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Selective and functional 5-hydroxytryptamine$_4$ receptor antagonism by SB 207266

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1 The pharmacology of a novel 5-HT$_4$ receptor antagonist, SB 207266 has been evaluated *in vitro* in the guinea-pig distal colon longitudinal muscle myenteric plexus (LMMP) and *in vivo* in the dog Heidenhain pouch.

2 SB 207266 is a highly potent antagonist of 5-HT-evoked, cholinergically-mediated contractions in the guinea-pig distal colon. Low concentrations (0.1 – 10 nM) produced a parallel shift to the right of the concentration-effect curve (apparent pA_2 10.6 ± 0.1) with no significant effect on the maximum response. With higher concentrations of SB 207266 (30 nM and above) the maximum response to 5-HT was reduced.

3 The antagonism seen with SB 207266 cannot be attributed to a non-selective effect since high concentrations (1 μM) had no effect on cholinergically-mediated contractions evoked by the nicotinic receptor agonist DMPP in the same preparation.

4 SB 207266 is not an irreversible antagonist since the effects of the compound were reversible upon washing of the tissue.

5 In the dog Heidenhain pouch, oral (0.1 – 100 μg kg^{-1}) and intravenous (0.1 – 100 μg kg^{-1}) administration of SB 207266 produced a dose-dependent antagonism of the contractions evoked by a bolus intravenous injection of 5-HT. An ID_{50} for SB 207266 of 1.3 μg kg^{-1} was obtained following i.v. administration and 9.6 μg kg^{-1} following oral administration.

6 The antagonistic effects of SB 207266 (0.1 – 100 μg kg^{-1}) in the dog Heidenhain pouch were long lasting since, following oral administration, the response to 5-HT was reduced for at least 135 min.

7 SB 207266 is a highly potent, highly selective and orally active 5-HT$_4$ receptor antagonist. This compound is the first orally active amide to be identified in this class of antagonists and as such is an important new tool in the evaluation of 5-HT$_4$ receptor function both *in vitro* and *in vivo*.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT$_4$ receptor; SB 207266; orally active; amide

Introduction

The 5-hydroxytryptamine$_4$ (5-HT$_4$) receptor has been identified in a variety of tissues in both the central nervous system (CNS) and the periphery. In the periphery, 5-HT$_4$ receptors predominate in the gastrointestinal tract where they are thought to stimulate motility at least partly via modulation of intramural cholinergic nerve pathways subserving peristalsis (Tonini *et al.*, 1991). Since the gut is by far the largest single source of 5-HT within the mammalian body, profound changes in function may be expected to occur as a result of even small changes in 5-HT turnover. Antagonists at the 5-HT$_4$ receptor may, therefore, find clinical utility in treating conditions of disturbed bowel function, such as irritable bowel syndrome.

Early characterization of the 5-HT$_4$ receptor was assisted by the use of non-selective agents (for review see Ford & Clarke, 1993) and by the development of two highly potent, highly selective 5-HT$_4$ receptor antagonists, namely GR 113808 (Grossman *et al.*, 1993) and SB 204070 (Wardle *et al.*, 1993; 1994; Gaster *et al.*, 1993). Despite the high potency and selectivity of these compounds *in vitro*, little is known about their activity *in vivo*. SB 204070, dosed intravenously, has been shown to act as a long lasting 5-HT$_4$ receptor antagonist in the dog Heidenhain pouch (Bingham *et al.*, 1995). However, since this compound and most of the other previously reported 5-HT$_4$ receptor antagonists are sterically unhindered esters they would be predicted to undergo rapid breakdown by esterases (see Bedford *et al.*, 1987) and as such, would be expected to display poor oral bioavailability.

More recently, the ester linkage of these compounds has been replaced by moieties which would be expected to confer increased *in vivo* stability. Preliminary results on two such compounds, namely the ketone RS-39604 (1-(4-amino-5-chloro-2-(3,5-dimethoxy) benzyloxphenyl)-3-[1-((2-methylsulphonylamino)ethyl)piperidin-4-yl]-1-propanone, Hegde *et al.*, 1995) and the amide SB 207266 (N-(1-Butyl-4-piperinylmethyl)-3,4-dihydro-2H-[1,3] oxazino [3,2-a] indole-10-carboxamide hydrochloride, Gaster *et al.*, 1995) have been published. We now describe detailed findings on the latter compound, SB 207266. The data suggests that SB 207266 is a potent and selective 5-HT$_4$ receptor antagonist which is orally active and long-lasting *in vivo*. Such a compound will prove to be a useful tool in the full evaluation of the functional role of the 5-HT$_4$ receptor *in vitro* and *in vivo* in both the CNS and periphery.

