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SB-243213; a selective 5-HT_{2C} receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety

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Abstract

SB-243213 (5-methyl-1-[[-2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindoline hydrochloride) is a new, selective 5-hydroxytryptamine (5-HT)$_{2C}$ receptor inverse agonist. SB-243213 has high affinity for the human 5-HT_{2C} receptor (pK_i 9.37) and greater than a 100-fold selectivity over a wide range of neurotransmitter receptors, enzymes and ion channels. In in vitro functional studies, SB-243213 acted as an inverse agonist at the human 5-HT_{2C} receptor with a pK_b of 9.8. In in vivo studies, SB-243213 was a potent inhibitor of central 5-HT_{2C} receptor-mediated function in rats, blocking meta-chlorophenylpiperazine-induced hypolocomotion with an ID_{50} of 1.1 mg/kg p.o. and a long duration of action (>8 h). In rats, SB-243213 exhibited anxiolytic-like activity in both the social interaction and Geller–Seifter conflict tests. Importantly, unlike diazepam, chronic administration of SB-243213 did not result in the development of either tolerance to the anxiolytic-like effects or withdrawal anxiogenesis. Furthermore, in rodents, SB-243213 did not affect seizure threshold, did not increase body weight or induce catalepsy, but attenuated the haloperidol-induced catalepsy. SB-243213 did not affect amphetamine-, MK-801- or phencyclidine-induced hyperactivity. In conclusion, SB-243213 may possess an improved anxiolytic profile compared to benzodiazepines. SB-243213 also modulates dopaminergic transmission, lacks pro-psychotic properties and may have utility in the treatment of schizophrenia and motor disorders.

Keywords: SB-243213; 5-HT_{2C} receptor; Anxiety; Tolerance; Withdrawal; Feeding; Seizures; Catalepsy

1. Introduction

5-Hydroxytryptamine (5-HT) mediates its actions through a wide family of receptors of which 5-HT_2 receptors form a distinct and closely related sub-group (Hoyer et al., 1994). The 5-HT_2 receptor family consists of three subtypes termed 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} which display close structural homology, pharmacology and signal transduction pathways (see Hoyer et al., 1994). These receptors are present in the rat and human brain where they are widespread but possess differential distributions (Wright et al., 1995; Abramowski et al., 1995; Pasqualetti et al., 1999).

The role of 5-HT_2 receptors in the brain has been the subject of considerable interest based on the actions of m-chlorophenylpiperazine (mCPP) and hallucinogens with 5-HT_{2C} and 5-HT_{2A} receptors, respectively (Kennett, 1993; Glennon et al., 1984). Indeed, such studies have implicated central 5-HT_{2C} receptors in the control of anxiety, depression and schizophrenia (Kahn and Wetzler, 1991; Kennett, 1993; Moreau et al., 1966; Canton et al., 1994).

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One of the roles in which 5-HT_2 receptors have been implicated is in the mechanism of action of atypical antipsychotics. The atypical neuroleptic clozapine, which displays high affinity for 5-HT_{2C} and 5-HT_{2A} receptors, is less likely to cause extrapyramidal side effects than typical neuroleptics and is thought to have greater efficacy against negative symptoms than other neuroleptics (Meltzer, 1996). Clozapine differs from typical neuroleptics in that it has higher antagonist activity for 5-HT_2 receptors than dopamine D_2 receptors, and this property may be responsible for its atypical profile (Meltzer et al., 1989). We have previously shown that 5-HT_{2C} receptor antagonists, but not 5-HT_{2A} or 5-HT_{2B} receptor antagonists, reverse catalepsy produced by haloperidol therefore implicating antagonist activity at the 5-HT_{2C} receptor as a factor contributing to the low frequency of side effects of clozapine (Reavill et al., 1999). However, other studies have shown that the 5-$HT_{2B/2C}$ receptor antagonist, SB-221284, increases dopamine efflux in the nucleus accumbens (Di Matteo et al., 1999) and potentiates phencyclidine-induced hyperactivity in rats (Barton et al., 1999), suggesting that 5-$HT_{2B/2C}$ receptor antagonism might facilitate dopaminergic function.

We have previously described the development of selective 5-HT_{2C} receptor antagonists including SB-206553 (Kennett et al., 1996) and SB-242084 (Kennett et al., 1997). Using these tools, we have demonstrated an involvement of the 5-HT_{2C} receptor in animal models of anxiety and depression and shown that such compounds do not possess either pro-convulsant or hyperphagic properties, which have been reported in mutant mice lacking the 5-HT_{2C} receptor (Tecott et al., 1995). Unfortunately these compounds were not suitable for progression into man for a number of reasons, including an interaction with cytochrome P450 enzymes. We have therefore developed SB-243213 (5-methyl-1-[[-2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindoline hydrochloride) as a selective 5-HT_{2C} receptor antagonist (Bromidge et al., 2000) which does not interact with cytochrome P450 enzymes at concentrations up to 10 μM. We now report the in vitro and in vivo pharmacological profile of SB-243213 including its profile in animal models of anxiety following acute and chronic administration. SB-243213 is currently in clinical development with potential for the treatment of a range of psychiatric diseases including anxiety, depression and schizophrenia.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley experimentally naive rats, 220–300 g, housed in groups of four–six under a 12 h light/dark cycle (lights on 07.00 h) with free access to food (Combined Rat and Mouse Diet, Special Diet Services, Witham, Essex, UK) and water, were used in all studies except the maximal electroshock seizure test where the animals were 70–102 g). All work was conducted in compliance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986 and was reviewed and approved by the SmithKline Beecham Procedures Review Panel.

2.2. Stable expression of human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors

The human 5-HT_{2C} and 5-HT_{2A} receptors (Wood et al., 1995) and the human 5-HT_{2B} receptor (Thomas et al., 1996) were expressed in HEK 293 cells.

2.3. Cell culture

HEK 293 cells were maintained in minimum essential medium (MEM) supplemented with (final concentration): l-glutamine (2 mM), foetal calf serum (10%), non-essential amino acids (1%) and geneticin (0.4 mg/ml). Cells were grown in an incubator at 37°C with oxygen containing 5% CO_2 and were harvested by trypsinisation.

2.4. Radioligand binding assays

In all assays (for details see Table 1), SB-243213 was dissolved in polyethylene glycol:dimethyl sulphoxide (1:1) at 1 mM and diluted to 0.1 mM using 5 mM HCl. Serial dilutions of SB-243213 in the same solution were carried out using a Biomek 2000 Workstation (Beckman). Diluted SB-243213 (0.05 ml) was mixed with 0.05 ml of radioligand, prepared in the incubation buffer and 0.4 ml of the homogenate of washed membranes, also in the working buffer. In dopamine binding assays, 0.1% (w/v) bovine serum albumen was included in the incubation buffer. After incubation at 37°C, samples were filtered using a Packard Filtermate (Packard, Pangbourne, Berks, UK) in Packard TopCount format. Filters were washed with 4×1 ml aliquots of ice-cold incubation buffer. Filters were dried and 0.04 ml of Microscint 20 (Packard) added and counted for radioactivity.

