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SB-272183, a selective 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor antagonist in native tissue

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1 A novel compound, SB-272183 (5-Chloro-2, 3-dihydro-6-[4-methylpiperazin-1-yl]-1[4-pyridin-4-yl]napth-1-ylaminocarbonyl]-1H-indole), has been shown to have high affinity for human 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors with pK_i values of 8.0, 8.1 and 8.7 respectively and is at least 30 fold selective over a range of other receptors.

2 [^{35}S]-GTPγS binding studies showed that SB-272183 acts as a partial agonist at human recombinant 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors with intrinsic activities of 0.4, 0.4 and 0.8 respectively, compared to 5-HT. SB-272183 inhibited 5-HT-induced stimulation of [^{35}S]-GTPγS binding at human 5-HT_{1A} and 5-HT_{1B} receptors to give pA_2 values of 8.2 and 8.5 respectively. However, from [^{35}S]-GTPγS autoradiographic studies in rat and human dorsal raphe nucleus, SB-272183 did not display intrinsic activity up to 10 μM but did block 5-HT-induced stimulation of [^{35}S]-GTPγS binding.

3 From electrophysiological studies in rat raphe slices *in vitro*, SB-272183 did not effect cell firing rate up to 1 μM but was able to attenuate (+)8-OH-DPAT-induced inhibition of cell firing to give an apparent pK_b of 7.1.

4 SB-272183 potentiated electrically-stimulated [^{3}H]-5-HT release from rat and guinea-pig cortical slices at 100 and 1000 nM, similar to results previously obtained with the 5-HT_{1B} and 5-HT_{1D} receptor antagonist, GR127935.

5 Fast cyclic voltammetry studies in rat dorsal raphe nucleus showed that SB-272183 could block sumatriptan-induced inhibition of 5-HT efflux, with an apparent pK_b of 7.2, but did not effect basal efflux up to 1 μM.

6 These studies show that, *in vitro*, SB-272183 acts as an antagonist at native tissue 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors.

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Keywords: SB-272183; 5-HT_{1A}; 5-HT_{1B}; 5-HT_{1D} receptors; recombinant cells; human; guinea-pig; rat

Abbreviations: α-1B, adrenergic-alpha 1B; aCSF, artificial cerebrospinal fluid; β2, beta2 adrenergic; DRN, dorsal raphe nucleus; h, human; 5-HT, serotonin; SSRIs, selective serotonin re-uptake inhibitors

Introduction

5-HT (serotonin) is one of the major neurotransmitters widely distributed in the brain and its actions are mediated *via* 14 distinct receptor subtypes (Barnes & Sharp, 1999). Central serotonergic dysfunction is implicated in the pathogenesis of several mood disorders such as anxiety, depression, panic and obsessive behaviour. It is proposed that the underlying pathology of depression is due to reduced brain 5-HT levels (Cowen, 1996) the latter which, under normal conditions, is regulated by autoreceptors and 5-HT re-uptake sites present on cell bodies in the raphe nuclei and on nerve terminals in projection areas. The autoreceptors present on cell bodies are predominantly of the 5-HT_{1A} receptor subtype which regulate serotonergic function by modulating the rate of cell firing (Craven *et al*., 1994; Haj-Dahmane *et al*., 1991; Sprouse & Aghajanian, 1987). Recent evidence also suggests the presence of 5-HT_{1B} and 5-HT_{1D} receptors in this brain region (Bonaventure *et al*., 1998a, b) within the somatodendritic area. Although their role is not totally clear, there have been several reports suggesting autoreceptor function for these 5-HT_{1B} and 5-HT_{1D} receptors in the raphe (Moret & Briley, 1997; Pineyro *et al*., 1996; Roberts *et al*., 1998; Stamford *et al*., 2000). The autoreceptors located on nerve terminals are of the 5-HT_{1B} receptor subtype and regulate 5-HT release within this region (Engel *et al*., 1986; Roberts *et al*., 1996).