Methods

Preparation of guinea-pig distal colon tissue

Distal colon (approximately 7 – 8 cm from the anus) was removed from young male Dunkin Hartley guinea-pigs (200 – 300 g) and placed in Krebs solution of the following composition (mM): NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 5.6.

Sections of longitudinal muscle-myenteric plexus (LMMP, 2 – 3 cm in length) were dissected as previously described (Wardle & Sanger, 1993) and mounted under a 0.5 g load in 10 ml tissue baths. Tissues were bathed with Krebs solution at 37°C, gassed with 5% CO_2 in O_2 and containing granisetron (1 μM) and methiothepin (100 nM) to inhibit responses mediated by 5-HT$_3$ and 5-HT$_1$-like and 5-HT$_2$ receptors respectively. Responses were recorded isotonically and displayed on a Lectromed MT8P multitrate chart recorder.

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Experimental protocol and concentration-effect curves.

Tissues were left to stabilize and sensitized to 5-HT as previously described (Wardle & Sanger, 1993). Sensitization was viewed as being complete when three consistent responses were obtained to 5-HT (1 μM). Agonist concentration-effect curves to 5-HT or BIMU 1 were constructed non-cumulatively by adding increasing concentrations of agonist at 15 min intervals. The agonist was left in contact with the tissue until a maximum response was obtained (usually 30 s). Two agonist concentration-effect curves were constructed in each tissue, the first in the absence, the second in the presence of the appropriate concentration of SB 207266. In the control situation, the first and second curve were superimposable. In all experiments, the antagonist was added to the reservoir, washed through to the tissues and left to equilibrate for a minimum of 45 min prior to construction of the second concentration-effect curve. Responses were expressed as a percentage of the maximum 5-HT-evoked contraction in each tissue obtained in the control concentration-effect curve. Results were expressed as mean $\pm$ standard error of mean (s.e.mean) of a number (n) of observations.

All agonist concentration-effect curves were fitted using the following three parameter logistic equation (a rearrangement of the classical Hill equation, see Jenkinson *et al.*, 1995) using Kaleidagraph (Synergy Software, PCS Inc. Reading, Pa, U.S.A.) on an Apple Macintosh II Ci computer.

$$E = \alpha/1 + (EC_{50}/[A])^n$$

α, [A] and n represent the maximum response, agonist concentration and curve mid point slope factor respectively. The EC_{50} is the concentration of agonist that produces 50% of the maximal response.

Where the maximum response to the agonist was not significantly reduced, affinity estimates for the antagonist (SB 207266) were expressed as pA_2 values, calculated according to the method of Arunlakshana & Schild (1959). As higher concentrations of SB 207266 reduced the maximum response, it would appear that something other than simple competitive antagonism was taking place. For this reason, the term 'apparent pA_2' will be used throughout this document.

Onset/recovery of effects of SB 207266

The rate of onset and time to wash out of the antagonistic effects of SB 207266 were investigated in the guinea-pig distal colon LMMP. These experiments were designed to investigate the nature (i.e. reversible or irreversible) of the antagonistic effect of SB 207266. Tissues were set up as previously described and exposed repeatedly to the approximate EC_{50} concentration of 5-HT (generally 1 nM) at 15 min intervals until consistent responses were obtained. Immediately after washout of 5-HT, SB 207266 was added to the bathing solution and left in contact with the tissues for 30 min, during which time tissues were exposed to the same concentration of 5-HT twice. The bathing solution was then replaced with compound-free Krebs solution and the tissues challenged with the same concentration of 5-HT every 15 min until responses returned to control level. The rate of recovery of the antagonistic effects of SB 207266 was calculated in terms of a $t_{1/2off}$. This value was defined as the time taken for the response to 5-HT to recover to 50% of its control value.

Dog Heidenhain pouch

Eight adult male beagle dogs, with previously prepared Heidenhain pouches (Bermudez *et al.*, 1990), were fasted overnight, restrained in Pavlov slings and the cephalic vein cannulated acutely for administration of 5-HT. Doses of 5-HT were chosen which elicited reproducible contractile activity in the Heidenhain pouch but which had no other overt physiological or behavioural effects (5 or 10 μg kg^{-1} i.v. depending upon the individual dog).