2.5. Phosphoinositide (PI) hydrolysis

Phosphoinositide (PI) studies were performed according to Wood et al. (1997a). Cells were plated into 24 well plates and incubated with [^{3}H]-myo-inositol. The cells were incubated at 37°C, 5% CO_2 for 48 h and transferred into serum-free inositol-deficient Dulbecco's modified Eagles's medium (DMEM) overnight. On the day of the assay, the cells were gently washed in DMEM medium and incubated for 30 min in the presence or

Table 1
Summary of receptor binding conditions[a]

Receptor	Host cell/ tissue	Assay buffer	Protein μg/assay	Radio-ligand	(nM)	Specific activity (Ci/mmol)	Non-specific definition	K_d (nM)
5-HT_{1A}	HEK293	2	50	[^{3}H]-8-OH-DPAT	1.0	120	Buspirone	1.0
5-HT_{1B}	CHO	2	70	[^{3}H]-5-HT	4.0	86	5-HT	4.0
5-HT_{1D}	CHO	2	150	[^{3}H]-5-HT	4.0	86	5-HT	4.0
5-HT_{1E}	CHO	2	120	[^{3}H]-5-HT	4.0	86	5-HT	24.0
5-HT_{1F}	CHO	2	140	[^{3}H]-5-HT	4.0	86	5-HT	24.0
5-HT_{2A}	HEK293	1	170	[^{3}H]-ketans	0.5	80	Mianserin	0.7
5-HT_{2B}	HEK293	2	160	[^{3}H]-5-HT	8.0	86	5-HT	11.0
5-HT_{2C}	HEK293	1	130	[^{3}H]-mesulergine	0.6	81	Mianserin	0.58
5-HT_4	Guinea Pig hippocampus	2	10	[^{125}I]-SB-207710	0.02	2000	SB-204070	1.0
5-HT_6	HeLa	4	40	[^{3}H]-LSD	2.0	83	methiothepin	3.1
5-HT_7	HEK293	2	250	[^{3}H]-5-CT	0.5	79	5-HT	0.5
D_2	CHO	3	220	[^{125}I]-iodosulpride	0.1	2000	YM-09151	1.3
D_3	CHO	3	60	[^{125}I]-iodosulpride	0.1	2000	YM-09151	2.4
$D_{4.4}$	CHO	3		[^{3}H]-YM09151			YM-09151	
ad-α_{1B}	CHO	1	120	[^{3}H]-prazosin	0.2	76	phentolamine	0.58
ad-β_2	CHO			[^{125}I]-iodocyano-pindolol				

[a] Incubation buffers were: (1) 50 mM Trizma (Sigma, UK) pH 7.7 at 25°C. (2) 50 mM Trizma (Sigma) pH 7.7 at 25°C, 5 mM $MgCl_2$, 500 nM pargyline and 10 mM ascorbate. (3) 50 mM Trizma (Sigma) pH 7.7 at 25°C, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$. (4) 20 mM HEPES, 10 mM $MgSO_4$. For Method references see Kennett et al. (1997).

absence of antagonist prior to a 20 min agonist exposure in the presence of 10 mM lithium chloride. The reaction was halted by aspiration of incubation media, and labelled inositol phosphates were isolated by solvent extraction from the aqueous phase followed by Dowex anion-exchange chromatography. The total [^{3}H]-labelled inositol phosphates were then determined by scintillation counting.

2.6. Microphysiometry

Functional studies at the human dopamine D2 receptor were performed according to Coldwell et al. (1999). Briefly, Chinese hamster ovary cells stably expressing the human dopamine D2 receptor were seeded at a density of 300,000 cells per well and changes in metabolic activity followed using the Cytosensor microphysiometer (Molecular Devices, Sunnyvale, California, USA), which detects changes in extracellular acidification. The effect of SB-243213 on extracellular acidification rate, i.e. agonist-like effect, and the effect on the response to the agonist quinpirole, were determined.

2.7. mCPP-induced hypolocomotion

Rats were dosed orally 1–8 h before the locomotion test with SB-243213 or vehicle, and injected i.p. 20 min before the test with mCPP or saline, in groups of four. At 0 h, they were each placed in automated locomotor activity cages (57×16.6×25.3 cm), made of black Perspex with a clear Perspex lid and sawdust covered floor, under red light for 10 min. During this time, locomotion was recorded by means of consecutively breaking two photocell beams traversing opposite ends of the box 3.9 cm above floor level (Kennett et al., 1994).

2.8. Social interaction

Anxiolytic and anxiogenic activity was evaluated in the rat social interaction model (File, 1980). Studies were performed according to Kennett et al. (1994). Active social interaction (sniffing, following grooming, biting, boxing and crawling over or under) was scored by an observer, blind with respect to treatments, by remote video monitoring and a computerised score pad.

2.9. Tolerance studies using the social interaction test

The testing arena was identical to the acute dosing social interaction study above. For the chronic pre-treatments, two groups of 30 rats were given SB-243213 (4.5 or 9 mg/kg p.o.) or vehicle twice daily at 8 a.m. and 8 p.m. for 14 days. These doses represent 15 and 30× the minimum effective dose in the rat social interaction test. Social interaction testing took place on day 15 after the initiation of dosing. Rats were housed singly in a room adjacent to the testing room on the eleventh day of dosing. On day 15, they were dosed p.o., 1 h before the test, with SB-243213 (0.3 mg/kg p.o.) or vehicle in treatment and weight matched (±5 g) pairs unfamiliar to each other

and returned to their home cages. Rats were then placed in the test arena and the time spent in active social interaction and the number of squares crossed were scored for 15 min via remote video monitoring. In all procedures, the observer was blinded with respect to treatments by the use of a vehicle containing 10 mg/ml barium sulphate to mask the presence of drug and by the independent coding of drug treatments prior to the experiment.

2.10. Withdrawal studies in the social interaction test

The social interaction test apparatus was identical to that described above, with the exception that the testing box was lit by red light (60 watts), corresponding to low light conditions being sensitive to anxiogenic effects (File, 1980). Two groups of 30 rats were given SB-243213 (4.5 or 9 mg/kg p.o.), diazepam (40 mg/kg p.o.) or vehicle twice daily at 8 a.m. and 8 p.m. for 14 days. Social interaction testing took place on day 16 or 17 after the initiation of dosing (48 or 72 h after the last pre-treatment dose. After the last drug treatment, rats were given substitute vehicle injections until 8 p.m. on the day preceding testing in the social interaction test. The vehicle control group of 10 rats was formed of rats chronically administered an equivalent number of vehicle doses as those forming groups withdrawn for 48 or 72 h (5 rats from each treatment regimen) from drug treatments. Rats were housed singly in a room adjacent to the testing room on the eleventh day of dosing. On days 14 and 15 they were individually exposed to the social interaction test box under red light for 10 min to allow habituation. On day 16 or 17, they were dosed p.o., 1 h before the test, with vehicle in pre-treatment and weight matched (±5 g) pairs unfamiliar to each other and returned to their home cages. Rats were then placed in the test arena and the time spent in active social interaction and the number of squares crossed were scored for 15 min by a remote observer blinded with respect to treatments as above.

2.11. Geller–Seifter test

The effects of SB-243213 on rats trained to associate pressing of a lever with a food pellet reward was performed as described in Kennett et al. (1997). Briefly, rats were introduced to a multiple schedule of reinforcement, a variable interval with the food reinforcement every 10–50 s, and a fixed ratio (FR) with one reinforcement every five lever presses. The FR component was signalled by a light and was contingent with a mild footshock. The unpunished variable interval component was used to detect non-specific effects such as sedation or stimulant properties.

2.12. Locomotor activity

Rat locomotor activity was monitored in an automated system as described in Reavill et al. (1998). Four separate experiments were carried out, either with SB-243213 alone, or with pre-treatment with SB-243213 prior to amphetamine, phencyclidine or MK-801. In the experiment where SB-243213 was tested alone, rats were injected with SB-243213 (1.0, 3.2 or 10 mg/kg p.o. at 2 ml/kg) or with vehicle and placed in locomotor activity boxes. Locomotor activity was monitored for 180 min in 10 min blocks.

