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SB-236057, a selective 5-HT$_{1B}$ receptor inverse agonist, blocks the 5-HT human terminal autoreceptor

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Abstract

A novel compound, SB-236057 (1′-ethyl-5-(2′-methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidine]) has been shown to have high affinity for human 5-hydroxytryptamine$_{1B}$ (5-HT$_{1B}$) receptors ($pK_i = 8.2$) and displays over 75 or more-fold selectivity for the human 5-HT$_{1B}$ receptor over other 5-HT receptors, including the human 5-HT$_{1D}$ receptor, and a range of other receptors, ion channels and enzymes. In functional studies using [^{35}S]GTPγS binding, SB-236057 displayed negative intrinsic activity ($pEC_{50} = 8.0$) at human 5-HT$_{1B}$ receptors stably expressed in Chinese Hamster Ovary (CHO) cells and caused a rightward shift of agonist concentration response curves consistent with competitive antagonism ($pA_2 = 8.9$). SB-236057 potentiated [^{3}H]5-HT release from electrically stimulated guinea pig or human cortical slices. SB-236057 also abolished the inhibitory effect of exogenously superfused 5-HT on electrically-stimulated release from slices of the guinea pig cortex. These studies using SB-236057 confirm that, in both the guinea pig and human cerebral cortex, the terminal 5-HT autoreceptor is of the 5-HT$_{1B}$ subtype.

Keywords: 5-HT (5-hydroxytrptamine, serotonin); SB-236057; 5-HT$_{1B}$ autoreceptor; 5-HT release; Human

1. Introduction

The family of 5-hydroxytryptamine (5-HT) receptors has been divided into seven distinct receptor types on the basis of operational, structural and transductional criteria (Hartig et al., 1996). One subgroup of this receptor family, the 5-HT$_1$ receptors, has been subdivided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$. The 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes can be pharmacologically identified with the selective 5-HT$_{1B/1D}$ partial agonist GR12-7935 (*N*-[4- methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′-methyl-4′-(5- methyl-1,2,4-oxadiazole-3-yl)[1,1′,-biphenyl]-carboxamide)(Skingle et al., 1996). Further pharmacological separation can be achieved with the 5-HT$_{1B}$ receptor selective partial agonist SB-216641 (*N*-[3-(2-dimethyl-amino)ethoxy-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-biphenyl-4-carboxamide) (Price et al., 1997) or the inverse agonist SB-224289 (2,3,6,7-tetra-hydro-1′-methyl-5-{2′-methyl-4′-[5-methyl-1,2,4-oxadiazole -3-yl)biphenyl-4-yl]carbonyl}furo[2,3-F]-indole-3-spiro-4′-piperidine oxalate) (Selkirk et al., 1998), the latter compound having reasonably high affinity for 5-HT$_{1B}$ receptors with 75-fold selectivity for the 5-HT$_{1B}$ receptor over other 5-HT receptors. However both GR127935 and SB-216641 are partial agonists at recombinant 5-HT$_{1B}$ receptors expressed in Chinese Hamster Ovary (CHO) cell lines (Watson et al., 1996; Price et al., 1997) and the inverse agonist, SB-224289, has relatively low potency in vivo (Hagan et al., 1997), precluding its development as a therapeutic agent in man. In native tissue no intrinsic activity at 5-HT$_{1B}$ receptors has been observed with any of these compounds (Skingle et al., 1996; Schlicker et al., 1997; Selkirk et al., 1998) and they have been used as antagonists to characterise both 5-HT auto- and hetero-receptors (Roberts et al., 1997, 1998; Schlicker et al., 1997, Selkirk et al., 1998).

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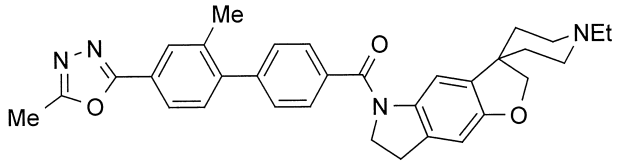

SB-236057

Fig. 1. Chemical structure of SB-236057.

As yet no studies have been reported on the effects of a selective 5-HT1B receptor inverse agonist at the human 5-HT terminal autoreceptor. We now report on a new compound, SB-236057 (1′-ethyl-5-(2′-methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidine]), which has high affinity, selectivity and inverse agonist activity at human 5-HT_{1B} receptors and increases the release of 5-HT from slices of guinea pig and human cortex (Fig. 1).