Effective antidepressant treatment can be achieved by the administration of selective serotonin re-uptake inhibitors (SSRIs) but the therapeutic benefits require several weeks of treatment before full clinical efficacy is manifest. This time lag in therapeutic benefit is thought to be due to SSRIs increasing 5-HT levels in the raphe which stimulate inhibitory 5-HT_{1A} receptors, reducing cell firing rate, which results in reduced post-synaptic 5-HT transmission (Artigas, 1993). Prolonged treatment of SSRIs is thought to cause desensiti-

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zation of these 5-HT_{1A} autoreceptors (Czachura & Rasmussen, 2000) and lead to an increase in extracellular 5-HT levels and thus to therapeutic efficacy. A major limitation to the therapeutic profile of SSRIs is, therefore, their slow on-set of action and current research is concentrating on addressing this shortcoming by seeking compounds which can acutely increase 5-HT levels in the brain.

One approach may be to block 5-HT_{1A} autoreceptors in conjunction with the re-uptake site and several clinical studies have been carried out using the 5-HT_{1A} receptor antagonist, pindolol, in combination with SSRIs (McAskill *et al*., 1998; Rasanen *et al*., 1999). There have been conflicting reports as to the success of this particular combination therapy but perhaps this is due to the beta adrenergic receptor activity of pindolol, which limits the level of dosing for therapeutic efficacy in the absence of vascular side effects.

Blockade of 5-HT_{1B} receptors at the nerve terminal may also prove to be a novel approach to this goal and previous studies have shown that 5-HT_{1B} inverse agonists can block rat, guinea-pig and human terminal autoreceptors *in vitro* (Middlemiss *et al*., 1999; Selkirk *et al*., 1998) and enhance extracellular 5-HT levels in freely-moving guinea-pigs (Roberts *et al*., 1999). Also, a recent study has shown that 5-HT_{1B} receptor blockade or deletion enhances the release of paroxetine-induced extracellular 5-HT release in ventral hippocampus and frontal cortex of freely-moving mice (Malagie *et al*., 2001).

Another approach for rapidly increasing extracellular 5-HT levels action would be to block 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors on cell bodies and nerve terminals. This combination would, firstly, attenuate inhibition of cell firing *via* 5-HT_{1A} receptors in the raphe, resulting in enhanced release of 5-HT at the terminal end, and, secondly, prevent negative feedback of release occurring *via* 5-HT_{1B} receptors at the terminal end of the neuron and perhaps 5-HT_{1D} receptors in this region. We now report on the *in vitro* pharmacology of the first compound that has high affinity and selectivity for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors, SB-272183 (5-Chloro-2, 3-dihydro-6-[4-methylpiperazin-1-yl]-1[4-[pyridin-4-yl]napth-1-ylaminocarbonyl]-1H-indole). This study investigates the functional activity of SB-272183 at human recombinant 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors and guinea-pig, rat and human native tissue 5-HT_{1A} and 5-HT_{1B} receptors using the techniques of [^{35}S]-GTPγS binding (membranes and autoradiography), [^{3}H]-5-HT release, cell firing and fast cyclic voltammetry.

Methods

Radioligand binding

Cloned receptors The radioligand binding affinity of SB-272183 was determined at the following receptors; human (h) 5-HT_{1A}, h5-HT_{1B}, h5-HT_{1D}, h5-HT_{1E}, h5-HT_{1F}, h5-HT_{2A}, h5-HT_{2B}, h5-HT_{2C}, 5-HT_4 (piglet), h5-HT_6, h5-HT_7, hD_2, hD_3, (h)adrenergic-alpha 1B(hα-1B) and hbeta2 adrenergic (hβ_2), as described by Kennet *et al*. (1996).

Native tissue receptors Radioligand binding to rat and guinea-pig native tissue 5-HT_{1A} and 5-$HT_{1B/D}$ receptors was carried out as described by Watson *et al*. (2000) and Scott *et al*. (2000) respectively.