In stimulant-induced hyperactivity experiments, rats were injected with SB-243213 (1.0, 3.2 or 10 mg/kg p.o.) or vehicle and returned to their home cages. Thirty min later, rats were placed in locomotor activity boxes for a 30 min period of habituation. Rats were then injected s.c. with either saline, dexamphetamine sulphate (0.4 mg/kg base), phencyclidine hydrochloride (0.94 mg/kg base) or MK-801 maleate (0.1 mg/kg base). Activity was monitored for a further hour in 5 min blocks. Data for the first 5 min was discarded to minimise handling artefacts.

2.13. Catalepsy

To test for catalepsy, rats were positioned so that their hindquarters were on the bench and their forelimbs rested on a 1 cm diameter horizontal steel bar, 10 cm above the bench. The length of time the rats maintained this position was recorded by stopwatch to a maximum of 120 s. This procedure occurred 30, 60 and 90 min after drug administration. Rats were judged to be cataleptic, and assigned a score of “1” if they maintained this position for 30 s or more; otherwise, they were assigned a score of “0”.

2.14. Food intake and body weight studies

In acute studies, rats were individually housed 2 days prior to the experiment with free access to their normal food and water. On day 3, at 13.00 h their food was removed and they were orally dosed with either vehicle or SB-243213 (1, 3 or 10 mg/kg). 1 h later, a weighed quantity of their normal food was added to their food hoppers and the amount remaining weighed 1, 2, 4 or 24 h later. Cumulative food intake was then calculated. In chronic studies, rats were group housed from days 1-10 and on the twelfth day were singly housed as part of the study on the effect of withdrawal from chronic administration of the drug. They were orally dosed with vehicle or SB-243213 (4.5 or 9 mg/kg p.o.) twice daily at 9.00 a.m. and 9.00 p.m. for 14 days. Body weight was recorded each morning and the total weight change over the 14 day period calculated.

2.15. Rat maximal electroshock test

The threshold for maximal (tonic hindlimb extension) electroshock seizures, in male Sprague–Dawley rats, was measured as described in Kennett et al. (1997).

2.16. Materials

Tris, polyethyleneimine, pargyline hydrochloride, 5-HT hydrochloride, dexamphetamine sulphate, haloperidol and other chemicals were purchased from Sigma (Poole, Dorset, UK). Mianserin HCl, buspirone HCl, phencyclidine hydrochloride and phentolamine mesylate were purchased from R.B.I. (Natick, MA, USA), while YM-09151 was a generous gift from Yamanouchi Pharmaceuticals (Tokyo, Japan). MK-801 maleate was obtained from Tocris. SB-243213 (5-methyl-1-[[-2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindoline hydrochloride) was synthesised in the Department of Discovery Chemistry at SmithKline Beecham. Dexamphetamine sulphate, phencyclidine hydrochloride and MK-801 maleate were dissolved in physiological saline and administered subcutaneously (s.c.) at 1 ml/kg. Haloperidol was sonicated with an equal weight of (+)-tartaric acid in water and administered intraperitoneally (i.p.) at 1 ml/kg. SB-243213 was ground with 1 drop BRIJ-35 and suspended in 1% methylcellulose in saline, and dosed periorally (p.o.) at a dose volume of 2 ml/kg. In stimulant experiments, SB-243213 was administered 60 min prior to dexamphetamine sulphate, phencyclidine hydrochloride or MK-801 maleate. All doses are quoted as the base equivalents. Diazepam and chlordiazepoxide, synthesised at SmithKline Beecham, was given in a similar manner and injection volumes of 2 ml/kg were used in all treatments. mCPP was dissolved in 0.9% NaCl and given in a 1 ml/kg volume i.p. 20 min before testing. Radiolabelled compounds were obtained as detailed in Kennett et al. (1997).

2.17. Data analysis and statistics

Data from receptor binding studies were analysed according to Wood et al. (1995) to give the inhibitory affinity constant (K_i) and expressed as the pK_i (negative $\log_{10}$ (K_i)). Data from in vitro functional studies were analysed using non-linear curve fitting to give EC_{50} and antagonist affinity, as the apparent dissociation constant K_B and Schild analysis to give pA_2 were derived according to Wood et al. (1997a).

The effect of treatments on mCPP-induced hypolocomotion was determined by one-way ANOVA and Newman–Keuls test as described in Kennett et al. (1997). All data are cited as the mean±s.e.m., unless otherwise indicated. For locomotor activity, data were analysed in *STATISTICA*®, version 6.0; StatSoft. Levene's test was used to test for homogeneity of variance and the data were transformed if necessary to obtain normally distributed data. Data were then analysed by one factor analysis of variance (ANOVA) and post hoc analysis carried out by Dunnett's *t*-test. In catalepsy studies, as the raw data were derived from a non-linear quantal scoring scheme, a logistic regression analysis in SAS-RA© (SAS Institute Inc.) or Statistica was used to analyse the data at the 90 min time point. Social interaction data were subjected to one-way ANOVA and Dunnett's *t*-test, while Geller–Seifter data were analysed by two-way ANOVA.

3. Results

3.1. Radioligand binding studies

SB-243213 shows high affinity for the human 5-HT_{2C} receptor with greater than 100-fold selectivity over 5-HT_{2A} and 5-HT_{2B} receptors (Table 2). In radioligand binding studies, SB-243213 showed little affinity (pK_i<6) for cloned human 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1E}, 5-HT_{1F} and 5-HT_7 receptors. It showed weak affinity (pK_i<6.5) for the cloned human 5-HT_{1D} and D_3 receptors and moderate affinity (pK_i 6.7) for the cloned human D_2 receptor (Table 2). SB-243213 also showed little affinity (<30% inhibition at 1 μM) for a wide range of neurotransmitter receptors, ion channels and enzymes (Table 2; CEREP report: (1) 2932 S 830 E).

3.2. Phosphoinositide (PI) hydrolysis

At the human 5-HT_{2C} receptor expressed in HEK 293 cells, 5-HT was a potent agonist with a pEC_{50} of 8.55±0.13 (*n*=6) and caused a maximal 250% increase in radiolabelled inositol phosphate release. SB-243213 did not induce any increase in radiolabelled inositol phosphate release on its own, but antagonised the effects of 5-HT (Fig. 1) with a pK_b of 9.80±0.21 (6). SB-243213 significantly attenuated the basal levels of PI hydrolysis from 16200±1940 (*n*=3) to 2340±407 at 100 nM SB-243213 (Fig. 1). In Schild analysis, SB-243213 appeared to be a competitive antagonist (pA_2 10.2) in that the slope was not different from unity (0.96).

At the human 5-HT_{2B} receptor expressed in HEK 293 cells, 5-HT was a potent agonist with a pEC_{50} of 7.91±0.15 (*n*=6) and caused a maximal 140% increase in radiolabelled inositol phosphate release over basal. SB-243213 did not induce any increase in radiolabelled inositol phosphate release on its own, but antagonised the effects of 5-HT with a pK_b of 7.0±0.1 (4).

