–120 and 121–180 min periods after being placed in the cage.

Restraint stress-induced diarrhoea in fasted rats

For each dose of antagonist tested, a treatment group of eight rats with a weight range of 152–170 g was used. The rats were fasted for 18–22 h with free access to water and dosed with SB-207266 or vehicle 30 min before being placed in the restraint cages. During a 180 min period of restraint, a rat was judged to have diarrhoea if there was excretion of loose stools and/or a predominantly watery form of faeces. The incidence of diarrhoea is presented as (number of rats with loose stool or diarrhoea) / (number of rats used) over the 180 min period of restraint.

Drugs

SB-207266 ((*N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2*H*-[1,3]-oxazino [3,2-*a*]indole-10-carboxamide; SmithKline Beecham) was dissolved and diluted in water for injection. Serotonin creatinine sulphate (5-HT) and 5-hydroxy-L-tryptophan (5-HTP) were obtained from Wako Pure Chemical Industries Ltd, dissolved in isotonic sodium chloride solution (Otsuka Pharmaceutical Factory, Inc.) and concentrations were calculated as free base.

Analysis of data

The number and weight of faecal pellets over time from each animal were determined and the mean and standard error calculated for each group. Kruskal–Wallis tests were performed at individual time points to identify overall group differences. If differences were detected, pairwise comparisons were carried out using a Mann–Whitney *U*-test.

By using a standard 5 or 1 significance level for each of the pairwise comparisons at each of the different time points, there is a high probability that at least one of these comparisons will incorrectly indicate a significant difference (Type I error). For this reason, the significance level of each test was adjusted to take into account the multiple testing (Bonferroni's inequality), thereby ensuring that the overall Type I error rate remains constant at 5 or 1% (e.g., to obtain an overall Type I error of 5% for six pairwise comparisons, we need $(1-\alpha)^6 = 0.05$ where $\alpha = 0.0085$ is the significance level of each individual test; Gardiner & Gettinby, 1998). ID_{50} values (dose which inhibits effects of procedures by 50% of the maximum procedural effect observed) were calculated for SB-207266 by least square method and when possible, 95% confidence limits obtained. In the fasted rats with diarrhoea, an overall χ^2 test was carried out to determine whether the number of incidences of loose stool/diarrhoea was statistically different between the groups.

Results

5-HTP-induced defecation in mice

In the present study, the control group of mice defecated most often during the first 60 min of observation; thereafter defecation was minimal or absent (Figure 1). After injection of 5-HTP 10 mg kg^{-1} s.c., there was a marked increase in the number and mean weight of formed faecal pellets excreted during the first 60 min of observation whereas in the remaining 120 min, the small numbers and mean weights of excreted faecal pellets did not differ significantly between the two groups of mice (Figure 1; Table 1).

SB-207266 1–1000 μg kg^{-1} p.o. dose-dependently prevented the ability of 5-HTP to increase defecation during the 60 min period following injection of 5-HTP. The ID_{50} (95% Confidence Limits) for this effect was 0.9 (0.3–2.4) μg kg^{-1} p.o., and maximal activity was observed at the 10 μg kg^{-1} p.o. dose of SB-207266 and above. SB-207266 did not reduce the incidence of defecation below that which was observed in the control mice which were not treated with 5-HTP, even at 100