[^{35}S]-GTPγS binding studies

Cloned receptors [^{35}S]-GTPγS binding studies in recombinant cells expressing human 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1D} receptors were carried out as described by Watson *et al*. (1996; 2000). In brief, membranes were pre-incubated in 20 mM HEPES containing 3 mM $MgCl_2$, 100 mM NaCl, 0.2 mM ascorbate and 10 μM GDP for 30 min at 30°C. The reaction was started by the addition of 100 pM [^{35}S]-GTPγS and incubated for a further 30 min. The reaction was stopped by filtration and radioligand bound determined by scintillation spectrometry.

Native tissue receptor autoradiography [^{35}S]-GTPγS autoradiography in rat dorsal raphe nucleus (DRN) was carried out as described by Sim *et al*. (1997). Brain sections (15 μM) were incubated in Tris buffer containing 2 mM GDP and 100 pM [^{35}S]-GTPγS with or without test compounds for 2 h at room temperature. Slides were dried and exposed to betamax film for 24–48 h. Films images were digitized with a Sony XC-77 video camera and analysed using Micro Computor Imaging Software (Imaging Research Inc., Canada). [^{35}S]-GTPγS autoradiography in human DRN was performed and analysed as described by Day *et al*. (2001). All human tissue was acquired with the permission of the donor or the donor's next of kin, and according to the legal and ethical guidelines set out in the Nuffield Report (Human Tissue; Ethical and Legal Issues, April 1995), the Medical Research Council Report (Interim Operational and Ethical Guidelines on Human Tissue and Biological Samples for use in research, November, 1999) and the Royal College of Pathologists Report (Guideleines for the Retention of Tissues and Organs at Post-Mortem Examination, March 2000). Donor and tissue information is described in Table 1a and b.

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

Electrically-stimulated [^{3}H]-5-HT release studies were carried out as described by Roberts *et al*. (1996). In brief, cross-chopped cortical slices were incubated with [^{3}H]-5-HT (100 nM) in the presence of pargyline and electrically stimulated in a Brandell superfusion 2000 unit at 3 Hz for 1 min. Test drugs were added to the superfusion medium after the first stimulation (S1), and 12 min before the second stimulation (S2). [^{3}H]-5-HT released was assessed by liquid scintillation counting.

Table 1(a) Donor details

Donor	*Sex*	*Age*	*Clinical diagnosis*	*Cause of death*
1	F	86	long-standing sensory motor neuropathy	multiple myelomas of the spine
2	F	49	breast cancer, liver metastases	breast cancer, liver metastases
3	F	68	ischaemic heart disease	acute cardiac death

(b) Tissue preparation

Donor	*Freezing delay* (h)	*Freezing method*
1	28	liquid N_2-cooled isopentane
2	38	brass plate on dry ice
3	16	brass plate in dry ice

Cell firing studies

Cell firing studies were carried out according to Corradetti *et al*. (1998). In brief, horizontal and coronal rat brain slices (350–400 μm) containing the DRN were prepared and immediately transferred to a submersion recording chamber perfused at 5 ml min^{-1} with artificial cerebrospinal fluid (aCSF). In the presence of 3 μM noradrenaline, extracellular single units (2–3 ms) were recorded using aCSF-filled glass microelectrodes connected to an Axoclamp 2B amplifier. Each single unit reflected the firing of an individual neurone.

Fast cyclic voltammetry in rat DRN

Rat brain slices (350 μM) containing DRN were perfused with aCSF (2.5–3 ml min^{-1}) and a bipolar stimulation electrode and carbon fibre microelectrode positioned in the region of the DRN. A triangular voltage scan (−1 V to 1.4 V to −1 V) was applied to the carbon fibre microelectrode and the generated current recorded at 525 mV. Slices were stimulated every 5 min with 20 pulses at 100 Hz and 10 mA (Roberts & Price, 2001).

