The effects of SB 204070, a highly potent and selective 5-HT₄ receptor antagonist, on guinea-pig distal colon

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1 The pharmacology of a novel 5-HT₄ receptor antagonist, SB 204070 has been evaluated in the guinea-pig isolated distal colon longitudinal muscle-myenteric plexus (LMMP).

2 SB 204070 is a highly potent antagonist of 5-HT-evoked cholinergically-mediated contractions in the guinea-pig distal colon. Low concentrations (10–100 pM) produced a shift to the right of the curve (apparent pA₂ 10.8 ± 0.1) with no significant effect on the maximum response. With higher concentrations of SB 204070 (300 pM and above), the maximum response to 5-HT was reduced.

3 When tested against the partial 5-HT₄ receptor agonist, BIMU 1, SB 204070 was active at similar low concentrations (10 pM and above) but produced a reduction in maximum, with no prior shift to the right of the curve, at all concentrations tested (10–300 pM).

4 The antagonism seen with SB 204070 is unlikely to be due to a non-selective effect since high concentrations (10 nM and 1 μM) of the compound had no effect on cholinergically-mediated contractions evoked by the nicotinic receptor agonist, DMPP, in the same preparation. SB 204070 is unlikely to be an irreversible antagonist since the effects of the compound could be reversed upon washing of the tissue.

5 Radioligand binding studies show that SB 204070 has a greater than 5000 fold selectivity for the 5-HT₄ receptor over 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, GABA_A, BDZ, TBPS, A₁ adenosine receptors, α₁, α₂, β₁, β₂ adrenoceptors and D₁, D₂ and D₃ dopamine receptors.

6 SB 204070 is a highly potent, highly selective 5-HT₄ receptor antagonist and as such is an important new tool in evaluating the functional role of the 5-HT₄ receptor.

Keywords: 5-HT (5-hydroxytryptamine); 5-HT₄ receptors; SB 204070; guinea-pig distal colon

Introduction

A novel 5-hydroxytryptamine (5-HT) receptor, positively coupled with adenylate cyclase was identified in mouse embryo colliculi neurones by Dumuis and co-workers as far back as 1988 (Dumuis *et al.*, 1988a,b). The receptor was tentatively named 5-HT₄ due to its inability to fit into the Bradley *et al.* (1986) classification. Since then, the 5-HT₄ receptor has been officially recognised (Humphrey *et al.*, 1993) and identified in a variety of tissues across many species (for review see Ford & Clarke, 1993).

Until recently, much of the characterization of the 5-HT₄ receptor has been based on the activity of agonists. Thus, the 5-HT₄ receptor is activated by indoleamine agonists (5-HT and 5-methoxytryptamine (5-MeOT)), by substituted benzamides (such as metoclopramide, renzapride, cisapride and zacopride) and by azabicycloalkyl benzimidazolones (such as BIMU 1 and BIMU 8). The antagonist profile of the 5-HT₄ receptor has been based largely on exclusion. Tropisetron was the first compound to be identified as having antagonist activity at the 5-HT₄ receptor (Dumuis *et al.*, 1988a). Tropisetron, however, possesses a 10 to 100 times higher affinity for the 5-HT₃ receptor than for the 5-HT₄ receptor (Clarke *et al.*, 1989). Furthermore, tropisetron has been shown to block both muscarinic receptors (Clarke *et al.*, 1989) and sodium channels (Scholtysik, 1987). An alternative antagonist, SDZ 205 557 has been shown to display around 50 fold selectivity for the 5-HT₄ receptor over the 5-HT₃ receptor in the guinea-pig isolated ileum (Buchheit *et al.*, 1992). However, since the 5-HT₃ receptors in guinea-pig yield lower antagonist affinity estimates than other species (Butler *et al.*, 1990), this selectivity appears to be an over-estimation (see Eglen *et al.*, 1992a).

More recently, compounds with much greater selectivity for the 5-HT₄ receptor have been reported. These include

RS-23597-190 (Eglen *et al.*, 1992b) and GR 113808 (Grossman *et al.*, 1993). RS-23597-190 has been shown to antagonize 5-HT₄ receptor-mediated relaxations in the rat oesophagus, yielding a pA₂ estimate of 7.8 (Eglen *et al.*, 1992b) and has selectivity over 5-HT₃ and dopamine receptors of greater than 125 fold. GR 113808 has been shown to act as a competitive antagonist of 5-HT₄ receptor-mediated responses in both the guinea-pig proximal colon and rat oesophagus, with pA₂ values of 9.2 and 9.5 respectively, with selectivity over a range of receptors of approximately 3000 fold (Grossman *et al.*, 1993).