The first dose of 5-HT was given 15 min after cannulation and was repeated at 30 min intervals until two consecutive, consistent responses were obtained. Fifteen minutes after the last control response, solutions of SB 207266 (0.1, 1, 3, 10, 100 μg kg^{-1}) or saline were given i.v. in a volume of 0.7–0.8 ml, or orally in a gelatine capsule (0.1, 1, 10, 30 and 100 μg kg^{-1}) in a volume of 0.15–0.2 ml.

Administration of 5-HT recommended 15 min after administration of the antagonist and therafter at 30 min intervals until the end of the observation period (105 min from the i.v. administration of the antagonist and 135 min following oral administration). A minimum of one week was left between each experiment for each dog. All husbandry and procedures were in accordance with the UK Animal (Scientific procedures) Act 1986 and were approved and monitored by trained veterinary staff.

During the experiments, Heidenhain pouch motility was measured by monitoring pouch pressure via a pressure transducer (LEC) and displayed on a chart recorder (LEC). The pressure signal was stored on video tape and either simultaneously integrated at 1 min intervals on line or played back at the end of each experiment and integrated in the same way (integrator made in house).

Duration of action studies on dog Heidenhain pouch

The duration of action of SB 207266 was investigated at a single dose of 10 μg kg^{-1}. The dogs were restrained and dosed i.v. with antagonist (10 μg kg^{-1}) or saline. The dogs were returned to their pen for 24 h before re-restraining in slings and acute cannulation of the cephalic vein as described above. Fifteen minutes later dogs were dosed twice with the standard dose of 5-HT at 30 min intervals.

Analysis of dog Heidenhain pouch results

Results were analysed by Student's t test for unpaired samples. A P value of less than 0.05 was taken as being significant. Results are expressed as mean $\pm$ s.e.mean of a number (n) of observations.

For each of the two control responses to 5-HT, motility was integrated (i.e. area under the pressure curve above baseline) every minute for a total of 3 min before and after administration of 5-HT, the difference calculated and a mean control difference obtained for each dog. This value was taken to be 100%. Responses to 5-HT after dosing with SB 207266 or saline were obtained in the same way and expressed as a percentage of the mean control response. From preliminary experiments a single time point was chosen when the effect of the antagonist was maximal (15 min for i.v. administration and 45 min for oral administration) and data obtained at these time points was used to calculate ID_{50} values using a logistic model and an iterative least squares curve fitting procedure in RS1 statistical software (BBN Software Products Corporation for use with VAX systems).

In studies where the duration of action of SB 207266 was investigated, the mean integrated response to 5-HT obtained after antagonist administration was expressed as a percentage of that obtained after saline administration in a separate experiment in the same dog.

To evaluate the effects of SB 207266 alone, the total integrated response was measured and compared 3 min before and 3 min after the injection of the antagonist.

Drugs

The following drugs were dissolved in 0.9% saline: 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma), 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP, Sigma), methiothepin hydrochloride (Roche), granisetron (SmithKline Beecham),

SB 207266, (N-(1-butyl-4-piperinyl-methyl)-3,4-dihydro-2*H*-[1,3] oxazino [3,2-a] indole-10-carboxamide hydrochloride, synthesized in house). BIMU 1 (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride) was a kind gift from Dr Carlo Rizzi, Boehringer Ingelheim, Italy.

Results

Effects of SB 207266 in the guinea-pig distal colon LMMP

In the presence of methiothepin (100 nM) and granisetron (1 μM), 5-HT (10 pM – 10 nM) evoked a monophasic concentration-dependent contraction with a pEC_{50} of 9.1 ± 0.1 ($n = 6$). This response has previously been shown to be antagonized by tropisetron (pA_2 6.4 ± 0.1, slope of Schild plot 1.3 ± 0.1), SDZ 205 557 (pA_2 7.8 ± 0.1, slope of Schild plot 1.1 ± 0.09) and SB 204070 (pA_2 10.6 ± 0.1 slope of Schild plot 0.9 ± 0.2, Wardle & Sanger, 1993; Wardle *et al.*, 1994).