Materials

SB-272183 (5-Chloro-2, 3-dihydro-6-[4-methylpiperazin-1-yl]-1[4-[pyridin-4-yl]napth-1-ylaminocarbonyl]-1H-indole) was synthesized by GlaxoSmithKline, Harlow, U.K. Drugs and reagents were purchased from Sigma-Aldrich (Poole, U.K.), Calbiochem (Nottingham, U.K.), Bio-Rad (Hemel Hempstead, U.K.), Fisons Scientific Equipment (Loughborough, U.K.), Research Biochemicals International (Poole, U.K.), Tocris Cookson Ltd. (Bristol, U.K.). Betamax hyperfilm was obtained from Amersham Pharmacia Biotech UK Ltd. (Little Chalfont, U.K.), phenisol film developer and hypam fixer were obtained from Ilford Imaging, U.K. Ltd. [^{35}S]-GTPγS and all radioligands used in the binding studies were supplied by Amersham Pharmacia Biotech UK Ltd. (Little Chalfont, U.K.) and NEN Life Science Products, Inc. (Boston, U.S.A.).

Data analysis

Data from radioligand binding studies were fitted by Inflexion (Bowen & Jerman, 1995) to yield IC_{50} values which were converted to pK_i values using the correction for radioligand concentration described by Cheng & Prusoff (1973). [^{35}S]-GTPγS binding studies were fitted by a 4-parameter logistic equation using GRAFIT (Erithacus Software Ltd.) to yield EC_{50} values.

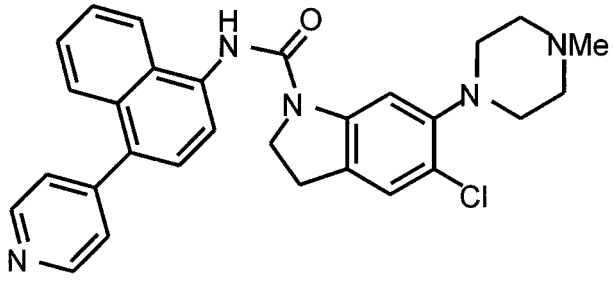
Results

Radioligand binding studies

SB-272183 displayed high affinity at human recombinant (h)5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Table 2) with 30 fold selectivity over 5-HT$_7$ and β_2 receptors and 100 fold selectivity over all other receptors tested (Table 3). The affinity at guinea-pig and rat native tissue 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors was slightly lower as compared to human recombinant receptors (Table 2).

[^{35}S]-GTPγS binding studies

Cloned receptors SB-272183 acted as a partial agonist at h5-HT$_{1A}$, h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors receptors with intrinsic activities, compared to 5-HT, of 0.4±0.1, 0.4±0.0 and 0.8±0.1 respectively (Figure 2 and Table 4). The intrinsic activity of SB-272183 at h5-HT$_{1A}$ receptors was equivalent to that of the 5-HT$_{1A}$ partial agonist, pindolol. In cells expressing h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors, SB-272183



SB-272183

Figure 1 Chemical structure of SB-272183 (5-Chloro-2, 3-dihydro-6-[4-methylpiperazin-1-yl]-1[4-pyridin-4-yl]napth-1-ylaminocarbonyl]-1H-indole).

Table 2 Affinity of SB-272183 for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors across species

	Human			*Rat*		*Guinea-pig*	
SB-272183	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{1A}$	5-HT$_{1B/1D}$	5-HT$_{1A}$	5-HT$_{1B/1D}$
(pK_i)	8.0±0.1	8.1±0.1	8.7±0.1	7.7±0.1	7.8±0.1	7.7±0.1	7.7±0.1

Data expressed as mean pK_i value±s.e.mean from at least three experiments.

Table 3 Selectivity profile for SB-272183 at human cloned receptors

SB-272183	*5-HT$_{1E}$*	*5-HT$_{1F}$*	*5-HT$_{2A}$*	*5-HT$_{2B}$*	*5-HT$_{2C}$*	*5-HT$_4$*	*5-HT$_6$*	*5-HT$_7$*	*D_2*	*D_3*	*α_{1B}*	*β_2*
pK_i	5.0±0.1	5.1±0.1	5.5±0.1	6.1±0.2	5.7±0.1	5.6±0.1	5.7±0.1	6.8±0.1	5.3±0.2	5.6±0.1	5.5±0.2	6.8±0.2

Data are expressed as mean pK_i value±s.e.mean from at least three experiments.