At the human 5-HT_{2A} receptor expressed in SH-SY5Y cells, 5-HT was a potent agonist with a pEC_{50} of 6.87±0.04 (*n*=6) and caused a maximal 400% increase in radiolabelled inositol phosphate release. SB-243213 did not induce any increase in radiolabelled inositol

Table 2
Affinities (pK_i) of SB-243213 at 5-HT_{2C} and other receptors. Data are mean±s.e.m. from (n) separate determinations[a]

Receptor	Mean pK_i±s.e.m. (n)	Receptor	Mean pK_i±s.e.m. (n)
Human 5-HT_{1A}	<5.3 (5)	Guinea pig 5-HT_4	6.17±0.02 (3)
Human 5-HT_{1B}	5.50±0.22 (6)	Human 5-HT_6	6.50±0.03 (3)
Human 5-HT_{1D}	6.32±0.03 (7)	Human 5-HT_7	5.64±0.19 (8)
Human 5-HT_{1E}	<5.4 (5)	Human adrenergic α_{1B}	<5 (3)
Human 5-HT_{1F}	5.35±0.22 (5)	Human adrenergic β_2	<5 (5)
Human 5-HT_{2A}	7.01±0.10 (11)	Human dopamine D_2	6.68±0.08 (14)
Human 5-HT_{2B}	7.20±0.11 (13)	Human dopamine D_3	6.50±0.06 (14)
Human 5-HT_{2C}	9.37±0.09 (14)	Human dopamine $D_{4.4}$	<6.4 (5)

[a] Inhibition of radioligand binding by a 1 μM (n=2) solution of SB-243213 was less than 30% for all of the following receptors: adenosine A_1 and A_2; adrenergic α_{1A}, α_{2A}, α_{2B}, α_{2C}, β_1, β_3; angiotensin 2; central benzodiazepine; calcitonin gene related peptide; central cannabinoid; dopamine D_1; endothelin A and B; $GABA_A$, AMPA, Kainate, NMDA; central histamine H_1, H_2; central imidazoline I_2; leukotriene LTB_4; muscarinic M_1, M_2; neurokinin NK_3; nicotinic; opiate δ, κ, μ; 5-HT_3, estrogen, progesterone, testosterone; calcium channel L type DHP site; sodium channel site 1 and site 2. Similarly 1 μM (n=2) SB-243213 inhibited the following enzymes by less than 30%: Sodium-potassium ATPase; elastase; phosphodiesterase I, II, III, IV, V; protein kinase C; EGF tyrosine kinase.

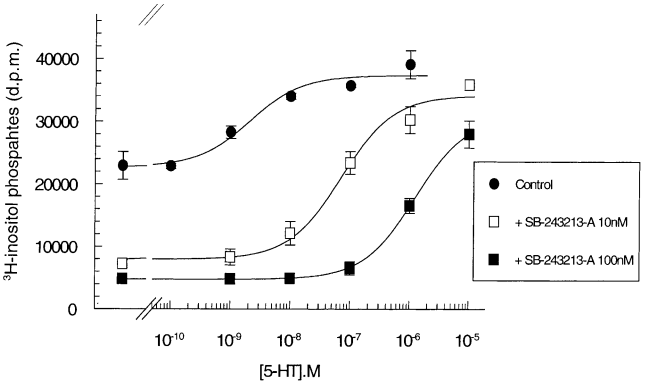

Fig. 1. Inhibition of 5-HT induced phosphoinositide hydrolysis at the human 5-HT_{2C} receptor by SB-243213 at 10 and 100 nM. Shown are the mean responses±s.e.m. for triplicate determinations from one experiment repeated three times.

phosphate release on its own, but antagonised the effects of 5-HT with a pK_b between 6.5–7.0. This effect could not be accurately quantified due to the limits of solubility for SB-243213.

3.3. Microphysiometry

The weak interaction with the cloned human dopamine receptors was studied in more detail in functional studies using the microphysiometer. At the human D_2 receptor, SB-243213 lacked agonist activity but acted as an antagonist with an apparent pK_b of 6.48±0.21 (n=3, data not shown).

3.4. Effect of SB-243213 on mCPP-induced hypolocomotion

The administration of mCPP (7 mg/kg, i.p., 20 min pre-test) to vehicle pre-treated rats resulted in a marked reduction in locomotor activity from 15.6±0.9 to 1.2±1.2 transits. Pre-treatment with SB 243213 p.o., 1 h pre-treatment, significantly ($F(5,70)=11.8$, $p<0.01$) and completely attenuated the effect of mCPP (Fig. 2). The dose producing 50% inhibition of the action of mCPP was calculated as 1.1±0.1 mg/kg, p.o. When SB 243213 was given alone to rats by the p.o. route in a separate experiment, it had no effect on locomotor activity per se under identical conditions (mean transits/10 min±SEM, n=6–8; vehicle 13.8±1.1; SB 243213 at 0.5 mg/kg, p.o., 19.5±0.8; 1.0 mg/kg, p.o., 19.3±2.7; 2.0 mg/kg, p.o., 17.0±2.6; 5.0 mg/kg, p.o., 18.5±1.8, no significant differences by one-way ANOVA). SB-243213 demonstrated a long duration of action, with complete inhibition of mCPP-induced hypolocomotion up to 8 h post-dose at 3.0 mg/kg. (data not shown).

3.5. Effect of acute administration of SB-243213 in rat social interaction test

SB-243213 (0.1-10 mg/kg, p.o. 1 h pre-test) dose-dependently and significantly increased the amount of time rats spent in social interaction over 15 min under brightly lit conditions and in an unfamiliar test box (high light unfamiliar), as did the positive control, chlordiazepoxide at 5 mg/kg, p.o., 1 h pre-test ($F(6,77)=12.1$, $p<0.01$, Fig. 3). The magnitude of the response to SB-243213 (3 mg/kg) was similar to that seen after chlordiazepoxide (5 mg/kg). Neither SB-243213 nor chlordiazepoxide had any effect on locomotor activity in the test at any dose tested (mean line crossings/15 min±s.e.m., n=12; vehicle 544±24; SB-243213 0.1, 1.0, 3.0 and 10 mg/kg, p.o., respectively, 485±22, 528±23, 572±24, 564±25, 578±23; chlordiazepoxide 5.0 mg/kg, p.o., 497±20.)

3.6. Effect of SB-243213 on responding in a rat Geller–Seifter procedure

SB-243213 significantly increased punished responding but not unpunished responding, compared

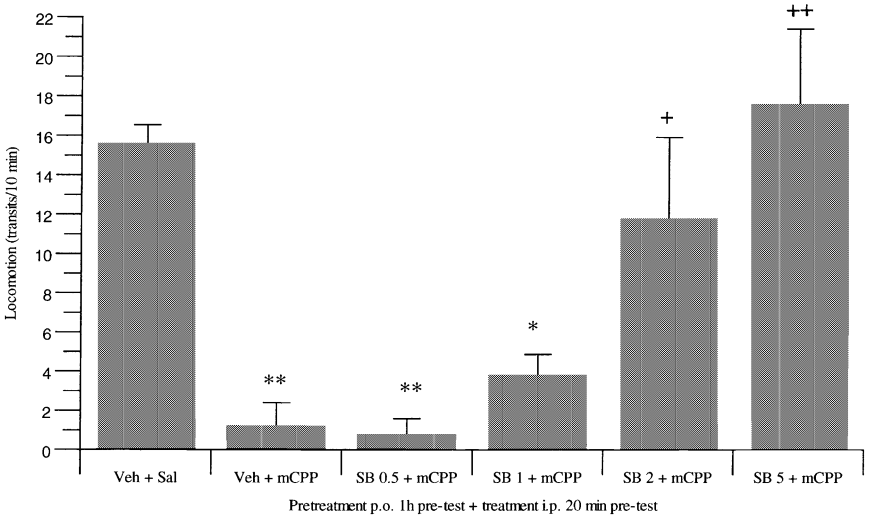

Fig. 2. Effect of SB 243213 on mCPP-induced hypolocomotion in rats. All data cited as means with SEM, n=6 per group. mCPP given at 7 mg/kg, i.p. Significantly different from vehicle+saline treated group, ** $p<0.01$, from vehicle+mCPP group, + $p<0.05$, ++ $p<0.01$ by Dunnett's t-test and one-way ANOVA.