2. Materials and methods

2.1. Preparation of membranes containing human 5-HT_{1B} and 5-HT_{1D} receptors

Human 5-HT_{1B} and 5-HT_{1D} receptors were stably transfected into Chinese hamster ovary (ACC098) cell lines as previously described (Watson et al., 1996). The cells were harvested by centrifugation, resuspended in HEPES buffer (20 mM) containing EDTA (10 mM), and homogenised with an Ultra-Turrax. The membranes were then washed in HEPES buffer (20 mM) containing EDTA (0.1 mM), centrifuged and stored as frozen aliquots at −80°C until use. These membranes were used in both radioligand binding and [^{35}S]GTPγS binding assays.

2.2. Radioligand binding studies

Radioligand binding studies were carried out on human cloned 5-HT_{1B} and 5-HT_{1D} receptors as follows. Membranes were resuspended in Tris buffer (Tris HCl (50 mM), $MgCl_2$ (10 mM), ascorbate (6 mM), pargyline (0.5 μM)), pH 7.4. Competition studies were performed (in single samples) using 10 × 0.5 log unit dilutions of competing ligand in the presence of [^{3}H]5-HT (4 nM), and non-specific binding was determined by the inclusion of 5-HT (10 μM). Membranes (~ 10^5 cells) were incubated in a final volume of 0.5 ml for 45 min at 37°C, and the reaction was stopped by rapid filtration through Whatman GF/B filters, presoaked with 0.3% polyethyleneimine, on a 96 well Tomtec harvester. This was followed by 4 × 1 ml washes with ice cold Tris buffer. Radioactivity was determined using liquid scintillation counting on a Packard Topcount.

2.3. Receptor selectivity studies

To determine the relative receptor selectivities of SB-236057, binding studies were also carried out on cloned human 5-HT_{1A}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_4, 5-HT_6 and 5-HT_7 receptors. Details of cell lines, the ligands and the concentrations used, together with the compounds included to define non-specific binding, are shown in Table 1.

2.4. [^{35}S]GTPγS binding studies

Membranes from CHO cells stably expressing human 5-HT_{1B} or 5-HT_{1D} receptors were prepared and [^{35}S]-GTPγS binding studies were carried out as described previously (Thomas et al., 1995). Briefly, membranes from 10^6 cells were pre-incubated at 30°C for 30 min in 20 mM HEPES buffer (pH 7.4) in the presence of $MgCl_2$ (3 mM), NaCl (100 mM), GDP (10 μM) and ascorbate (0.2 mM), with or without compounds. The reaction was started by the addition of 10 μl of [^{35}S]GTPγS (100 pM, assay concentration) followed by a further 30 min incubation at 30°C. Non-specific binding was determined using non-radiolabelled GTPγS (20 μM) added prior to the membranes. The reaction was terminated by rapid filtration through Whatman GF/B grade filters followed by 5 × 1 ml washes with ice cold HEPES (20 mM)/$MgCl_2$ (3 mM)

Table 1
Summary of conditions used in binding assays to assess human 5-HT receptor selectivity of SB-236057

Receptor	Cell line/source	Radioligand	Concentration	NSB (10 μM)
5-HT_{1A}	HEK 293	[^{3}H]8-OH DPAT	1 nM	Buspirone
5-HT_{1B}	CHO	[^{3}H]5-HT	4 nM	5-HT
5-HT_{1D}	CHO	[^{3}H]5-HT	4 nM	5-HT
5-HT_{1E}	CHO	[^{3}H]5-HT	4 nM	5-HT
5-HT_{1F}	CHO	[^{3}H]5-HT	4 nM	5-HT
5-HT_{2A}	HEK 293	[^{3}H]Ketanserin	0.5 nM	Mianserin
5-HT_{2B}	HEK 293	[^{3}H]5-HT	8 nM	5-HT
5-HT_{2C}	HEK 293	[^{3}H]Mesulergine	0.6 nM	Mianserin
5-HT_4	guinea-pig/piglet hippocampus	[^{125}I]SB-207710	1.3 nM	SB-205008
5-HT_6	HeLa	[^{3}H]LSD	2 nM	Methiothepin
5-HT_7	HEK 293	[^{3}H]5-CT	0.5 nM	5-HT

NSB: compound used to define non-specific binding used at 10 μM.

buffer. Radioactivity was measured using liquid scintillation spectrometry.