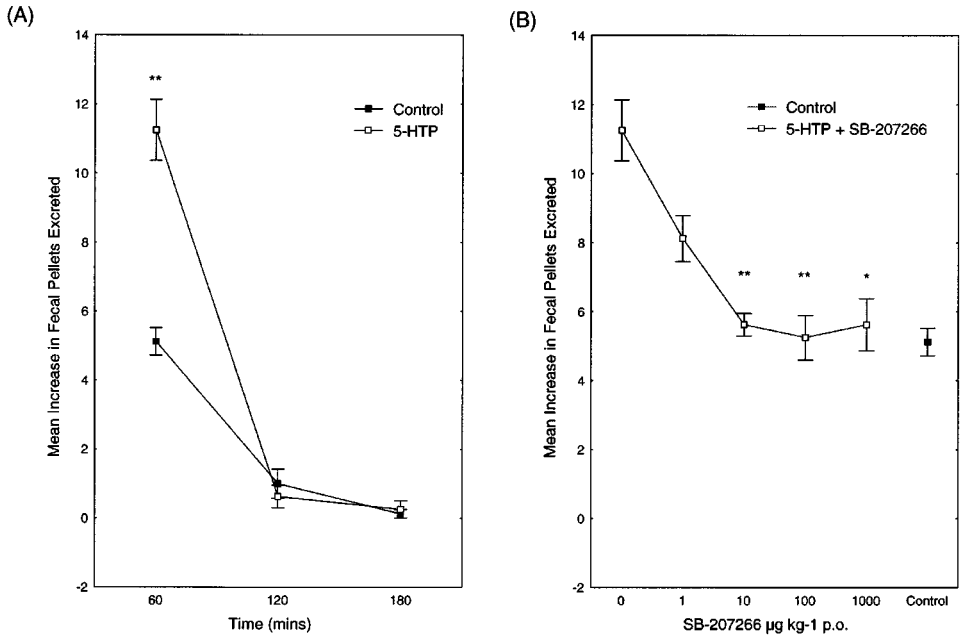

Figure 1 Prevention by SB-207266 of 5-HTP-induced defecation in conscious, fed mice. The number of faecal pellets excreted were monitored (A) during three 60 min periods in control mice and in mice injected with 5-HTP 10 mg kg^{-1} s.c. The ability of SB-207266 to inhibit the response to 5-HTP (B) is then shown using data obtained during the first 60 min after injection of 5-HTP 10 mg kg^{-1} s.c., after predosing orally with vehicle control or with different doses of SB-207266; the mean number of faecal pellets excreted by the mice not treated with 5-HTP are also shown, for comparison with the effects of the high doses of SB-207266. Data are expressed as the mean (±s.e.mean) total number of pellets excreted during each of the three 60 min periods and were analysed using Mann–Whitney *U*-tests. The Kruskal–Wallis *P*-values were <0.01 in the first time period and >0.05 in the other two time periods. $^{*}P<0.0085$ or $^{**}P<0.0017$ (the Bonferroni adjusted 5% and 1% significance levels) when (A) the effects of 5-HTP are compared with the control mice or (B) when the effects of the different doses of SB-207266 on the response to 5-HTP are compared with the effects of 5-HTP after predosing with the vehicle for SB-207266 (0 μg kg^{-1} p.o.); $n=8$ for each observation.

Table 1 The effects of 5-HTP 10 mg kg^{-1} s.c. + SB-207266 on the mean weight of faecal pellets excreted by mice over a 180 min observation period

Observation period (min)	*Control pellet weights* (mg)	*Mean pellet weights* (mg) *after 5-HTP + SB-207266* (μg kg^{-1} p.o.) *0*	*1*	*10*	*100*	*1000*
0–60	110.8±12.3	241.8±27.0++	172.4±25.6	147.4±23.8	123.4±22.1*	113.0±14.4**
61–120	19.3±8.0	12.7±6.4	6.9±5.4	12.3±8.1	36.5±16.8	10.6±3.9
121–180	1.7±1.7	4.1±4.1	10.1±5.0	11.9±7.1	8.2±4.3	7.8±3.9

Data are expressed as means±s.e.mean ($n=8$ each). The Kruskal–Wallis *P*-values were <0.01 in the first time period and >0.05 in the other two time periods. $^{++}P<0.0017$ (the Bonferroni adjusted 1% signifucance level) when the effects of 5-HTP alone are compared with the solvent controls (Mann–Whitney *U*-test); $^{*}P<0.0085$ or $^{**}P<0.0017$ (the Bonferroni adjusted 5% and 1% significance levels) when the effects of each dose of SB-207266 are compared back with the effects of 5-HTP and solvent alone.

times the dose which completely prevented the effect of 5-HTP (Figure 1). A similar pattern of activity was observed with SB-207266 when measuring the mean weight of faecal pellets excreted after dosing with 5-HTP and SB-207266 or its solvent (Table 1).