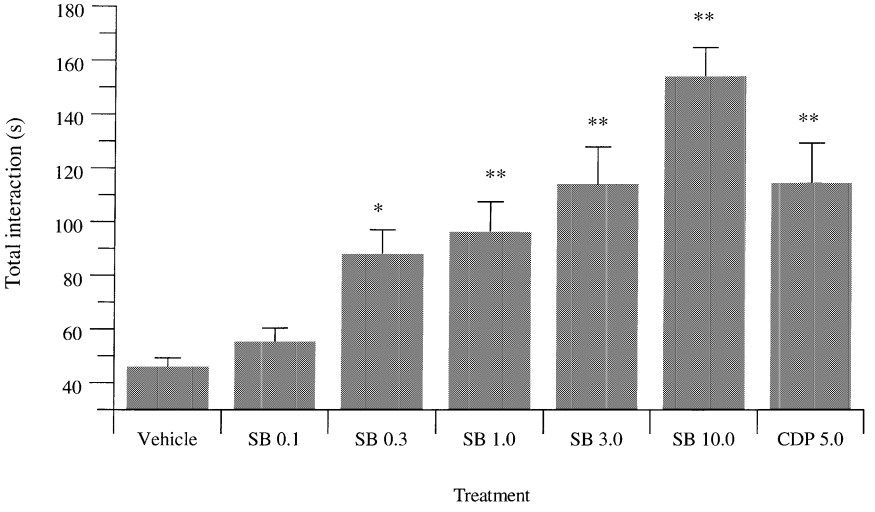

Fig. 3. Effect of SB-243213 on social interaction test in rats under "high light unfamiliar conditions". All data are means±SEM, n=10. Significantly different from vehicle treated group, * $p<0.05$, ** $p<0.01$, Dunnett's t-test and one-way ANOVA.

with the effects of vehicle treatment on two days preceding the test day when given at doses of 0.5–20 mg/kg p.o. 1 h pre-test (Table 3). Diazepam (5 mg/kg, p.o., 1 h pre-test) had a greater effect to that observed following administration of 2 mg/kg of SB-243213 (Table 3).

3.7. Tolerance study

Administration of SB-243213 (0.3 mg/kg) to chronic vehicle pre-treated rats significantly increased time spent in social interaction ($F(1,52)=99.1$, $p<0.01$, Fig. 4). Chronic pre-treatment with SB-243213 had no effect on social interaction one day later and did not alter the response to acute SB-243213 ($F(2,52)=0.2$, n.s.). No treatment combination was observed to alter locomotor activity in the test (data not shown).

Diazepam (2 mg/kg p.o. 1 h pre-test) also increased the time spent in social interaction of chronic vehicle pretreated rats ($F(5,103)=6.3$, $p<0.01$) and rats treated chronically with diazepam. However, this response to 2 mg/kg diazepam was reduced in rats chronically pretreated with diazepam at 10, 20 and 40 mg/kg p.o., with this reduction being significant at 10 and 40 mg/kg (Table 4). None of these treatments affected locomotion scores.

3.8. Withdrawal study

In rats chronically treated for 14 days with either 4.5 or 9 mg/kg p.o., b.i.d. SB-243213, there was no effect following 48 h or 72 h withdrawal on the amount of time rats spent in active social interaction compared with

Table 3
Effect of SB 243213 on rat responses in a modified Geller–Seifter test[a]

Treatment (p.o., 1 h)	Dose (mg/kg)	Percentage change in number of lever presses	
		Unpunished (VI)	Punished (FR)
SB-243213	0.2	+5±1	+12±3
	0.5	+4±2	+24±3**
	1.0	+5±1	+43±3***
	2.0	+9±1	+35±3***
	5.0	-2±1	+41±8***
	10.0	+4±2	+31±7***
	20.0	+6±2	+30±4***
Diazepam	5.0	-19±14	+119±45***

[a] All data cited as means±SEM, n=6–8 per group. Significantly different from vehicle treated group, ** $p<0.01$, *** $p<0.001$ by two-way ANOVA (treatments×subjects).

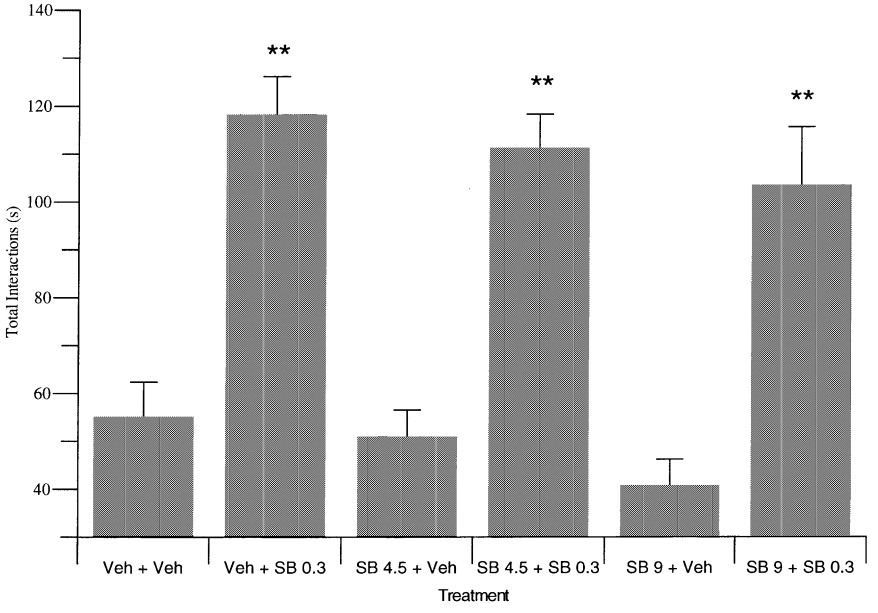

Fig. 4. Effect of 243213 (0.3 mg/kg, p.o., 1 h pre-test) on behaviour of rats chronically pre-treated with SB-243213 (4.5 or 9 mg/kg, p.o. b.i.d.×14 days, last dose 48 h pre-test). All data cited as means with SEM, n=8–10 per group. Significantly different from relevant vehicle treated group ** $p<0.01$ by Newman–Keuls test and two-way ANOVA.

Table 4
Effect of diazepam (2 mg/kg, p.o., 1 h pre-test) on the social interaction of rats chronically pre-treated with diazepam (5, 10, 20 or 40 mg/kg p.o., b.i.d.×14 days)[a]

Chronic pretreatment (mg/kg p.o. b.i.d.×14)	Treatment (mg/kg p.o. 1 h pre-test)	Mean±s.e.m. time spent in social interaction (s)	Locomotion (mean±s.e.m. number of squares crossed)
Vehicle	Vehicle	48.5±4.0	495.3±16.7
Vehicle	Diazepam 2	100.3±8.6**	492.0±30.0
Diazepam 5	Diazepam 2	85.3±10.2**	499.0±15.5
Diazepam 10	Diazepam 2	69.9±6.7[b]	480.5±16.0
Diazepam 20	Diazepam 2	79.4±7.0*	480.6±21.0
Diazepam 40	Diazepam 2	67.5±4.6[b]	484.2±11.9

[a] All groups comprised of 18–20 rats. Significantly different from vehicle+vehicle treated group, * $p<0.05$, ** $p<0.01$, from vehicle+diazepam treated group.