In the present study we describe details of another highly potent and selective 5-HT₄ receptor antagonist, the benzodioxan derivative, SB 204070. The pharmacology of this compound has been characterized in the guinea-pig distal colon (Wardle & Sanger, 1992; 1993). Preliminary results have previously been reported (Wardle *et al.*, 1993; Gaster *et al.*, 1993).

Methods

Preparation of guinea-pig distal colon tissue

Distal colon (discarding the terminal 7–8 cm) 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, CaCl₂ 2.5, KH₂PO₄ 1.2, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25.0, 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% CO₂ in O₂ and containing granisetron (1 μM) and methiothepin (100 nM) to inhibit responses mediated by 5-HT₃ and 5-HT₁-like and 5-HT₂ receptors

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respectively. Responses were recorded isotonicly and displayed on a LECTROMED MT8P multitrace chart recorder.

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 μ M). Agonist concentration-effect curves 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), then washed out. Two agonist concentration-effect curves were constructed in each tissue, the first in the absence, the second in the presence of SB 204070. In all experiments, the antagonist was left to equilibrate with the tissues for a minimum of 45 min. 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 $\pm$ s.e.mean of a number (*n*) of observations.

All agonist concentration-effect curves were fitted to the following equation 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. EC_{50} values from individual curves were converted to $\log_{10}$ values prior to calculation of the arithmetic mean.

Where the maximum response to the agonist was not significantly reduced, affinity estimates for the antagonist (SB 204070) were expressed as pA_2 values, calculated according to the method of Arunlakshana & Schild (1959). However, as higher concentrations of SB 204070 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 paper.

Onset/recovery of effects of SB 204070

The rate of onset and time to wash out of the antagonistic effects of SB 204070 were investigated in the guinea-pig distal colon LMMP. 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 204070 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 drug-free Krebs and the tissues challenged with the same concentration of 5-HT every 15 min until responses returned to control level. The rate of recovery from the antagonistic effects of SB 204070 was calculated as the time taken for the response to 5-HT to recover to 50% of its control value (t_{off}).

Radioligand binding studies

Radioligand binding studies were performed as described by Sanger & Nelson (1989), except that [3 H]-idazoxan (0.5 nM), [3 H]-SCH 23390 (0.1 nM), and [125 I]-iodosulpiride (0.1 nM) were used to label the α_2 adrenoceptor, dopamine D_1 and dopamine D_2 receptors respectively. Iodosulpiride (100 μ M) was used to define non-specific binding in this latter assay. In addition the following assays were used: 5-HT $_{1D}$ [3 H]-5-HT (4 nM) in guinea-pig cortex in the presence of 100 nM 8-OH-DPAT and 100 nM pizotifen (Waeber *et al.*, 1989); 5-HT $_{2C}$, [3 H]-mesulergine (2 nM) in pig choroid plexus tissue in the

presence of 30 nM spiperone (Pazos *et al.*, 1985); 5-HT $_3$, [3 H]-granisetron in pooled rat hippocampus and entorhinal cortex, non-specific binding defined by 100 μ M metoclopramide (Nelson & Thomas, 1990); 5-HT $_{1E}$, [3 H]-5-HT (10 nM) in CHO cells expressing human 5-HT $_{1E}$ clones using the conditions of Hamblin & Metcalf (1991) and defining non-specific binding with 10 μ M 5-HT; dopamine D_3 , [125 I]-iodosulpiride (200 pM) in CHO cells expressing human D_3 clones, non-specific binding defined by 100 μ M iodosulpiride (Bowen *et al.*, 1993); β_1 adrenoceptor, [125 I]-iodocyanopindolol (0.024 nM) on cloned human β_1 receptors expressed in CHO cells, non-specific binding defined by 30 nM isoprenaline; β_2 adrenoceptor, [125 I]-iodocyanopindolol (12 pM) on cloned human β_2 receptors expressed in CHO cells, non-specific binding defined with 30 nM isoprenaline; GABA $_A$, [3 H]-muscimol (10 nM) in rat cortex, non-specific binding defined by 100 μ M bicuculline (Peters *et al.*, 1988); adenosine A_1 , [3 H]-phenylisopropyladenosine in rat cortex, non-specific binding defined by 100 μ M 2-chloroadenosine (Schwabe & Trost, 1980).