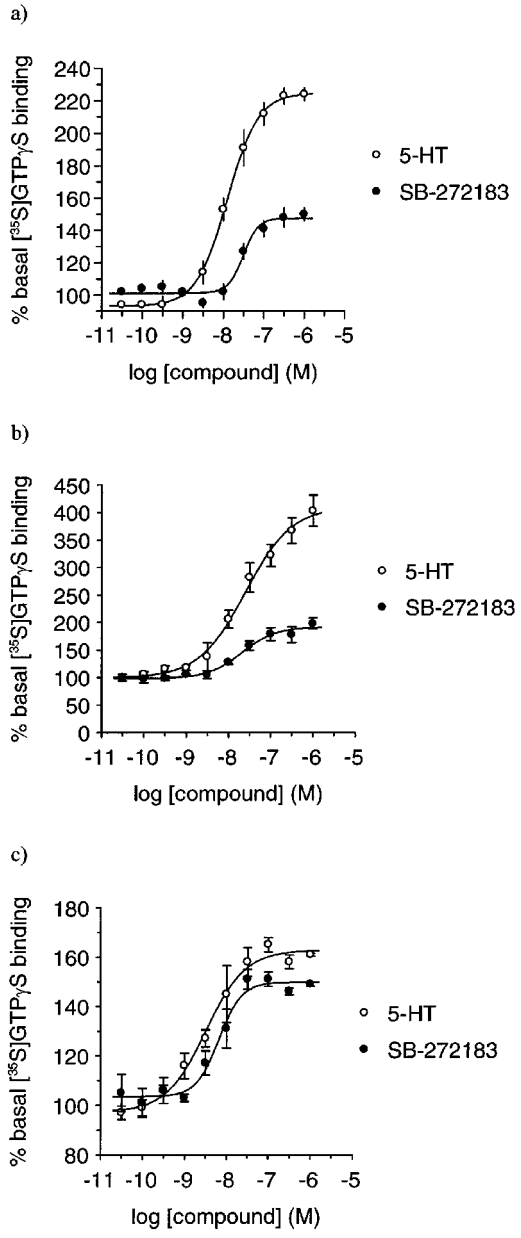


Figure 2 [^{35}S]-GTPγS binding to HEK293 cells expressing h5-HT$_{1A}$ receptors (a) and CHO cells expressing h5-HT$_{1B}$ (b) and h5-HT$_{1D}$ (c) receptors. Data are expressed as per cent of basal [^{35}S]-GTPγS binding and are the mean$\pm$s.e.mean from three individual experiments, each performed in duplicate. All curves were fitted by a 4-parameter logistic equation.

had intrinsic activities comparable to the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor antagonist, GR127935. Antagonist studies showed that SB-272183 inhibited 5-HT-induced stimulation of [^{35}S]-GTPγS binding in h5-HT$_{1A}$ and h5-HT$_{1B}$ receptor expressing cells in a concentration-related manner to give pA$_2$ values of 8.2$\pm$0.2 and 8.5$\pm$0.1 respectively (Figure 3). Antagonist studies were not possible in h5-HT$_{1D}$ receptor expressing cells due to the high intrinsic activity of SB-272183.

Receptor autoradiography From studies in rat mid-brain sections, 5-HT (10 μM) stimulated [^{35}S]-GTPγS binding, in the