Low concentrations of SB 207266 (0.1 – 10 nM, $n = 6$, Figure 1) caused a concentration-dependent, surmountable antagonism of the 5-HT response, yielding mean dose-ratios of 5 ± 2, 46 ± 18 and 204 ± 69 at 0.1, 1 and 10 nM respectively. The slope of the Schild plot, calculated over this concentration-range was 0.9 ± 0.1 (Figure 2). When this value was constrained to unity, the apparent pA_2 for SB 207266, calculated over the concentration range of 0.1 to 10 nM was 10.6 ± 0.1. At higher concentrations of SB 207266 (30 and 100 nM), rightward displacements of the concentration-effect curve to 5-HT were associated with a reduction in the maximum response. At all concentrations tested, SB 207266 was a silent antagonist and displayed no detectable intrinsic activity.

SB 207266 was also examined as an antagonist against the responses mediated by BIMU 1, a benzimidazolone derivative reported to possess 5-HT_4 receptor agonist activity (see Tonini *et al.*, 1991). In the guinea-pig distal colon, BIMU 1 was a partial agonist relative to 5-HT with an intrinsic activity of 0.6 ± 0.04 and a pEC_{50} of 7.1 ± 0.1, $n = 4$, Figure 3). SB 207266, at all concentrations tested (0.1 – 10 nM, $n = 4$), produced a concentration-dependent reduction in the amplitude of the maximum response to 5-HT with no prior shift to the right of the curve.

Onset and recovery of antagonistic effects of SB 207266

A 30 min incubation with SB 207266 (0.1, 1, 10 and 100 nM, $n = 6$) caused maximum antagonism of the contraction evoked by a pEC_{50} concentration of 5-HT (1 nM) of 80 ± 6%, 96 ± 3%, 100 ± 0% and 100 ± 0% respectively (Figure 4). Longer incubations with SB 207266 revealed no further antagonism (results not shown). Upon washout, the responses to 5-HT

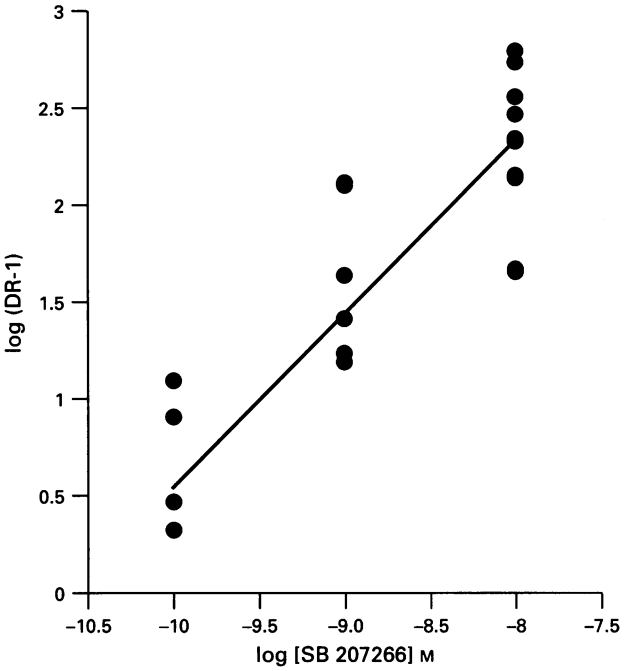

Figure 2 Schild regression analysis on the effects of SB 207266 (0.1 to 10 nM) in guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μM).

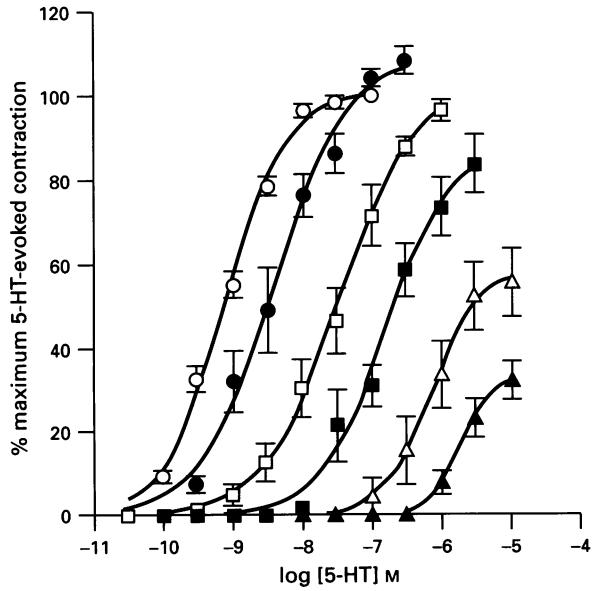

Figure 1 Concentration-response curves to 5-HT in the absence (○) and presence of 0.1 nM (●), 1 nM (□), 10 nM (■), 30 nM (△), and 100 nM (▲) SB 207266 in guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μM). Each point represents the mean ± s.e.mean of 6 observations.