2.5. In vitro [^{3}H]5-HT release studies in brain slices

2.5.1. Guinea pig cortex

Electrically evoked [^{3}H]5-HT release studies were performed in guinea pig cortex as previously described (Selkirk et al., 1998). Male guinea pigs (400–500 g) were killed by cervical dislocation, decapitated and the brains removed. The whole cortex was rapidly dissected out and cross-chopped into 300 μm × 300 μm slices using a McIlwain chopper. The slices were incubated in Krebs buffer containing [^{3}H]5-HT (100 nM) in the presence of pargyline (10 μM) at 37°C for 15 min. Slices were then gently washed twice in buffer and resuspended in 10 ml Krebs buffer bubbled with a mixture of 95% O_2/5% CO_2 and 100 μl aliquots transferred to a Brandel Suprafusion 2000 Apparatus. The composition of the Krebs solution was the following (in mM): NaCl (118), KCl (4.8), $CaCl_2$ (1.3), $MgSO_4$ (1.2), $NaHCO_3$ (25), NaH_2PO_4 (1.2), glucose (10), L-ascorbate (0.06), Na_2EDTA (0.03). Slices were superfused with oxygenated Krebs solution in the presence of the 5-HT uptake blocker paroxetine (10 μM), at a rate of 0.5 ml/min.

After 32 min of superfusion ($t = 0$), samples were collected every 4 min for a period of 80 min. Transmitter release was stimulated electrically with a Brandel Stimulator using a 2 ms biphasic square wave pulse, 20 mA in amplitude. Slices were stimulated at a frequency of 1 Hz for 2 min at $t = -30$ min to aid tissue equilibration, and again at $t = 12$ min (S1) and $t = 56$ min (S2) at a frequency of 1 Hz for 2 min for reversal of 5-HT inhibition studies, and 3 Hz for 1 min to measure the effects of the antagonists alone on [^{3}H]5-HT release. Agonists were superfused at $t = 44$ min and removed at $t = 64$ min. Antagonist superfusion started at $t = 24$ min and continued for the remainder of the experiment. At the end of the experiment the amount of radioactivity in the slices and superfusate samples were determined by liquid scintillation spectrometry.

Fractional release (FR) for each sample was calculated as the amount of radioactivity in a sample expressed as a fraction of the total radioactivity present in the slices at the time of that collection. Basal levels of [^{3}H]5-HT release were calculated as the mean percent FR per 4 min sample of the two samples either side of the S1 and S2 electrical stimulation collection periods, and were designated B1 and B2 respectively. S1 and S2 collection periods were for two 4 min samples during and immediately following the electrical stimulation. The effects of drugs on the stimulated release were calculated as (S2-B2)/(S1-B1) ratios of FR, and the results were expressed as this ratio.

2.5.2. Human cortex

Studies on the electrically-evoked release of [^{3}H]5-HT from human cerebral cortical slices, prepared from tissue obtained from 3 patients who were undergoing neurosurgery for epilepsy, were carried out as previously described (Schlicker et al., 1997). The study was approved by the local ethics committee.

Briefly cortical slices (0.3 mm thick, diameter 2 mm) were incubated with modified Krebs solution containing [^{3}H]5-HT (0.1 μM) for 60 min at 37°C and superfused at 0.5 ml/min with a medium containing 6-nitroquipazine (3.2 μM) and idazoxan (1 μM) for 120 min at 37°C. Tritium overflow was evoked by two 2 min periods of electrical stimulation (3 Hz, 100 mA, 2 ms) after 60 min and 100 min of superfusion (S1 and S2). SB-236057, when studied, was present in the medium throughout superfusion. At the end of the superfusion, the cortical slices were solubilised with SolueneR and the radioactivity in the tissues and supernatants was determined by liquid scintillation counting.

Tritium efflux was calculated as the fraction of the tritium content in the tissues at the beginning of the respective collection period (FR). To quantify the effects of SB-236057 on basal efflux, the FR was determined in the sample collected from 55–60 min of superfusion expressed as a percent of tissue tritium. To quantify the effect of SB-236057 on evoked tritium overflow, the difference between total and basal tritium efflux during S1 and the subsequent 13 min was determined and expressed as a percent of tissue tritium.