Restraint stress-induced defecation in fed rats

In the control group of unrestrained or restrained rats, measurements were made 30 min after oral administration of an equivalent volume of water (10 ml kg^{-1}) used to dissolve SB-207266. For the un-restrained rats, defecation occurred rarely during the first 120 min of observation, with most defecation being recorded during the third, final hour of the experiment (Figure 2). Restraint stress significantly increased the number of excreted faecal pellets, this increase occurring during the first and second 60 min periods of observation. During the third hour of restraint stress, the number of excreted pellets tended to decline and was similar to the control values (Figure 2). A similar pattern of change was observed when measuring the mean weights of the faecal pellets excreted (Table 2). SB-207266 1–1000 μg kg^{-1} p.o. did not prevent the stress-induced increase in faecal pellet defecation or the mean weight of these pellets during the first 60 min of observation. However, SB-207266 dose-dependently reduced or prevented the increase in faecal pellet output and mean faecal pellet weight during the second hour of observation (Figure 2; Table 2). For the incidence of defecation, the ID_{50} for this effect was approximately 0.8 μg kg^{-1} p.o. At this time-point, the number of faecal pellets excreted after administration of the highest dose of SB-207266 tested (1000 μg kg^{-1} p.o.) did not differ significantly from the value recorded using non-stressed rats (Figure 2). A similar, approximately dose-dependent effect of SB-207266 was also observed during the third 60 min period of observation (Figure 2; Table 2) and at this time-point, the number of faecal pellets excreted after dosing with SB-207266