Statistics and data analysis for radioligand binding studies

The concentration of inhibitor required to inhibit 50% of specific binding (IC_{50}) was derived using the four parameter logistic function 'Allfit' (DeLean *et al.*, 1978) and corrected to inhibitory affinity constant (K_i) according to Cheng & Prusoff (1973). For all binding studies, *n* values were greater than or equal to 2.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (5-HT), 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP), isoprenaline hydrochloride and 2-chloroadenosine (+)-bicuculline (Sigma), 8-hydroxy-n,n-dipropylaminotetralin hydrogen bromide (8-OH-DPAT, RBI), spiperone (Janssen), methiothepin hydrochloride (Roche), granisetron, renzapride, iodosulpiride hydrochloride, metoclopramide, and pizotifen hydrogen maleate (SmithKline Beecham), SB 204070 ((1-butyl-4-piperidinyl)methyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride, synthesized in house). BIMU 1 was a kind gift from Dr Carlo Rizzi, Boehringer Ingelheim, Italy.

All drugs were dissolved in 0.9% w/v saline and diluted with Krebs solution. Glass was used at all times in the making up and dilution of SB 204070 since the compound may adhere to certain types of plastic (see Discussion).

Radiochemical sources were as follows (specific activity Ci mmol $^{-1}$): [3 H]-5-HT (20), [3 H]-8-OH-DPAT (161), [125 I]-iodocyanopindolol (2200), [3 H]-granisetron (61), [3 H]-prazosin (80), [3 H]-ter-butyl-bicyclphosphorothionate (TPBS, 127) – New England Nuclear; [3 H]-mesulergine (80), [3 H]-idazoxan (43), [3 H]-SCH 23390 (73), [3 H]-muscimol (19), [3 H]-flunitrazepam (84), [3 H]-phenylisopropyladenosine (50), [125 I]-iodosulpiride (2000) – Amersham International.

Results

Effects of SB 204070 in the guinea-pig distal colon LMMP

In the presence of methiothepin (100 nM) and granisetron (1 μ M), 5-HT (10 pM–10 nM) evoked a monophasic concentration-dependent contraction with a pEC_{50} of 9.2 ± 0.1 (*n* = 38). 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) and SDZ 205 557 (pA_2 7.8 ± 0.1 , slope of Schild plot 1.1 ± 0.1), suggesting that the response is 5-HT $_4$ receptor-mediated (Wardle & Sanger, 1993).

Low concentrations of SB 204070 (10, 30 and 100 pM, $n = 6$, Figure 1a) caused concentration-dependent, surmountable antagonism of the 5-HT curve, yielding apparent pA_2 values of 11.1 ± 0.2 , 10.7 ± 0.2 and 10.7 ± 0.1 respectively. At higher concentrations (0.3, 1 and 3 nM), rightward displacements of the concentration-effect curve to 5-HT were associated with a reduction in the maximum response. SB 204070 did not display agonism at any concentration tested.

SB 204070 was also examined as an antagonist of the responses mediated by two other classes of 5-HT₄ receptor agonists, namely the substituted benzamide, renzapride and the benzimidazolone derivative, BIMU 1. Renzapride was a full agonist in the guinea-pig distal colon (intrinsic activity cf.

5-HT = 1.0 ± 0.02 , $n = 4$) with a pEC_{50} of 7.2 ± 0.1 . SB 204070 (10–300 pM, $n = 4$, Figure 1b) evoked a concentration-dependent reduction in the maximum response to renzapride, with no prior shift to the right of the curve. Similar results were obtained with the partial agonist, BIMU 1 (intrinsic activity cf. 5-HT = 0.6 ± 0.03 , $pEC_{50} = 6.9 \pm 0.2$, $n = 4$, Figure 1c).

Onset and recovery of antagonistic effects of SB 204070

A 30 min incubation with SB 204070 (30 pM, 100 pM, 300 pM and 1 nM, $n = 6$) caused maximum antagonism of the contraction evoked by a pEC_{50} dose of 5-HT of $50 \pm 3\%$, $84 \pm 2\%$, $100 \pm 0\%$ and $100 \pm 0\%$ respectively (Figure 2).

Figure 1 Concentration-response curves to 5-HT (a), renzapride (b) and BIMU 1 (c) in the absence (○) and presence of 10 pM (●), 30 pM (□), 100 pM (■), 300 pM (△), 1 nM (▲) and 3 nM (◇) SB 204070 in the guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μ M). Each point represents the mean $\pm$ s.e. mean of 6, 4 and 4 observations respectively.