2.6. Data analysis

Receptor binding data were generated as single data points, and GTPγS data were generated in duplicate within each experiment. Each experiment was carried out at least 3 times. Curve fitting of the meaned data was generated by a four parameter logistic equation, using GRAFIT (Erithacus Software). pK_i data were calculated from the IC_{50}, using the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

[^{35}S]GTPγS binding data was expressed as percent stimulation above basal [^{35}S]GTPγS binding, i.e., in the absence of test compounds.

[^{3}H]5-HT release from guinea pig cortical slices was expressed as the S2/S1 ratio (see above) and is the mean of 4 independent experiments. Statistical significance was determined using analysis of variance post hoc Dunnett's *t*-test using the control as standard for the antagonist potentiation studies and the 5-HT [30 nM] as standard for the abolition of inhibition studies.

[^{3}H]5-HT release from human cortical slices was expressed as the percent of tissue tritium (see above) and is the mean of a total number of 6–7 slices per experimental condition (2–3 slices in each of 3 independent experiments, each based on tissue from one individual patient). For comparison of means, Student's *t*-test was used; if two experimental values were compared to the same control, the Bonferroni correction was used.

Table 2
Receptor binding affinities, expressed as pK_i, of SB-236057 at human cloned 5-HT receptors

Receptor/Tissue	pK_i
5-HT_{1A}	< 5
5-HT_{1B}	8.2 ± 0.1
5-HT_{1D}	6.3 ± 0.1
5-HT_{1E}	< 5
5-HT_{1F}	< 5
5-HT_{2A}	< 5.2
5-HT_{2B}	< 5
5-HT_{2C}	< 5.3
5-HT_4	5.4
5-HT_6	< 5
5-HT_7	< 5

Data are the mean ± S.E.M of 8–9 separate experiments (5-HT_{1B} and 5-HT_{1D}) or the mean of at least 3 experiments (other 5-HT receptors).

2.7. Drugs

SB-236057 (1′-Ethyl-5-(2′-methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidine]), paroxetine HCl, buspirone, mianserin, SB-205008 and methiothepin were synthesised by SmithKline Beecham Pharmaceuticals (Harlow, UK). 5-HT (5-hydroxytryptamine creatine sulphate), GTPγS (Guanosine-5′-*O*-3-thiotriphosphate), GDP (Guanosine 5′-diphosphate, Tris salt) and pargyline (*N*-methyl-*N*-propargylbenzylamine; *N*-methyl-*N*-2-propynylbenzylamine) were obtained from Sigma (Poole, UK). 6-Nitroquipazine was obtained from RBI (Natick, USA) and idazoxan from Reckitt and Colman (Hull, UK). [^{3}H]5-HT (90 Ci/mmol) and [^{35}S]GTPγS (900–1200 Ci/mmol) and all other radiolabels used were purchased from Amersham International plc (Buckinghamshire, UK). The [^{3}H]5-HT used for experiments in human cortical slices was purchased from NEN (Dreieich, Germany) and had a specific activity of 29.7 Ci/mmol.

3. Results

3.1. Radioligand binding studies

Competition studies were performed to determine the affinity of SB-236057 at a number of 5-HT receptors (Table 2). Specific binding with [^{3}H]5-HT (defined with 10 μM 5-HT) represented over 90% of total binding in both human 5-HT_{1B} and 5-HT_{1D} receptor-expressing systems. SB-236057 displayed 75 fold higher affinity (pK_i = 8.2) at the human 5-HT_{1B} receptor expressed in CHO cells