[b] $p<0.05$ by Newman–Keuls test and 1-way ANOVA.

chronic vehicle treated controls under the "low-light familiar" conditions used (Fig. 5). The effect of 24 h withdrawal was not studied as, at these high doses, SB-243213 still displayed biological activity after 24 h (30.9% inhibition of the mCPP hypolocomotion response with 9 mg/kg SB-243213). In contrast, 24 h withdrawal from chronic diazepam (40 mg/kg p.o. b.i.d.×14 days) significantly reduced time spent in social interaction ($F(5,54)=8.3$, $p<0.01$, Fig. 5). However, rats 72 h withdrawn from chronic treatment with SB-243213 4.5 mg/kg had reduced locomotion ($F(5,54)=3.0$, $p<0.05$). This was not seen in rats 72 h withdrawn from the chronic 9 mg/kg regimen or 48 h withdrawn from the 4.5 or 9 mg/kg regimen and so was not a consistent effect. Further, this reduction in locomotor activity was not associated with a reduction in time spent in social interaction.

3.9. Effect of SB-243213 on total activity and transits in male rats

Activity was analysed for the periods 60–120 min after oral administration of SB-243213 (1–10 mg/kg, p.o.). Analysis of the $\log_{10}$ transformed data showed there was no treatment effect ($F(3,28)=0.689$; $p=0.566$, Table 5). There was a large variance in the 1 mg/kg SB-243213 treated group but this probably represents biological variation. For transits, analysis of the untransformed data by ANOVA showed there was no effect of SB-243213 ($F(3,28)=0.516$; $p=0.675$).

Table 5
Effect of SB-243213 on spontaneous and stimulant-induced activity[a]

Treatment (mg/kg)	Total beam breaks	Transits
Vehicle	222±73	3±1
SB 1	542±240	7±5
SB 3	136±35	3±1
SB 10	256±83	5±2
Vehicle/Saline	198±119	5±2
Vehicle/Amp 0.4	1663±354**	34±10**
SB 1 /Amp 0.4	2000±345**	51±18**
SB 3 /Amp 0.4	1443±186**	22±4**
SB 10 /Amp 0.4	1864±199**	34±11**
Vehicle/Saline	115±37	2±1
Vehicle/PCP 0.94	522±123**	5±2
SB 1 /PCP 0.94	561±99**	2±1
SB 3 /PCP 0.94	517±68**	4±1
SB 10 /PCP 0.94	696±78**	10±3**
Vehicle/Saline	210±64	2±1
Vehicle/MK-801 0.1	2343±463**	28±14*
SB 1 /MK-801 0.1	2832±666**	120±46**
SB 3 /MK-801 0.1	4142±1068**	256±114**
SB 10 /MK-801 0.1	3834±941**	201±81**

[a] All data are means±s.e.m., n 6–8 per group.

3.10. Effect of SB-243213 on amphetamine-induced increase in total activity and transits

For total activity, ANOVA revealed a significant treatment effect on total activity over the final 55 min

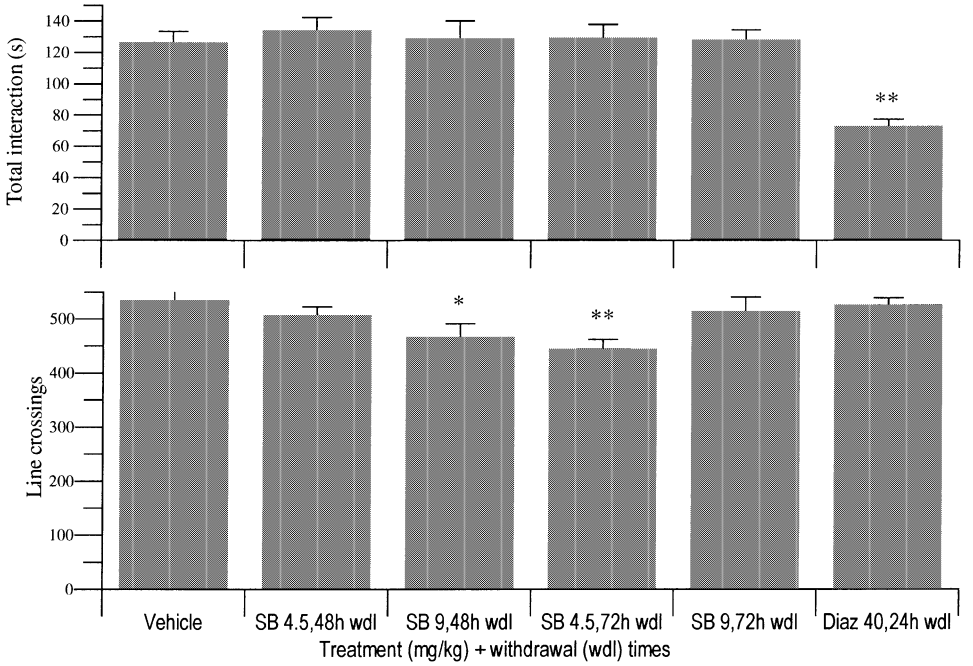

Fig. 5. Lack of withdrawal anxiogenesis of SB-243213. Shown is the effect on behaviour in a rat 15 min social interaction test conducted under low-light familiar conditions following chronic treatment with either SB-243213 or diazepam. All data cited as means with SEM, n=10. Significantly different from vehicle treated group *$p<0.05$, **$p<0.01$.

($F(4,35)=7.82$; $p=0.00013$). Dunnett's *t*-test showed that amphetamine significantly increased activity compared to the control group ($p<0.01$). SB-243213 (1–10 mg/kg, p.o.) did not potentiate or reduce amphetamine-induced hyperactivity (Table 5). For transits, after $\log_{10}$ transformation, ANOVA revealed a significant treatment effect on total activity over the final 55 min ($F(4,35)=6.040$; $p=0.000835$). Dunnett's *t*-test showed that amphetamine significantly increased activity compared to the control group ($p<0.01$) and that this effect of amphetamine was neither attenuated nor potentiated by SB-243213.

3.11. Effect of SB-243213 on phencyclidine-induced increase in total activity and transits

For total activity, analysis of the $\log_{10}$ transformed data showed a significant overall treatment effect for the final 55 min test period ($F(4,35)=11.687$; $p=0.000004$). Post hoc comparisons showed that phencyclidine increased activity compared with vehicle-treated rats ($p<0.05$) whereas no dose of SB-243213 (1-10 mg/kg, p.o.) antagonised or potentiated phencyclidine-induced hyperactivity (Table 5). For transits, after $\log_{10}$ transformation, there was a significant overall treatment effect for the final 55 min test period ($F(4,35)=4.331$; $p=0.0060$). Post hoc comparisons showed that phencyclidine did not increase activity compared with the vehicle-treated rats and no dose of SB-243213 affected activity seen after phencyclidine (Table 5).

3.12. Effect of SB-243213 on MK-801-induced increase in total activity and transits

For total activity, analysis of the $\log_{10}$ transformed data revealed an overall treatment effect for the final 55 min treatment period ($F(4,35)=19.33$; $p=0.000001$). Post hoc comparisons showed that MK-801 significantly increased locomotor activity when compared to vehicle ($p<0.01$). SB-243213 (1–10 mg/kg, p.o.) did not reverse or potentiate the MK-801 effect (Table 5). For transits, analysis of the $\log_{10}$ transformed data revealed an overall treatment effect for the final 55 min treatment period ($F(4,35)=7.99$; $p=0.0001$). Post hoc comparisons showed that MK-801 significantly increased locomotor activity when compared to vehicle ($p<0.05$). SB-243213 (3.2 mg/kg), but not other doses, potentiated the MK-801 effect ($p<0.05$, Table 5).