Figure 2 Onset and recovery of the antagonistic effects of SB 204070 in the guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. A standard pEC_{50} concentration of 5-HT was added to tissues until stable responses were produced. At time $t = -30$ min, various concentrations of SB 204070 (30 pM (●), 100 pM (○), 300 pM (■) and 1 nM (□)) were added to the bath. Following a 30 min incubation with SB 204070 the antagonist was washed from the bath and 5-HT added at 15 min intervals until responses returned to control levels. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μ M). Each point represents the mean $\pm$ s.e. mean of 4 observations.

Figure 3 Concentration-response curves to 1,1-dimethyl-4-phenylpiperazinium (DMPP) in the absence (○) and presence of 10 nM (●) and 1 μ M (■) SB 204070 in the guinea-pig distal colon longitudinal muscle-myenteric plexus preparation. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μ M). Each point represents the mean $\pm$ s.e. mean of 6 observations.

Table 1 *In vitro* binding profile of SB 204070

Receptor	Affinity (pK _i) (range)
5-HT _{1A}	< 6.0
5-HT _{1D}	< 5.0
5-HT _{1E}	< 5.0
5-HT _{2A}	< 6.0
5-HT _{2C}	(6.9–7.1)
5-HT ₃	(6.4–6.8)
Dopamine D ₁	< 6.0
Dopamine D ₂	< 5.0
Dopamine D ₃	< 6.0
Adrenoceptor α_1	< 6.0
Adrenoceptor α_2	< 6.0
Adrenoceptor β_1	< 6.0
Adrenoceptor β_2	< 6.0
GABA _A	< 4.0
BDZ	< 5.0
TBPS	< 5.0
A ₁	< 4.5

Longer incubations with SB 204070 revealed no further antagonism (results not shown). Upon washout, the responses to 5-HT recovered to control levels with t_{off} values of 36 ± 5 min, 47 ± 5 min and 70 ± 7 min for concentrations of SB 204070 of 100 pM, 300 pM and 1 nM respectively.

Selectivity studies

In the guinea-pig distal colon the nicotine receptor agonist DMPP (3–300 μM , $n = 4$) evoked concentration-dependent, cholinergically-mediated contractions. These responses were not affected by SB 204070 (10 nM and 1 μM) at concentrations significantly greater than those required to antagonize the cholinergically-mediated contractions evoked by 5-HT in the same preparations (Figure 3).

The effects of SB 204070 in a number of radioligand binding systems were studied *in vitro*. Results are summarized in Table 1. SB 204070 had little or no affinity for the 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E} or 5-HT_{2A} receptors. Weak affinity was observed for the 5-HT_{2C} (pK_i 6.9–7.1) and 5-HT₃ (pK_i 6.4 $\pm$ 6.8) sites. In the following binding systems: dopamine D₁, D₂ and D₃, α_1 , α_2 , β_1 and β_2 adrenoceptors, GABA_A, BDZ, TBPS and adenosine A₁, no inhibition of binding was observed for SB 204070 at concentrations up to and including 1 μM .

Discussion

The tools currently available for the study of the 5-HT₄ receptor, including tropisetron, SDZ 205 557 and DAU 6285 are limited by selectivity and bioavailability. For example, tropisetron has affinity for the 5-HT₄ receptor in the micromolar range (Dumuis *et al.*, 1988a; Kaumann *et al.*, 1990), but antagonizes 5-HT₃ receptor-mediated responses at nanomolar concentrations (Richardson *et al.*, 1985). Both SDZ 205 557 and DAU 6285 show a greater affinity for the 5-HT₄ receptor over the 5-HT₃ site, but with pA₂ estimates in the range of 7.4 for SDZ 205 557 (Buchheit *et al.*, 1992) and 7.0 for DAU 6285 (Turconi *et al.*, 1991; Baxter *et al.*, 1992), potency values are still relatively low. More recently, two highly potent and selective 5-HT₄ receptor antagonists, GR 113808 (Grossman *et al.*, 1993) and SB 204070 (Wardle *et al.*, 1993; Gaster *et al.*, 1993) have been identified. At the present time the bio-availability of the former compound, GR 113808 is unknown, and based on its structure, may be speculated to be low. In the present investigation we describe a more detailed study of the latter compound, SB 204070. This compound has previously been shown to have good bio-availability when dosed sub-cutaneously (Banner *et al.*, 1993) and as such represents a useful tool in the evaluation

of 5-HT₄ receptor-mediated responses both *in vitro* and *in vivo*.