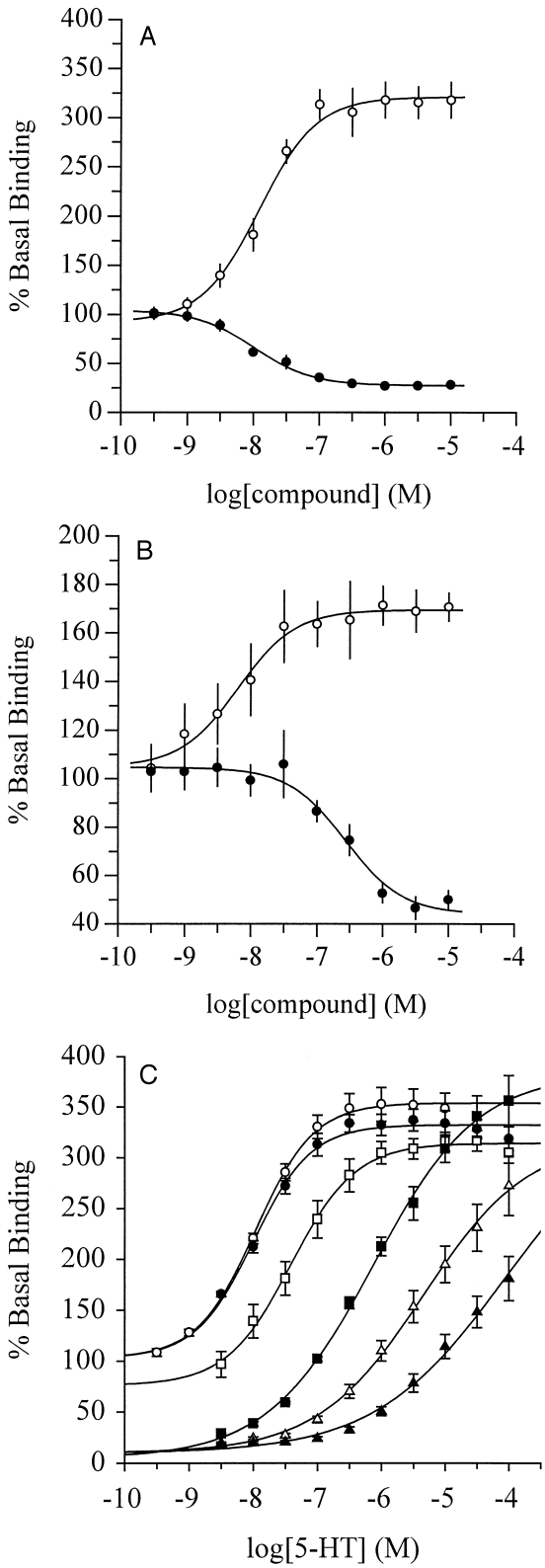

Fig. 2. Stimulation of [^{35}S]GTPγS binding in CHO cell membranes expressing h5-HT_{1B} and h5-HT_{1D} receptors. Data are expressed as percent stimulation above basal binding within each experiment. (A) and (B) show concentration response curves to 5-HT (○) and SB-236057 (●) at h5-HT1B (A) and h5-HT1D (B) receptors respectively. Antagonist shifts of the 5-HT concentration response curve (○) are shown with 1 nM (●); 10 nM (□); 100 nM (■); 1 μM (△) and 10 μM (▲) SB-236057 at h5-HT1B receptors (c). Each data point is the mean of duplicate samples from 3–5 independent experiments and the bars illustrate the standard error of this mean. All curves were fitted with a 4-parameter logistic equation.

Table 3
Receptor binding affinities (pK_i) and [^{35}S]GTPγS binding study results (pEC_{50}, percent stimulation (stim) or inhibition (inhibit), for 5-HT and SB-236057)

	Human 5-HT_{1B} receptors				Human 5-HT_{1D} receptors			
	pK_i	pEC_{50}/pIC_{50}	E_{max}	pA_2	pK_i	pEC_{50}/pIC_{50}	E_{max}	app. pA_2
5-HT	8.4 ± 0.1	7.8 ± 0.1	225% stim	–	8.5 ± 0.1	8.0 ± 0.1	70% stim	–
SB-236057	8.2 ± 0.1	8.0 ± 0.1	75% inhibit	8.9	6.3 ± 0.1	6.6 ± 0.9	55% inhibit	6.8 ± 0.3

All values are expressed as the mean ± the standard error of the mean.

than at the human 5-HT_{1D} receptor ($pK_i = 6.3$). SB-236057 was also greater than 100-fold selective for the human 5-HT_{1B} receptor over the other human 5-HT receptor subtypes tested (Table 2) and for 55 other receptor and enzyme systems (see Section 4).