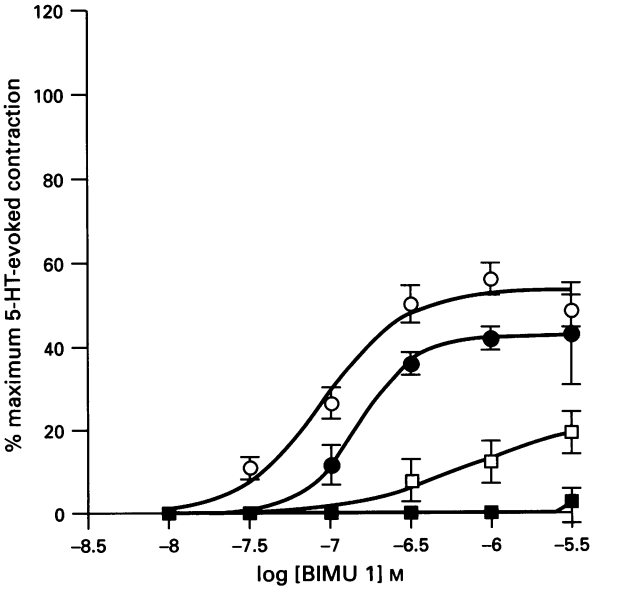

Figure 3 Concentration-response curves to BIMU 1 in the absence (○) and presence of 0.1 nM (●), 1 nM (□) and 10 nM (■) SB 207266 in guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μM). Each point represents the mean ± s.e.mean of 4 observations.

recovered to control levels with estimated $t_{1/2off}$ values of 10±3 min, 29±4 min, 58±3 min and 71±7 min for concentrations of SB 207266 of 0.1, 1, 10 and 100 nM respectively.

Selectivity of action of SB 207266 in the guinea-pig distal colon

In the guinea-pig distal colon the nicotine receptor agonist, DMPP (3–300 μM, $n=4$) evoked concentration-dependent, cholinergically-mediated contractions with a pEC_{50} value of 5.4±0.03. These responses were not affected by 1 μM SB 207266 (pEC_{50} 5.4±0.05, maximum response 95±5% of control curve, data not shown), a concentration significantly greater than that required to antagonize the cholinergically-mediated contractions evoked by 5-HT in the same preparation.

Dog Heidenhain pouch intravenous dosing studies

In the dog Heidenhain pouch, i.v. administration of SB 207266 (0.1–100 μg kg^{-1}) had no effect on basal motility when measured over a 3 min observation period (e.g. mean integrated response before and after administration of 100 μg kg^{-1} SB 207266 calculated to be 12.8±3.6 and 10.4±1.3, respectively). Over this dose-range, SB 207266 had no other observable effects (e.g.: behaviour, respiration – monitored throughout the experiment).

Fifteen min after intravenous administration, SB 207266 (100 and 10 μg kg^{-1}) abolished the response to 5-HT (5 or 10 μg kg^{-1}, i.v.) and reduced the responses to 5-HT in a dose-dependent fashion at 3 and 1 and 0.1 μg kg^{-1}. An ID_{50} of 1.3 μg kg^{-1} (confidence limits 0.1–14.0 μg kg^{-1}) was calculated for the 15 min time period (Figure 5).

The recovery from antagonism was also dose-dependent. At 100 μg kg^{-1} SB 207266, the response to 5-HT was effectively abolished for the duration of the experiment (105 min). With 10 μg kg^{-1} there was a small recovery towards the end of the experiment and at 3 μg kg^{-1} the response to 5-HT remained similar at all time points. At 1 μg kg^{-1} and 0.1 μg kg^{-1} SB 207266, the response to 5-HT returned to control levels within 75 min (Table 1).