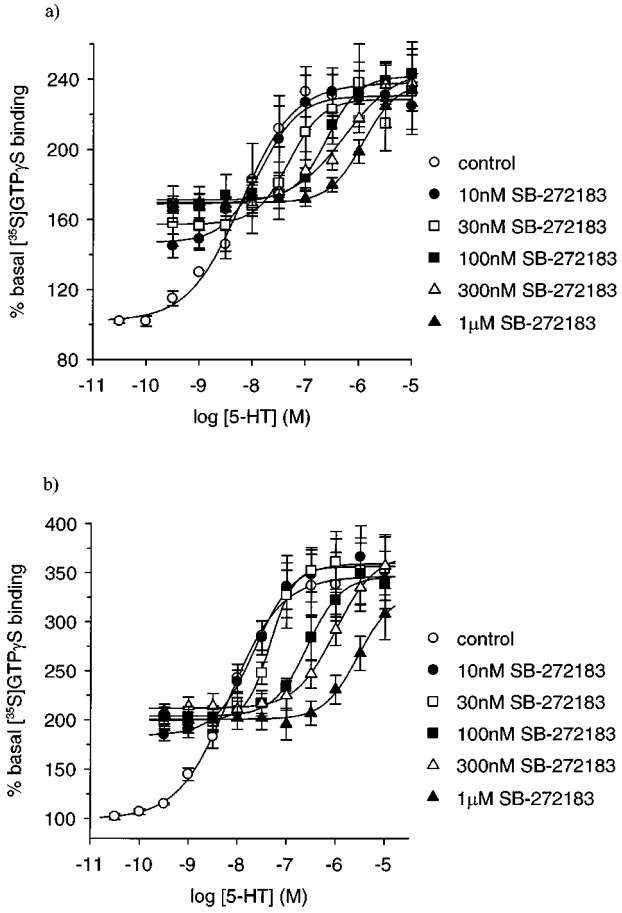


Figure 3 [^{35}S]-GTPγS binding to HEK293 cells expressing h5-HT$_{1A}$ receptors (a) and CHO cells expressing h5-HT$_{1B}$ receptors (b). Data are expressed as per cent of basal [^{35}S]-GTPγS binding and are the mean$\pm$s.e.mean from three individual experiments, each performed in duplicate. All curves were fitted by a 4-parameter logistic equation.

Table 4 Summary of potencies and intrinsic activity of test compounds at h5-HT1A, 1B and 1D receptors

	5-HT$_{1A}$		*5-HT$_{1B}$*		*5-HT$_{1D}$*	
Compound	pEC$_{50}$	I.A.	pEC$_{50}$	I.A.	pEC$_{50}$	I.A.
5-HT	8.0$\pm$0.0	1.0[a]	7.7$\pm$0.1	1.0[b]	8.4$\pm$0.1	1.0[c]
SB-272183	7.7$\pm$0.0	0.4$\pm$0.1	7.7$\pm$0.1	0.4$\pm$0.0	8.2$\pm$0.1	0.8$\pm$0.1
(+)8-OH-DPAT	8.5$\pm$0.1	0.9$\pm$0.1				
Pindolol	7.3$\pm$0.1	0.4$\pm$0.1				
GR127935			8.2$\pm$0.4	0.5$\pm$0.1	8.4$\pm$0.4	0.9$\pm$0.1

Data are mean$\pm$s.e.mean from at least three individual experiments, performed in duplicate. I.A.: intrinsic activity compared to 5-HT. [a]E$_{max}$=123% stimulation, [b]E$_{max}$=300% stimulation, [c]E$_{max}$=63% stimulation.