3.2. [^{35}S]GTPγS binding studies

In both human 5-HT_{1B} and 5-HT_{1D} receptor expressing cell lines, 5-HT produced a concentration-dependent stimulation of [^{35}S]GTPγS binding, with a maximal stimulation of 225% and 70% above basal levels respectively (Fig. 2a and b). Consistent with its receptor binding affinity, 5-HT had similar potencies at human 5-HT_{1B} and 5-HT_{1D} receptors (Table 3). SB-236057 suppressed basal binding by 75% at the human 5-HT_{1B} receptor, and by 55% at the human 5-HT_{1D} receptor. SB-236057 was more potent at 5-HT_{1B} ($pEC_{50} = 8.0$) than at 5-HT_{1D} ($pEC_{50} = 6.6$) receptors, Antagonist studies were carried out to determine the affinity of SB-236057 at human 5-HT_{1B} receptors. At 5-HT_{1B} receptors, SB-236057 (1–10,000 nM) produced parallel rightward shifts of the 5-HT concentration response curve (Fig. 2c). Schild analysis of the data based on pEC_{50}s of individual curves gave a slope not significantly different from unity and a pA_2 of 8.9 (not shown). At 5-HT_{1D} receptors, SB-236057 (1 μM) caused a parallel rightward shift in the concentration response curve to 5-HT with an apparent pA_2 of 6.8 (data not shown).

3.3. [^{3}H]5-HT release studies

3.3.1. Guinea pig cortical slices

At a frequency of 1 Hz, superfusion of 5-HT (30 nM) significantly inhibited electrically stimulated [^{3}H]5-HT release, decreasing the S2/S1 ratio from 0.96 ± 0.02 to 0.56 ± 0.04. SB-236057 reversed the inhibitory effects of exogenous 5-HT in a concentration dependent manner, statistical significance being attained at 100 nM and 1000 nM ($P < 0.05$) (Fig. 3a). SB-236057 (up to 1000 nM) had no effect on basal [^{3}H]5-HT release (data not shown).

At the higher frequency of 3 Hz, SB-236057 increased electrically-evoked [^{3}H]5-HT release from guinea pig cortical slices in a concentration dependent manner with a statistically significant potentiation at 100 nM and 1000 nM (Fig. 3b).

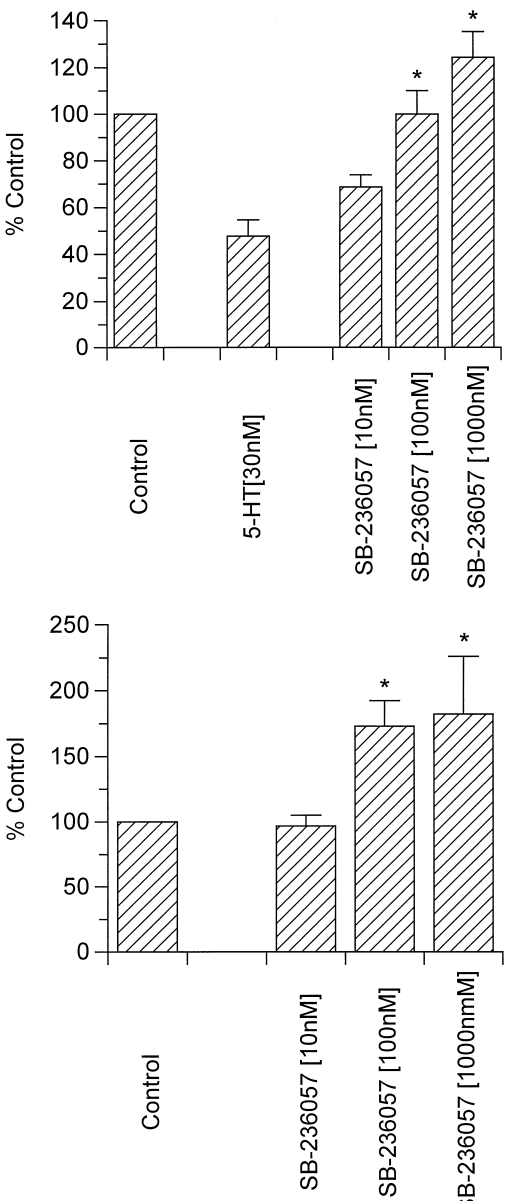

Fig. 3. Electrically evoked [^{3}H]5-HT release from guinea pig cortical slices. Data are shown for the antagonism of 5-HT inhibition of [^{3}H]5-HT release by SB-236057 at 1 Hz, 20 mA for 2 min (top) and the increase of [^{3}H]5-HT release by SB-236057 at 3 Hz, 20 mA for 1 min (bottom), Data is expressed as the percent control for 4 independent experiments and the bars represent the standard error of this mean. Statistical significance is given as * = $P < 0.05$.