The activity of SB 204070 has been investigated against 5-HT₄ receptor-mediated contractions in the guinea-pig distal colon (Wardle & Sanger, 1992; 1993). SB 204070 alone had no effects on resting tone, suggesting that it is devoid of agonist effects at the 5-HT₄ receptor. When examined against 5-HT-evoked contractions, low concentrations of SB 204070 shifted the curve in an apparently competitive manner (mean apparent pA₂ value over this concentration-range: 10.8 ± 0.1). Higher concentrations produced a further shift to the right of the curve with a concomitant reduction in the maximum response.

There are many plausible mechanisms of non-surmountable antagonism including multiple receptor subtypes, allosteric receptor modulation, irreversible antagonism and pseudo-irreversible antagonism (see Bond *et al.*, 1989).

The possibility that SB 204070 mediates its effects through multiple receptors is unlikely. Thus, radioligand binding studies indicate a lack of affinity of SB 204070 for other 5-HT receptors and other classical neurotransmitter receptors. When compared to activity at the 5-HT₄ receptor in functional systems, binding studies indicate that SB 204070 has a greater than 5,000 fold selectivity for the 5-HT₄ receptor over 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, GABA_A, BDZ, TBPS, A₁ adenosine receptors, α_1 , α_2 , β_1 , β_2 adrenoceptors and D₁, D₂ and D₃ dopamine receptors. These findings suggest that SB 204070 is highly selective for the 5-HT₄ receptor and that the non-surmountable effects are unlikely to be due to antagonism of a non-5-HT₄ site.

It has previously been shown that, in the guinea-pig distal colon, 5-HT₄ receptor-mediated contractions are evoked indirectly via acetylcholine release (Wardle & Sanger, 1993). Thus, the possibility that the apparent non-surmountable antagonism observed with SB 204070 was due to a non-selective effect was investigated against DMPP-evoked, cholinergically-mediated contraction in the same preparation. SB 204070, at concentrations of up to 10,000 fold greater than those required to block the 5-HT₄ receptor had no significant effect on DMPP-evoked contractions, suggesting that the reduction in maximum seen with SB 204070 is unlikely to be due to either a local anaesthetic or anticholinergic effect of the antagonist.

The possibility of an allosteric binding site or of pseudo-irreversible antagonism are difficult to evaluate in the present system. However, irreversible or pseudo-irreversible antagonism are preferred mechanism in explaining the non-surmountable activity of SB 204070 because they are intrinsically less complicated than allosteric modulation. Thus, this latter mechanism involves the postulation of a second binding site, in addition to the 5-HT₄ receptor, which in turn mediates a change in receptor state. While this possibility cannot be ruled out, the observed effects of SB 204070 can be better accounted for by a simpler explanation.

The apparent non-surmountable effects of SB 204070 in the guinea-pig distal colon may be explained if the compound behaved as an irreversible antagonist at the 5-HT₄ receptor. Such an effect, however, is unlikely since, upon repeated washing of the compound from the bathing solution, the responses to a pEC₅₀ concentration of 5-HT were restored to control levels for all concentrations of SB 204070 tested. This observation strongly suggests that the effects of SB 204070 are reversible in this system. Further evaluation of the washout profile of SB 204070 against a complete concentration-effect curve to 5-HT was not possible to obtain. Thus, control experiments, in which three consecutive concentration-effect curves were constructed to 5-HT, indicated that the third curve was not super-imposable on the first two, an effect probably due to fatigue of the tissues (unpublished observation).

The most likely explanation for the reduction in the maximum response to 5-HT observed with higher concentrations

of SB 204070 is that, in this preparation, SB 204070 is a slowly reversible or 'persistent' antagonist and acts in a pseudo-irreversible manner (Rang, 1966). Thus, since the 5-HT₄ 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 204070 may be attributed to the occupation and removal of spare receptors in a manner similar to that expected for an irreversible antagonist (Kenakin, 1993). Furthermore, in this model, BIMU 1 acts as a partial agonist relative to 5-HT and, by definition, must occupy all of the available receptors to produce its maximum response (Kenakin, 1993). Thus, when tested against BIMU 1, SB 204070, at all concentrations, produced an immediate reduction in maximum, without any prior shift to the right of the curve.