Dog Heidenhain pouch oral dosing studies

SB 207266 (0.1–100 μg kg^{-1}) had no effect on basal motility when administered orally (e.g. mean integrated response before and after administration of 100 μg kg^{-1} SB 207266 calculated to be 14.0±3.1 and 16.1±3.7 respectively). Fifteen min after oral administration of SB 207266 (100, 30 and 10 μg kg^{-1}) there was no effect on the contractile response to 5-HT (5 or 10 μg kg^{-1}, i.v.); however, 45 min after administration, there was a dose-dependent reduction in the 5-HT-evoked response, an effect that was maintained for the duration of the experiment (135 min, Table 2). A lower dose of SB 207266 (1 μg kg^{-1}) showed no significant antagonism of the 5-HT-evoked response 45 min after administration but did produce a small but maintained antagonism 75 min after administration. An ID_{50} of 9.6 μg kg^{-1} (confidence limits 0.7–128 μg kg^{-1}) was calculated from data obtained 45 min after administration of SB 207266 (Figure 5).

Discussion

The pharmacology of a novel 5-HT_4 receptor antagonist, SB 207266, has been investigated *in vitro* against 5-HT_4 receptor-mediated contractions in the guinea-pig distal colon (Wardle & Sanger, 1993) and *in vivo* against 5-HT_4 receptor-mediated contractions in the dog Heidenhain pouch (Bermudez *et al.*, 1990).

In the guinea-pig distal colon, SB 207266 alone had no effects on resting tone, suggesting that it is devoid of agonist effects at the 5-HT_4 receptor. When examined against 5-HT-evoked contractions, low concentrations of SB 207266 shifted the curve in an apparently competitive manner (mean apparent

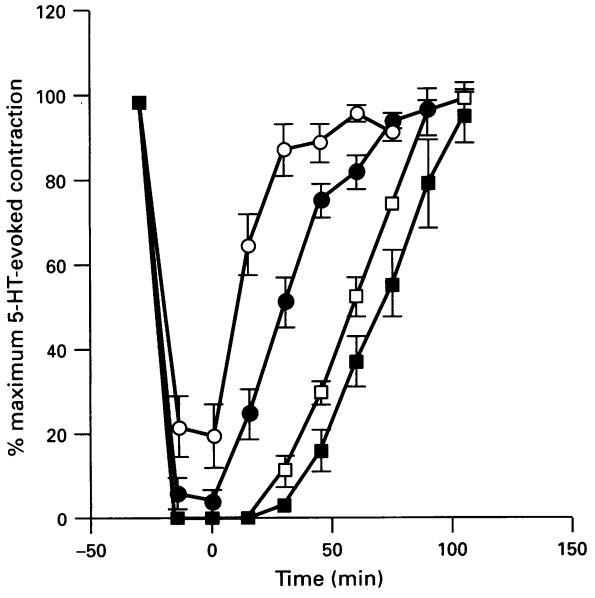

Figure 4 Onset and recovery of the antagonistic effects of SB 207266 in the guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. Tissues were dosed with a standard pEC_{50} concentration of 5-HT until stable responses were produced. At time $t=-30$ min various concentrations of SB 207266 (0.1 nM (○), 1 nM (●), 10 nM (□) and 100 nM (■)) were added to the bath. Following a 30 min incubation with SB 207266 (during which time 5-HT was given twice) the antagonist was washed from the bath and tissues dosed at 15 min intervals until responses to 5-HT returned to control levels. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μM). Each point represents the mean± s.e.mean of 4 observations.