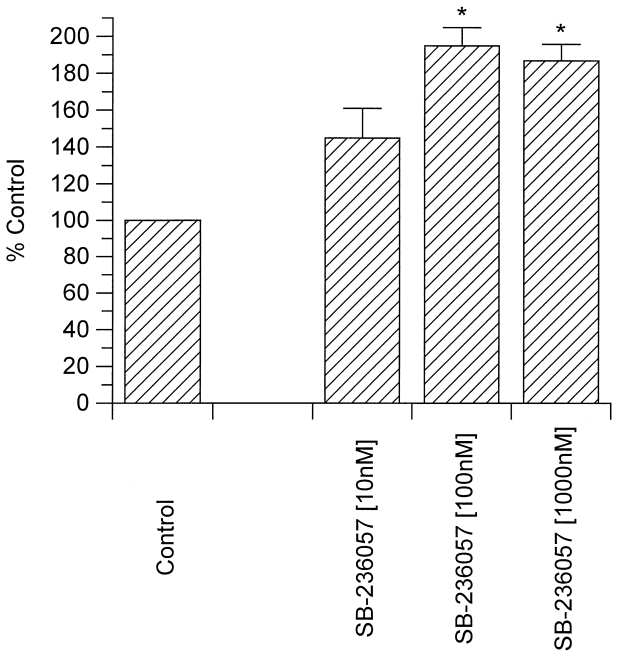

Fig. 4. Electrically evoked [^{3}H]5-HT release from human cortical slices. Data are shown for the increase of [^{3}H]5-HT release by SB-236057 at 3 Hz, 20 mA for 1 min. Data is expressed as the mean percent control. Each data point is the mean of measurements on a total number of 6–7 slices (2–3 slices per condition), in each of 3 independent experiments, each based on tissue from one individual patient; the bars represent the standard error of this mean. Statistical significance is given as * = $P <$ 0.05.

3.3.2. Human cortical slices

In slices from human cortex, SB-236057 (10–1000 nM) did not affect basal tritium efflux (data not shown), but increased electrically (3 Hz)-evoked [^{3}H]5-HT release in a concentration dependent manner (Fig. 4). The percent fractional release was increased by 45%, 96% and 87% over control values by 10 nM, 100 nM and 1000 nM SB-236057, respectively, reaching statistical significance ($P < 0.05$) at 100 nM and 1000 nM concentrations.

4. Discussion

In this series of studies we have evaluated the pharmacological profile of the novel 5-HT$_{1B}$ receptor selective compound, SB-236057, and utilised this ligand to determine the effects of a 5-HT$_{1B}$ receptor inverse agonist on [^{3}H]5-HT release from guinea pig and human cortical slices.

SB-236057 is a potent ($pK_i = 8.2$) and selective (75-fold) ligand for the human 5-HT$_{1B}$ receptor as compared to a number of other 5-HT receptors. Moreover in a comprehensive selectivity screen, SB-236057 has been shown to be more then 100-fold selective for the human 5-HT$_{1B}$ receptor as compared to 55 other receptors and enzymes (Novascreen Task Order 1319). Thus, SB-236057 is a more potent and selective 5-HT$_{1B}$ ligand when compared to another compound developed in our laboratories, SB-216641, which was only 25-fold selective for human 5-HT$_{1B}$ versus 5-HT$_{1D}$ receptors (Price et al., 1997) but is similar to SB-224289, which displayed 75-fold selectivity between these two 5-HT receptors (Selkirk et al., 1998).

In in vitro functional studies in CHO cells using GTPγS radioligand binding as a functional assay, SB-236057 behaved as a potent receptor inverse agonist at human 5-HT$_{1B}$ receptors (pEC_{50} 8.0). Furthermore, in receptor antagonist studies at human 5-HT$_{1B}$ receptors, using the same functional assay, SB-236057 was a competitive antagonist ($pA_2 = 8.9$) with the slope of a Schild plot not significantly different from unity. SB-236057 is weaker as an antagonist at human 5-HT$_{1D}$ receptors (apparent $pA_2 = 6.8$) and therefore is a suitable tool compound to investigate the actions of a 5-HT$_{1B}$ receptor inverse agonist at the terminal 5-HT autoreceptor in native tissue.