3.13. Effect of SB-243213 in catalepsy test and on haloperidol-induced catalepsy

Doses of 10 and 32 mg/kg of SB-243213 resulted in one rat out of six in each group showing a cataleptic response. This was not statistically different from the vehicle treated control group. In contrast, in the positive control group, all six haloperidol treated rats showed a cataleptic response (Fig. 6). Haloperidol alone caused catalepsy in all 6 rats at the 90 min time point. SB-243213 (0.1–10 mg/kg, p.o.) reduced this catalepsy with a maximum effect seen at 1 and 3.2 mg/kg p.o. (Fig. 6).

3.14. Effects of SB-243213 on the food intake of freely-feeding rats

Acute administration of SB-243213 (1, 3 or 10 mg/kg p.o.) had no effect on food intake over 1, 2, or 4 h while there was a small and non-significant (9%) increase in 24 h food intake only in the 3 mg/kg p.o. treated group, an effect not seen at the higher dose of 10 mg/kg (data not shown). Chronic administration of the SB-243213 (4.5 and 9 mg/kg p.o. b.i.d.×14 days) did not have any effect on body weight gain over the period studied, except that chronic administration of the higher dose (9 mg/kg p.o.) slightly, but significantly, reduced weight gain from 108.7±2.1 to 97.9±2.1 ($p<0.05$, Dunnett's *t*-test).

3.15. Effect of SB-243213 on convulsant activity in the rat maximal electroshock seizure threshold test

The administration of the known pro-convulsant agent picrotoxin, 2 mg/kg i.p. 30 min pre-test, significantly lowered the current producing tonic hindlimb extensor seizures in 50% of rats in the test (CC_{50} mA±s.e.m., n=12–13; vehicle 48.5±2.1; picrotoxin 25.8±1.0). SB-243213 (10–100 mg/kg, p.o., 1 h pre-test) had no effect compared to 1% methyl cellulose vehicle control (SB-243213 dose, CC_{50} mA±s.e.m., n=12–13; vehicle 43.3±0.7, 10 mg/kg p.o., 44.2±1.0; 30 mg/kg p.o.,

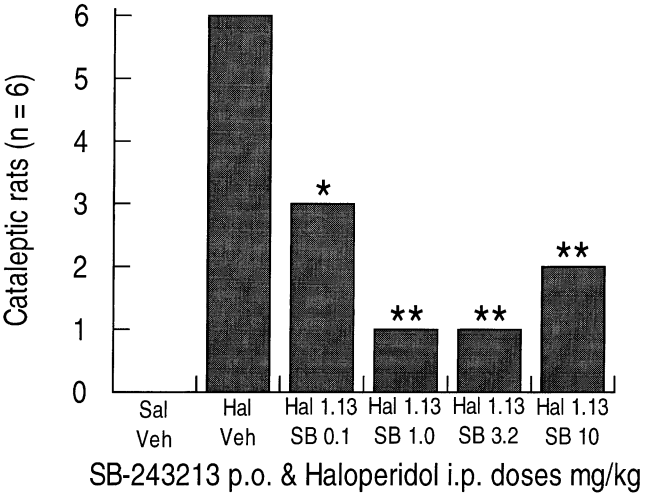

Fig. 6. Effect of SB-243213 on haloperidol-induced catalepsy in rats. Each bar shows the number of cataleptic rats 90 min after administration of haloperidol and pre-treatment with SB-243213. * $p<0.05$; ** $p<0.01$ compared to vehicle/haloperidol group by logistic regression analysis, n=6 per group.

49.2±1.9; 100 mg/kg p.o., 48.3±3.6). There was a small but significant increase in seizure threshold at 30 mg/kg, p.o.(13% change from control, $p<0.01$, Litchfield and Wilcoxon).

4. Discussion

Receptor binding and functional studies show that SB-243213 is a high affinity and selective inverse agonist at the human 5-HT_{2C} receptor. SB-243213 displays at least 100 fold selectivity over a wide variety of neurotransmitter receptors, including the 5-HT_{2A} and 5-HT_{2B} receptors. In functional studies, SB-243213 appeared as a competitive antagonist in Schild analysis, but it also acted as an inverse agonist in that it lowered basal phosphoinositide hydrolysis (Wood et al., 1997b).

In in vivo studies, consistent with previous studies on other 5-HT_{2C} receptor antagonists (Kennett et al., 1997), SB-243213 was a potent, orally active 5-HT_{2C} receptor antagonist with a long duration of action in inhibiting the mCPP-induced hypolocomotor response in rats. SB-243213 also displayed anxiolytic-like activity in the rat social interaction and Geller–Seifter tests. The anxiolytic activity of SB-243213 was similar to that of chlordiazepoxide in the social interaction test but was lower than that seen to diazepam in the Geller–Seifter test, probably reflecting the sensitivity of this test to the sedative effects of benzodiazepines. This further confirms the suggestion that reduction in 5-HT_{2C} receptor function may be anxiolytic (Kennett et al., 1998), consistent with studies in mutant mice lacking the 5-HT_{2C} receptor (Tecott, 1996). However, other characteristics of mutant mice lacking the 5-HT_{2C} receptor include a susceptibility to seizures and an increased food intake (Tecott et al., 1995). Despite this and consistent with previous findings (Kennett et al., 1997), we have found no evidence in the rat, using SB-243213, that 5-HT_{2C} receptor antagonism is associated with either hypophagic or pro-convulsant effects. A lack of hypophagic effects associated with 5-HT_{2C} receptor blockade has also been suggested from studies with a non-selective 5-HT_{2C} antagonist, S16924 (Cussac et al., 2000). In the present study, we saw no increase in body weight following chronic treatment with SB-243213, indeed there was a small but inconsistent reduction in body weight. It is therefore possible that some of the characteristics of the 5-HT_{2C} knock-out mice may arise from developmental or neuroadaptive changes or that there are species and strain differences in the response to the absence of 5-HT_{2C} receptor stimulation.

Major problems are associated with chronic use of benzodiazepine-type anxiolytics in the clinic, including the development of tolerance to their anxiolytic effects and a withdrawal anxiogenesis appears following cessation of chronic drug administration. These effects have been modelled in animals, showing that both tolerance to the anxiolytic effects of benzodiazepines and withdrawal anxiety can be demonstrated using the social interaction test (Andrews et al., 1997; Fernandes et al., 1999). In the present study, using the social interaction test, we have demonstrated the development of tolerance to the anxiolytic effects of diazepam and the presence of withdrawal anxiety following cessation of chronic diazepam treatment. In contrast, however, there was no evidence of tolerance development to the anxiolytic-like response to SB-243213 in this model, nor did 48 h withdrawal from chronic SB-243213 have any effect on social interaction under the conditions used. The lack of tolerance development to SB-243213 at doses up to 30 times its minimum effective dose compares very favourably with the marked tolerance to the anxiolytic effect of 2 mg/kg diazepam — a dose only 8 times the minimum effective dose (unpublished data). There was a small reduction in locomotor activity seen following withdrawal from SB-243213. However, this was not related to dose or time after discontinuation and, further, was not associated with a reduction in time spent in social interaction which may be expected with sedative effects. These results suggest that in the clinic, selective 5-HT_{2C} receptor blockade may have an improved anxiolytic profile, compared to benzodiazepines, as 5-HT_{2C} receptor blockade may not be associated with the development of tolerance and withdrawal anxiogenesis.