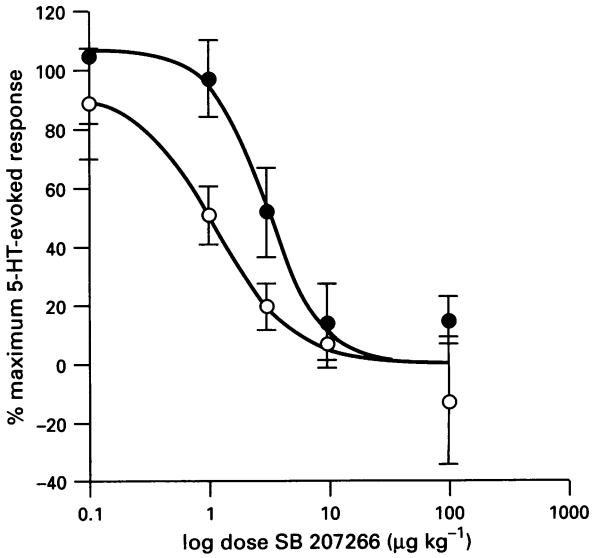

Figure 5 Dose-response curves showing the effects of intravenous (i.v. ○,) and oral (p.o., ●) SB 207266 in the dog Heidenhain pouch on the contractile response to 5-HT. For each dose of SB 207266 data were taken 15 min following i.v. administration and 45 min following p.o. administration. Responses to bolus injections of 5-HT are expressed as a percentage of the mean of two control responses. Data are expressed as mean±s.e.mean with a minimum of 4 dogs in each group. ID_{50} values of 1.3 μg kg^{-1} (confidence limits 0.1–14.0 μg kg^{-1}) and 9.6 μg kg^{-1} (confidence limits 0.7–128 μg kg^{-1}) were calculated for i.v. and oral administration respectively.

Table 1 The effects of intravenous (i.v.) SB 207266 (0.1–100 $\mu g\ kg^{-1}$) on the contractile response of the dog Heidenhain pouch to bolus injections of 5-HT

		Time (min)			
Dose (i.v.)	*Control*	*15*	*45*	*75*	*105*
Saline ($n=7$)	100	126.2 ± 15.7	138.9 ± 26.4	106.2 ± 13.2	146.3 ± 28.8
0.1 $\mu g\ kg^{-1}$ ($n=4$)	100	88.9 ± 18.9	83.9 ± 16.2	92.4 ± 10.2	80.3 ± 42.8
1 $\mu g\ kg^{-1}$ ($n=7$)	100	50.9 ± 9.7**	54.3 ± 12.8**	90.6 ± 24.9	94.6 ± 31.8
3 $\mu g\ kg^{-1}$ ($n=4$)	100	19.7 ± 7.9***	14.9 ± 14.8**	27.9 ± 22.4**	20.3 ± 17.5**
10 $\mu g\ kg^{-1}$ ($n=8$)	100	7.0 ± 5.7***	13.4 ± 10.0***	19.3 ± 5.6***	32.4 ± 12.4**
100 $\mu g\ kg^{-1}$ ($n=7$)	100	−12.6 ± 21.8***	10.7 ± 10.9***	9.83 ± 6.8***	2.64 ± 5.5***

Responses, obtained at the times indicated after administration of saline or SB 207266, are expressed as a percentage of the mean of two control integrated responses. Data are expressed as mean ± s.e.mean where n = number of dogs. Results were analysed by Student's unpaired t test: P values of *0.05, **0.01 and ***0.001 when compared with time-matched saline controls.

Table 2 The effects of oral (p.o.) SB 207266 (0.1–100 $\mu g\ kg^{-1}$) on the contractile response of the dog Heidenhain pouch to bolus injections of 5-HT

		Time (min)				
Dose (p.o.)	*Control*	*15*	*45*	*75*	*105*	*135*
Saline ($n=4$)	100	133.0 ± 20.6	103.9 ± 3.5	96.9 ± 9.9	88.5 ± 17.4	85.1 ± 13.2
0.1 $\mu g\ kg^{-1}$ ($n=4$)	100	92.5 ± 18.4	104.9 ± 23.0	81.7 ± 6.3	93.8 ± 13.1	47.2 ± 16.3
1 $\mu g\ kg^{-1}$ ($n=3$)	100	103.3 ± 11.4	97.5 ± 13.0	68.6 ± 12.1	65.9 ± 23.9	49.9 ± 16.0
10 $\mu g\ kg^{-1}$ ($n=6$)	100	91.0 ± 8.7*	52.1 ± 15.0*	52.8 ± 19.6	26.8 ± 23.0	37.7 ± 12.9*
30 $\mu g\ kg^{-1}$ ($n=3$)	100	67.1 ± 17.4*	13.2 ± 14.6**	18.2 ± 11.2**	6.7 ± 10.9**	5.4 ± 6.6**
100 $\mu g\ kg^{-1}$ ($n=4$)	100	80.0 ± 39.0	15.0 ± 8.0***	13.0 ± 11.0***	20.0 ± 11.0**	26.0 ± 16.0*

Responses, obtained at the times indicated after administration of saline or SB 207266, are expressed as a percentage of the mean of two control integrated responses. Data are expressed as mean ± s.e.mean where n = number of dogs. Results were analysed by Student's unpaired t test: P values of *0.05, **0.01 and ***0.001 when compared with time-matched saline controls.

pA_2 of 10.6 ± 0.1), whereas higher concentrations produced a further shift to the right of the curve with a concomitant reduction in the maximum response.