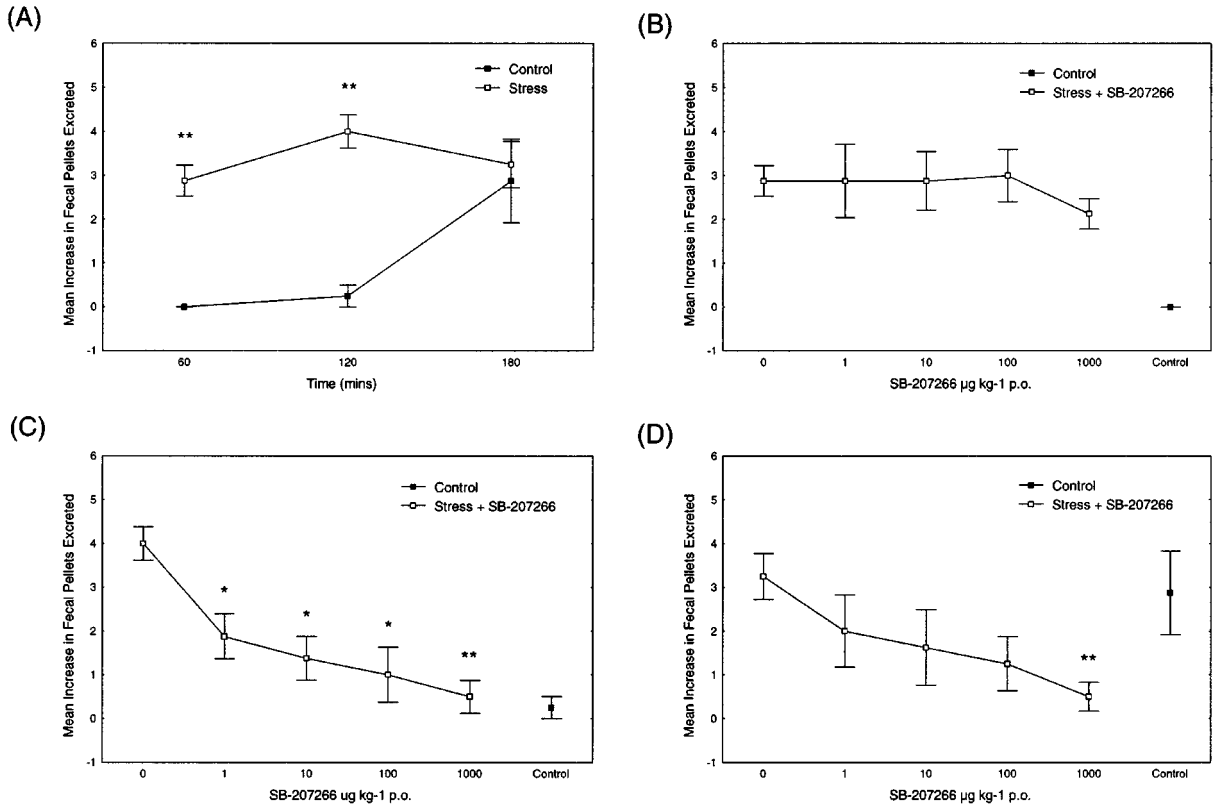

Figure 2 Increased defecation by fed rats caused by restraint stress and the time- and dose-dependent inhibition of this increase by SB-207266. The number of faecal pellets excreted were monitored during three 60 min periods in (A) control rats and in rats exposed to restraint stress. The effects of different doses of SB-207266 on the stress-induced defecation is then shown during the first (B), second (C) and third (D) of those 60 min time periods; the mean number of faecal pellets excreted by the unstressed, control rats are also shown, for comparison with the effects of the high doses of SB-207266. Data are expressed as the mean (±s.e.mean) total number of pellets excreted during each of the three 60 min periods and were analysed using Mann–Whitney *U*-tests. The Kruskal–Wallis *P*-values were <0.01 for the first two time periods and 0.06 in the third period. $*P<0.0085$ or $**P<0.0017$ (the Bonferroni adjusted 5% and 1% significance levels) when (A) the effects of Restraint Stress are compared with the control mice or (B–D) when the effects of the different doses of SB-207266 on the response to stress are compared with the effects of stress after predosing with the vehicle for SB-207266 (0 μg kg^{-1} p.o.); $n=8$ for each observation.

Table 2 The effects of stress + SB-207266 on the mean weight of faceal pellets excreated by fed rats over a 180 min observation period

Observation period	*Control weights* (mg)	*Mean pellet weights* (mg) *during stress + SB-207266* (μg kg^{-1} po)				
		0	*1*	*10*	*100*	*1000*
0–60	0.0±0.0	999.3±199.8++	892.9±296.9	881.3±164.4	783.3±164.0	800.4±157.5
61–120	76.0±76.0	1227.0±115.5++	786.9±198.0	406.1±155.9*	425.1±241.7	187.6±132.1**
121–180	536.8±190.9	907.6±153.7	685.1±284.3	463.5±204.3	338.8±185.6	238.6±156.9

Data are expressed as mean±s.e.mean ($n=8$ each). The Kruskal–Wallis *P*-values were <0.01 in the first two periods and >0.05 in the third time period. $++P<0.0017$ (the Bonferroni adjusted 1% significance level) when the effects of stress alone are compared with the solvent controls (Mann–Whitney *U*-test); $*P<0.0085$ or $**P<0.0017$ (the Bonferroni adjusted 5% and 1% significance levels) when the effects of each dose of SB-207266 are compared back with the effects of stress and solvent alone.

1000 μg kg^{-1} p.o. tended to be less than the value recorded using non-stressed rats; an inference confirmed to some extent by the weak statistical evidence ($P=0.07$; Figure 2).

Restraint stress-induced diarrhoea in fasted rats

After injection of the vehicle for SB-207266, restraint stress induced the appearance of watery diarrhoea/loose stools in each of eight rats tested. Although this effect tended to be reduced by SB-207266 1 (six of eight rats showed evidence of diarrhoea/loose stools), 10 (five of eight rats), 100 (four of eight rats) or 1000 (four of eight rats) μg kg^{-1} p.o., this overall tendency was not statistically significant ($P>0.05$).

Discussion

It is established that 5-HT$_4$ receptor antagonism can prevent the ability of exogenously-applied 5-HT to sensitize the peristaltic reflex in isolated preparations of intestine (see Introduction for references). Similarly, 5-HT$_4$ receptor antagonism prevented or reduced the ability of 5-HTP to increase the incidence of defecation of formed faecal pellets in conscious, fed mice (Banner *et al*, 1996; Sanger *et al*, 1998) and the current experiments with 5-HTP are supportive of this finding. In these experiments, we used low doses of the 5-HT precursor 5-HTP rather than exogenous 5-HT to stimulate gastrointestinal function, because we wanted to study disturbances in defecation behaviour mediated predominantly