In neurotransmitter release studies, SB-236057 reversed the inhibitory effects of 5-HT on electrically-evoked (1 Hz) [^{3}H]5-HT release from slices of the guinea pig cortex. In this respect, these studies confirm that the terminal 5-HT autoreceptor in the guinea pig is of the 5-HT$_{1B}$ subtype, a conclusion previously supported by the use of the 75-fold selective 5-HT$_{1B}$ receptor inverse agonist, SB-224289 (Selkirk et al., 1998). Moreover, at the higher frequency of 3 Hz stimulation, SB-236057 potently increased 5-HT release from guinea pig cortex slices. This effect is probably due to blockade of the effect of endogenous 5-HT released by the electrical stimulus. However it is possible that the inverse agonist properties of this compound seen in recombinant systems are also reflected in native tissue, uncoupling the terminal 5-HT$_{1B}$ autoreceptor from its G-protein (Lefkowitz et al., 1993) and thereby negating a constitutive inhibitory effect of this receptor on the release of 5-HT (see below).

Similar results of SB-236057 were seen in slices of the human brain. SB-236057, like the receptor partial agonist SB-216641 in earlier studies (Schlicker et al., 1997), increased [^{3}H]5-HT release in the human cortex when the slices were electrically stimulated at a frequency of 3 Hz.

The ability of 5-HT$_{1B}$ receptor antagonists such as SB-236057 to increase [^{3}H]5-HT release from electrically-stimulated slices of both the guinea pig and human cortex could be due to the presence of endogenous 5-HT in the superfusion fluid or to the inverse agonist properties of the compound. In both the guinea pig and human cortex, the latter explanation is unlikely to be true since partial receptor agonists at the 5-HT$_{1B}$ receptor such as GR 127935 (Watson et al., 1996) or SB-216641 (Price et al., 1997) have also been shown to increase [^{3}H]5-HT release from electrically-stimulated slices. Indeed, one study of the effects of the non-selective inverse agonist, methiothepin (Watson et al., 1996), carried out in human brain synaptosomes has shown that this drug can block the actions of exogenously applied 5-HT but does not, per se, increase 5-HT release (Maura et al., 1993). In this preparation, the

human superfused synaptosome, any 5-HT released by the stimulus (elevated K^+ ions) is efficiently removed from the vicinity of the terminal 5-HT autoreceptor by the rapid perfusion of the synaptosomes, thereby obviating the ability of endogenous 5-HT to stimulate the terminal 5-HT autoreceptor. Thus the results of the present study on SB-236057, together with studies of a partial receptor agonist at the human 5-HT_{1B} receptor, SB-216641 (Schlicker et al., 1997) and the non-selective inverse agonist at the 5-HT_{1B} receptor, methiothepin (Schlicker et al., 1985; Galzin et al., 1992; Maura et al., 1993), argue that the increase in 5-HT release elicited by 5-HT_{1B} receptor antagonists is probably due to blockade of the effect of endogenous 5-HT released by the nerve stimulus. In addition the larger potentiation of release of 5-HT seen at higher frequencies of stimulation is consistent with antagonism of increased autoreceptor tone by a greater biophase concentration of 5-HT. Presumably the same degree of constitutive coupling would be present at 1 Hz or 3 Hz, therefore the same degree of inverse agonism, i.e., potentiated 5-HT release would have been observed. Since this was not the case the former explanation is likely to be correct.

Thus, in conclusion, SB-236057 has been shown to be a potent, selective inverse agonist at the human 5-HT_{1B} receptor and to block the 5-HT terminal autoreceptor in both the guinea pig and human cortical slice preparation. Since SB-236057 increases 5-HT release from electrically-stimulated slices, it is likely that blockade of the 5-HT_{1B} autoreceptor will enhance 5-HT release if endogenous tone is present. Such an action is seen in vivo after chronic but not acute blockade of the 5-HT re-uptake site by selective serotonin re-uptake blockers (Gardier et al., 1996; Gundlah et al., 1997; Sharp et al., 1997). The ability of 5-HT_{1B} receptor antagonists/inverse agonists to elicit such an effect both in vitro (present study) and in vivo (Roberts et al., 1997, 1998) may indicate that this class of drug may mimic acutely the actions of chronically administered selective serotonin reuptake inhibitors.

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