It has previously been shown that, in the guinea-pig distal colon, 5-HT_4 receptor-mediated contractions are evoked indirectly via acetylcholine release (Wardle & Sanger, 1993). Thus, the possibility that the apparent non-surmountable antagonism observed with SB 207266 was due to a non-selective effect was investigated against DMPP-evoked, cholinergically-mediated contraction in the same preparation. SB 207266, at concentrations of up to 10,000 fold greater than those required to block the 5-HT_4 receptor had no significant effect on DMPP-evoked contractions, suggesting that the reduction in maximum seen with SB 207266 is unlikely to be due to either a local anaesthetic or anti-cholinergic effect of the antagonist.

Similarly, radioligand binding studies previously published on SB 207266 indicate a lack of affinity of the antagonist for both other 5-HT receptors and other classical neurotransmitter receptors (Gaster *et al.*, 1995). These findings suggest that SB 207266 is highly selective for the 5-HT_4 receptor and that the non-surmountable effects are unlikely to be due to antagonism of a non-5-HT_4 site.

The non-surmountable antagonism observed with SB 207266 in the present study is also unlikely to be due to irreversible antagonism since, upon repeated washing of the compound from the bathing solution, the responses to a pEC_{50} concentration of 5-HT were restored to control levels for all concentrations of SB 207266 tested.

Thus, as has previously been suggested for SB 204070 (Wardle *et al.*, 1994), the most likely explanation for the apparent non-surmountable effects of SB 207266 in the guinea-pig distal colon is that the compound acts as a pseudoirreversible antagonist at the 5-HT_4 receptor. Since the 5-HT_4 receptor-mediated response in the guinea-pig distal colon has previously been proposed to be a well coupled system (Wardle & Sanger, 1993), the initial shift to the right of the curve seen with the low concentrations of SB 207266 may be attributed to the occupation and removal of spare receptors in a manner similar to that expected for an irreversible antagonist (Kenakin, 1993). This idea is supported by the experiments involving the 5-HT_4 receptor partial agonist, BIMU 1. Thus, under conditions where no receptor reserve exists, SB 207266, at all concentrations tested, produced an immediate reduction in the maximum response, without any prior shift to the right of the curve. Such an observation is consistent with the idea of pseudoirreversible antagonism. For full discussion of this concept, see Wardle *et al.* (1994).

In the conscious dog Heidenhain pouch, systemic administration of 5-HT elicits a cholinergically mediated, contractile response which has previously been shown to be blocked by

the selective 5-HT_4 receptor antagonist SB 204070 (Bingham *et al.*, 1995). In the present study SB 207266 dose-dependently reduced the contractile response to 5-HT when given by both the intravenous and oral routes and the potency and efficacy by both routes were comparable. As has previously been reported for SB 204070 (Bingham *et al.*, 1995), SB 207266 had no effects on spontaneous contractile activity at any concentration tested, indicating a lack of 5-HT_4 receptor-mediated tone in this preparation and no intrinsic 5-HT_4 receptor agonist activity. This latter observation supports findings from the *in vitro* study of a lack of intrinsic activity of this compound.

These experiments *in vitro* and *in vivo* imply that SB 207266 exerts its effects at the level of the enteric nervous system following systemic absorption. It exerts its effects rapidly, onset of antagonism was achieved within 15 min of administration by the intravenous route and between 15 and 45 min by the oral route and lasted for at least the duration of the experiment at the higher doses. Recovery from the antagonistic effects of SB 207266 was dose-dependent; at the lower doses (0.1–1 $\mu g\ kg^{-1}$) the response to 5-HT had returned to control levels within 105 min of intravenous administration confirming the findings from the *in vitro* study that SB 207266 is not an irreversible antagonist.

The present results suggest that the amide, SB 207266 is a highly potent, highly selective 5-HT_4 receptor antagonist which displays good oral bioavailability, a rapid rate of onset and a long duration of action. This compound will prove to be a useful tool in understanding the pathophysiological role of the 5-HT_4 receptor.

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