via the enteric nervous systems, rather than simply watery diarrhoea and/or muscle spasm. Differences between the *in vivo* actions of these two substances were illustrated by Sanger & McClelland (1986) who showed that whereas exogenous 5-HT directly contracted rat gastric smooth muscle, 5-HTP stimulated enteric cholinergic function and increased gastric motility. Similarly in human volunteers, atropine prevented the induction of prolonged intestinal contractility caused by intravenous injection of 5-HTP, but had no effect on the relatively short-lasting contraction evoked by 5-HT (Haverback & Davidson, 1958). Sanger & McClelland (1986) suggested that since 5-HT-containing neurones exist within the enteric nervous system and since enteric sensory nerves are likely to be activated by the local release of 5-HT from EC cells it is, therefore, more likely that stimulation of enteric neuronal function by exogenous 5-HTP will mimic the effects of small changes in endogenous gastrointestinal 5-HT release. It follows that if 5-HT is involved in the mechanisms of stress-induced defecation and/or in the mechanisms of functional bowel disorders (Sanger, 1996), the use of 5-HTP may be more relevant to such conditions.

Our data also supports the conclusion that 5-HT_4 receptors have little or no role to play in normal gut function (see Introduction) since even the high doses of SB-207266 did not reduce the level of defecation to below that observed in normal mice not exposed to 5-HTP. Thus, there would appear to be a clear role for the 5-HT_4 receptor, not necessarily in normal gut physiology, but in mediating the ability of inappropriate levels of 5-HT to disturb gut function.

In the present experiments, different patterns of defecation were observed in control groups of fed rats or mice. Compared with the mice which defecated mostly during the first 60 min of observation, declining thereafter, rats defecated less frequently and instead, the overall number of faecal pellets slowly increased over the 3 h observation period. Compared with the control rats, the first 2 h of restraint stress substantially increased the numbers of faecal pellets excreted and their mean weight; this increase tended to decline during the third hour, so that the overall level of pellet excretion no longer differed from the controls at this time point. Although not measured, it seems likely that the increased weight of the faecal pellets is predominantly the consequence of an increased fluid content, previously demonstrated by others in response to stress (Barclay & Turnberg, 1987) or by 5-HTP (Banner *et al*., 1996). SB-207266 did not immediately prevent these effects of stress and this is consistent with a report that 5-HT_4 receptor antagonism did not affect the increased defecation of rats caused by 1 h exposure to wrap restraint stress (Yamamoto *et al*., 1998). However, SB-207266 did prevent the continuation of this increased defecation caused by maintaining the exposure to restraint stress for up to 180 min. These data suggest that the effects of restraint stress on the gut are mediated initially by mechanisms other than those which involve the 5-HT_4 receptor, but that the 5-HT_4 receptor subsequently plays a critical role in the mechanisms by which the effects of continued stress are sustained. After 3 h of stress, the apparent reduction in the level of defecation caused by SB-207266, relative to the untreated control rats, may be attributed to the loss of so many faecal pellets earlier in the experiment and hence to an inability to match previous control levels of defecation at this time point.

The ability of stress to increase intestinal fluid secretion (Barclay & Turnberg, 1987) becomes more obvious in fasted rats, in which watery diarrhoea and/or the excretion of loose stools are evoked by restraint stress. In this model, previously identified by Miyata *et al*. (1992), 5-HT_4 receptor antagonism tended to reduce the severity of the diarrhoea but did not totally prevent the effect. Nevertheless, the trend is consistent with a previous study in which the 5-HTP-evoked increase in faecal pellet fluid content was reduced by 5-HT_4 receptor antagonism (Banner *et al*., 1996). It may also be consistent with the present data in which SB-207266 significantly reduced the increased weight of faecal pellets caused by 5-HTP or by the effects of stress in fed rats.