Non-surmountable antagonism is a common phenomenon in 5-HT receptor systems (see Leff & Martin, 1988; Martin, 1990) and in particular with highly potent compounds. It is relevant to note that, in the guinea-pig proximal colon, the potent 5-HT₄ receptor antagonist GR 113808 (pA₂ 9.2), has also been reported to cause a small suppression of the maximum response to 5-HT at concentrations approximately 100 fold higher than its pA₂ value (Grossman *et al.*, 1993). Such an effect is consistent with that seen in the present study. Furthermore, in the present study, tissues were exposed to the agonist for a period of 30 s, during which time the response to the agonist reached a plateau. Considering the possible slow offset of SB 204070 from the receptor, it is likely that re-equilibration between SB 204070 and 5-HT is incomplete in this short time period, an effect contributing to the observed reduction in maximum response. If the depression in the maximum response is simply due to a problem of drug-receptor kinetics, then it should be possible to protect against agonist curve depression by co-incubation with an excess of a simple competitive antagonist, such as SDZ 205,557. Such an investigation is currently under way.

Radio-ligand binding studies with an iodinated derivative of SB 204070 (SB 207710, Brown *et al.*, 1993) are currently ongoing in piglet hippocampus. Preliminary data suggest a binding affinity of 9.9 ± 0.1 (T. Young, personal communication). This value is clearly lower than that predicted by the present study. The reason for this difference is at present unclear. While it may reflect differences in the 5-HT₄ receptor in the two models, an alternative explanation is that of tissue accumulation of SB 204070 in the functional studies. Thus, the compound may become concentrated in the tissue elements close to the receptor phase, disturbing equilibrium. Such a suggestion is supported by the apparent high lipophilicity of SB 204070 (see below) and by the washout data presented in the present study.

While studying the antagonistic effect of SB 204070 it was noted that when the compound was diluted using plastic

pipettes, variations were observed in the potency of SB 204070. This variation could be eliminated by carrying out all dilutions in glass test tubes and using glass pipettes (as was the case in the present results). The reason for this is unclear, but may reflect adsorption and release of SB 204070 from the plastic throughout the dilution procedure. Such a possibility is currently being investigated further.

The antagonistic effects of SB 204070 were investigated against each of the three chemical classes of 5-HT₄ receptor agonists (indoles; 5-HT, 4-amino-5-chloro-2-methoxy substituted benzamides; renzapride and azabicycloalkyl benzimidazolone derivatives; BIMU 1). Since the mechanism of antagonism of SB 204070 in this preparation cannot be described as simple competition, a comparison of pA₂ values against different agonists was not possible. What can be concluded from the present data, however, is that, when tested against each of the three types of 5-HT₄ receptor agonists, SB 204070 is consistently active at very low concentrations (10 pM and above). From this it may be suggested that the potency of SB 204070 in the guinea-pig distal colon is agonist-dependent.

In conclusion, SB 204070 has been evaluated for its ability to antagonize 5-HT₄ receptor mediated-contractions in the guinea-pig distal colon LMMP. This compound is shown to be a highly potent, highly selective 5-HT₄ receptor antagonist in this preparation. The nature of the antagonism is complex. While the binding of the compound *in vitro* is reversible, dissociation from the receptor appears to be slow, thus preventing the attainment of equilibrium between 5-HT and the receptor within the time course of the experiment. The simplest explanation for the observed results is that SB 204070 behaves as a pseudo-irreversible antagonist. It is worth pointing out that the non-surmountable nature of the antagonism observed with SB 204070 suggests a need for caution when using this ligand for comparison of 5-HT₄ receptors in different tissues or in the discrimination of putative 5-HT₄ receptor subtypes. Indeed, the behaviour of SB 204070 and its apparent affinity may be influenced by the receptor reserve of the particular preparation under investigation (see Baxter *et al.*, 1994). Notwithstanding this caveat, the high potency and selectivity of SB 204070 suggest that it will be a useful tool in the study of the physiology and pathophysiology of the 5HT₄ receptor. We eagerly await results from other functional studies to see if the nature of this antagonism is a peculiarity to the model currently used and from whole animal studies to see how this observation manifests itself *in vivo*.

The authors wish to thank all members of the Neuroscience Research at SmithKline Beecham involved in the radio-ligand binding studies on SB 204070.

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(Received December 9, 1993)

Revised March 14, 1994

Accepted March 16, 1994)