5-HT neurones innervate both the dopamine cell bodies in the substantia nigra pars compacta and the ventral tegmental area, and their projection terminal fields in the striatum, nucleus accumbens and frontal cortex (Azmitia and Segal, 1978). Therefore, 5-HT mechanisms are well placed to modulate dopaminergic processes. Recently the advent of new antagonists with improved selectivity for the subtypes of the 5-HT_2 receptor have facilitated research into the specific effects of 5-HT_{2C} receptor antagonism on ascending dopaminergic systems. Thus Di Matteo et al. (1999) have shown that SB-242084 (100 fold selective for 5-HT_{2C} over 5-HT_{2A} and 5-HT_{2B} receptors) increases both phasic and tonic dopaminergic neuronal firing and dopamine release in the mesolimbic system. As a result of these studies, they suggested that 5-HT_{2C} receptor antagonists may be useful antidepressant drugs, as previous hypotheses have suggested that increased dopamine transmission in the mesolimbic system is responsible for the clinical effects of antidepressants (Cervo and Samanin, 1988). Conversely, according to the classical dopamine hypothesis of schizophrenia, enhanced dopamine mechanisms in the mesolimbic axis may produce or exacerbate psychosis as antagonism of increased dopamine activity with typical neuroleptics is thought to underlie their antipsychotic action (Carlsson, 1978).

It has also been suggested that 5-HT_{2C} receptor blockade may be pro-psychotic. Thus, Barton et al. (1999) demonstrated a potentiation of phencyclidine-induced

hyperactivity and dopamine efflux in the nucleus accumbens by the 5-$HT_{2C/2B}$ receptor antagonist SB-221284. Conversely, in another study, the selective 5-HT_{2C} receptor antagonist SB-242084 did not potentiate MK-801 hyperactivity (O'Neill et al., 1999). However, as phencyclidine is known to interact with cytochrome P450 enzymes (Owens et al., 1993) and as SB-221284 is a potent inhibitor of cytochrome P450 (unpublished data), a drug interaction at the level of this enzyme may explain the results of Barton et al. (1999). Data from the present studies indicate that 5-HT_{2C} receptor antagonism by SB-243213 does not potentiate stimulant-induced hyperactivity since the only significant effect was seen in one measure of MK-801-induced hyperactivity. This is consistent with previous reports using the selective 5-HT_{2C} receptor antagonist SB-242084 which did not potentiate MK-801 hyperactivity (O'Neill et al., 1999). These studies therefore indicate that SB-243213 is unlikely to exacerbate psychotic symptoms in man.

Serotonergic mechanisms are known to influence neuroleptic-induced catalepsy (Balsara et al., 1979) and 5-HT_2 receptor antagonism has been suggested to confer a favourable side-effect profile to neuroleptics (Meltzer et al., 1989). There is clinical evidence to support this as the mixed 5-$HT_{2A/2C}$ receptor antagonist, mianserin, has been shown to reduce neuroleptic-induced akathisia (Poyurovsky and Weizman, 1997; Poyurovsky et al., 1999) and possibly reduce psychosis and neuroleptic-induced dysphoria (Poyurovsky et al., 1999). Other workers have attempted to discover if 5-HT_2 receptor antagonists modulate catalepsy. Kalkman et al. (1998), in a study with SB-200646, failed to show attenuation of loxepine-induced catalepsy. However, SB-200646 has only moderate affinity (pK_i=6.9) and selectivity for the 5-HT_{2C} receptor (Kennett et al., 1994). Bligh-Glover et al. (1995) showed that ritanserin attenuated the catalepsy induced by low doses (0.25-0.375 mg/kg) but not high doses (0.75 mg/kg) of haloperidol. However, while ritanserin has a high affinity for 5-HT_{2C} receptors (Reavill et al., 1999), it is a non-selective compound, particularly with respect to 5-HT_{2A} receptors. The recent discovery of high affinity and more selective 5-HT_2 receptor antagonists has enabled further investigation of the role of these receptors in catalepsy. The 5-HT_{2A} receptor antagonist, MDL-100907 has 100 fold selectivity for the 5-HT_{2A} over the 5-HT_{2C} receptor (5-HT_{2A} K_i=0.85 nM; 5-HT_{2C} K_i=87 nM, Kehne et al., 1996), although other data have shown little selectivity (Reavill et al., 1999). MDL-100907 has been in clinical development for schizophrenia, and has an "atypical" profile in preclinical tests (Kehne et al., 1996). It has been shown, however, that MDL-100907 failed to attenuate haloperidol-induced catalepsy at doses that were active in blocking the 5-HT_{2A} receptor in the rat (Reavill et al., 1999) and, Kalkman et al. (1998), have shown that MDL-100151 (R-(±)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol), the racemate of MDL-100907, is cataleptogenic at a dose of 0.3 mg kg^{-1}. This may suggest that there is a critical window of 5-HT_{2C} over 5-HT_{2A} selectivity which is necessary to cause reversal of haloperidol-induced catalepsy (Reavill et al., 1999). It is notable that another selective 5-HT_{2C} receptor antagonist, SB-228357, had a pronounced anti-cataleptic profile (Reavill et al., 1999). This compound has a high affinity for the 5-HT_{2C} receptor and is 100 and 10 fold selective over the 5-HT_{2A} and 5-HT_{2B} receptor, respectively (Reavill et al., 1999). Evidence has recently been provided that 5-HT_{2C} receptor antagonism may produce a favourable outcome in states of motor disturbance. Thus, oro–facial dyskinesias elicited by stimulation of subthalamic 5-HT_{2C} receptors is blocked by the 5-HT_{2C} receptor antagonist, SDZ SER 082 (Eberle-Wang et al., 1996), and injection of the 5-HT_{2C} receptor antagonist SB-206553 (5-methyl-1-(3-pyridylcarbamoyl)-2,3-dihydropyrrolo[2,3-f]indole), into the substantia nigra zona reticulata produces an anti-parkinsonian effect in the rat (Fox et al., 1998). Taken together, these data suggest that 5-HT_{2C} receptor antagonism may have positive effects in treating neuroleptic-induced extrapyramidal side-effects and other conditions.

In conclusion, SB-243213 is a selective 5-HT_{2C} receptor antagonist which has inverse agonist properties in recombinant systems. In vivo, it has a long duration of action and is active in two pre-clinical models of anxiety with different motivational basis, namely the social interaction and Geller–Seifter models. SB-243213 has an improved anxiolytic profile compared to benzodiazepines, suggesting that 5-HT_{2C} receptor blockade may be free from tolerance and dependence liabilities. There is no evidence for an increase in weight gain or for pro-convulsant activity associated with SB-243213 treatment, two properties associated with 5-HT_{2C}-receptor knock-out mice. There was no evidence that SB-243213 had pro-psychotic effects, but there is clear evidence that 5-HT_{2C} receptor blockade may modulate striatal dopaminergic neurones, which suggests that a 5-HT_{2C} receptor antagonist may be useful in the treatment of motor disorders caused by altered dopaminergic function, including those induced by chronic neuroleptic treatment. Given that disinhibition of the mesolimbic dopaminergic system may underlie the mechanism of action of antidepressants (Cervo and Samanin, 1988) and given the close association between depression and anxiety disorders, selective 5-HT_{2C} receptor antagonists may also have therapeutic utility in depression (Kennett, 1993; Di Matteo et al., 2000).

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