A preliminary report has previously indicated that selective 5-HT_4 receptor antagonism might prevent the increased defecation caused by wrap restraint stress over 2 h (Pichat *et al*., 1996) and in addition, a mixed 5-HT_3 + 5-HT_4 receptor antagonist has been shown to exert inhibitory activity in a similar model of stress (Kadowaki *et al*., 1996). Our data with SB-207266, therefore, confirms and greatly extends the conclusions derived from these experiments. This ability of SB-207266 to inhibit the effects of stress on gut function is likely to be related to a reported stress-induced increase in the availability of 5-HT at the 5-HT_4 receptors within the gut (see below for Discussion). However, alternative possibilities exist. Firstly, SB-207266 could have reduced the increased incidence of defecation simply by inducing constipation. Selective 5-HT_3 receptor antagonists also inhibit stress-induced defecation (Miyata *et al*., 1993; 1998), but since these agents cause constipation in normal individuals (see Sanger, 1999 for references), a true role for the 5-HT_3 receptor in the mechanisms of disturbed defecation cannot be claimed. Recent data using alosetron, for example, indicates that this compound may reduce the symptom of diarrhoea in female diarrhoea-predominant IBS patients, but in this study, there was also a high incidence of constipation (Mangel & Northcutt, 1999). By contrast, the current experiments with SB-207266 and more especially, the failure to detect a constipation-like behaviour with a number of different 5-HT_4 receptor antagonists in several different experimental paradigms (see Introduction), strongly suggests that the predominant role of the 5-HT_4 receptor is not physiological, but pathological. Interestingly, this conclusion is also supported in a different way by the current experiments, in which SB-207266 was found not to prevent the increase in defecation during the first 60 min period of restraint, a time at which the level of stress might be at its highest and/or at which other mechanisms may be operating to increase defecation. Thus, this compound did not cause a generalized suppression of gut function, but acted specifically to remove the pathology mediated *via* the 5-HT_4 receptor.

A second possible mechanism by which 5-HT_4 receptor antagonism might prevent stress-induced defecation is related to the finding that 5-HT_4 receptor antagonism may cause a small reduction in rodent anxiety-like behaviour caused by a novel social interaction between different rats (Kennett *et al*., 1997). However, although immobilization stress can increase the availability of 5-HT within specific rat brain areas (e.g., Nakahara & Nakamura, 1999), 5-HT_4 receptor antagonism does not affect the more robust anxiety-like behaviour caused by punished performance (Kennett *et al*., 1997), a procedure more likely to match the level of stress experienced by restraint. Nevertheless, to confirm this suggestion, further experiments are now required to examine the effects of 5-HT_4 receptor antagonism on the defecation behaviour in rats exposed to the different models of anxiety-like behaviour used by Kennett *et al*. (1997).

The ability of 5-HT_4 receptor antagonism to prevent stress-induced defecation is consistent with the ability of 5-HT_4 receptor antagonism to prevent stress-induced increases in intestinal allodynia (Pichat *et al*., 1996) and possibly, with the involvement of the 5-HT_4 (and 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} but not 5-HT_3) receptor in the mechanisms by which restraint

stress increases ACTH secretion (Jorgensen *et al*., 1998). These observations are also consistent with studies in rats and humans which have shown that the urinary excretion of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) is greatly increased by stressful procedures; urinary 5-HIAA is thought to originate mostly from the large amounts of 5-HT contained with gastrointestinal enterochromaffin (EC) cells (Anthony & Lance, 1967; Kundrotas & Gregg, 1977). The mechanisms by which stress releases 5-HT from EC cells are not well understood but could involve the actions of circulating adrenaline or of spinal efferent noradrenergic neurones projecting from the spinal cord to the EC cells of the gut (Burks & Long, 1966; Larsson *et al*., 1979; Pettersson, 1979). The current experiments indicate that the consequences of this release on 5-HT_4 receptor and on gut function are profound. Perhaps critically, if rats are then exposed to repeated periods of stress, the responsiveness to 5-HT at the 5-HT_4 receptors themselves may also alter. Thus, Goldhill *et al*. (1998) reported that the 5-HT-induced, 5-HT_4 receptor-mediated increase in short circuit current in rat isolated colonic mucosa becomes either hyper- or hypo-responsive after repeated restraint stress over 3 days.

Increases in 5-HT blood plasma concentrations have been reported in patients with IBS (Bearcroft *et al*., 1998) and in a single pilot study, 5-HT_4 receptor antagonism by SB-207266 reduced the transit time along the small intestine in diarrhoea-predominant IBS patients (Houghton *et al*., 1999). Taken together with the current experiments, these data again highlight the suggestion that whilst the 5-HT_4 receptor plays little or no role in normal gut physiology, it does play a role in mediating at least some pathological changes in intestinal function. 5-HT_4 receptor antagonism by SB-207266 may, therefore, provide a means of desensitizing the bowel during disturbances in gut function caused by inappropriate activity of 5-HT.

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