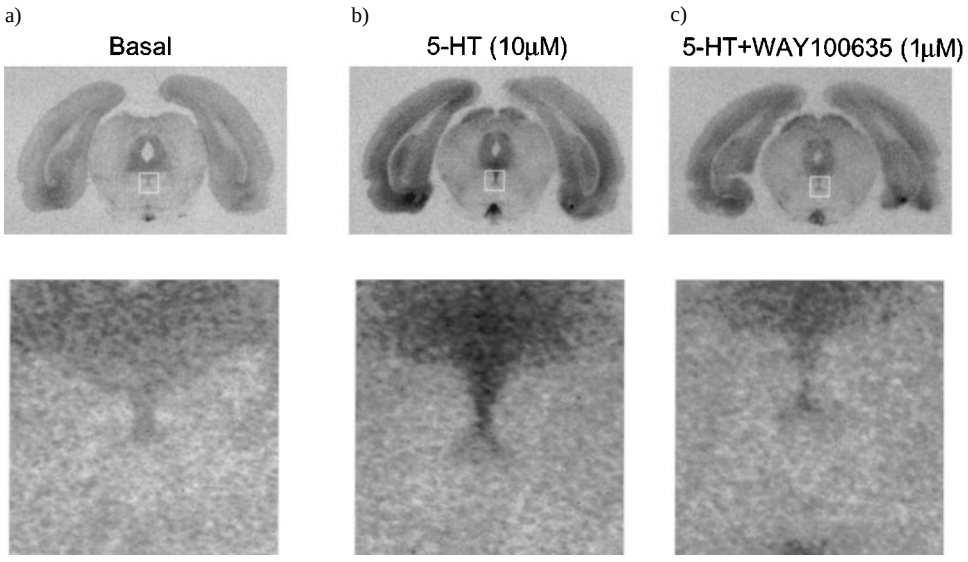


Figure 4 (a) [^{35}S]-GTPγS autoradiography in rat mid-brain sections cut at the level of the DRN. Lower panels represent enhanced images of the DRN. (b) shows 5-HT-induced stimulation of [^{35}S]-GTPγS binding to the DRN and (c) shows blockade of stimulated binding by WAY100635. Autoradiograms are representative of duplicate sections from three individual rats.

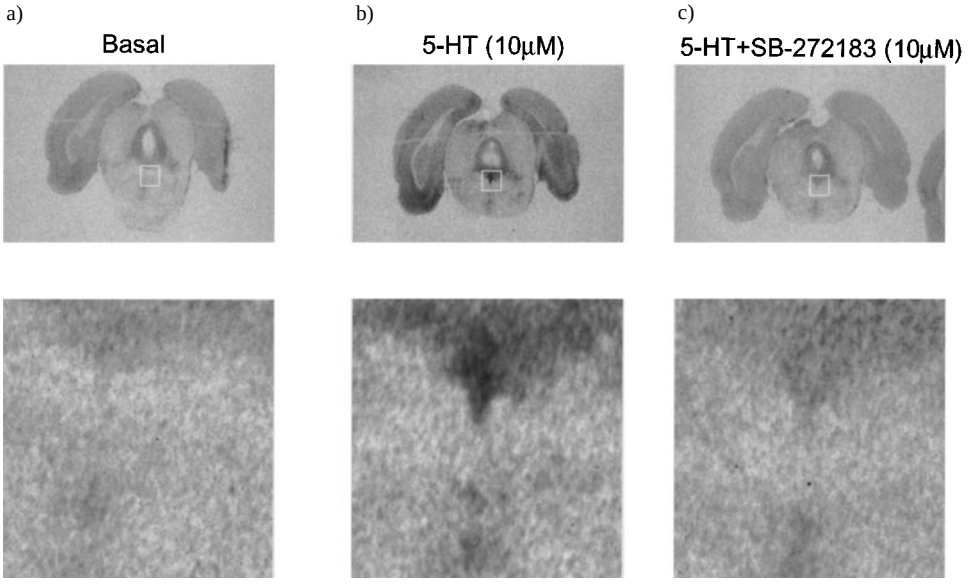


Figure 5 (a) [^{35}S]-GTPγS autoradiography in rat mid-brain sections cut at the level of the DRN. Lower panels represent enhanced images of the DRN. (b) shows 5-HT-induced stimulation of [^{35}S]-GTPγS binding to the DRN and (c) shows blockade of stimulated binding by SB-272183. Autoradiograms are representative of duplicate sections from three individual rats.

DRN, by 130% (Figures 4–6). The distribution of stimulation correlated well with the distribution of 5-HT$_{1A}$ receptors labelled by [^{3}H]-8-OH-DPAT as previously shown by Marcinkiewicz *et al*. (1984). The selective 5-HT$_{1A}$ receptor antagonist, WAY100635 (1 μM), blocked the 5-HT response (Figure 4) but did not show intrinsic activity in this assay system (data not shown). These data suggest that the 5-HT response is mediated, predominantly, *via* 5-HT$_{1A}$ receptors. SB-272183 did not show evidence of intrinsic activity up to 10 μM but did block the 5-HT-induced stimulation of [^{35}S]-GTPγS binding (Figures 5 and 6).

Similar results were obtained from studies in human mid-brain sections. 5-HT (1 μM) stimulated [^{35}S]-GTPγS binding in the DRN, which was blocked by WAY100635 (30 nM) (Figures 7 and 9b). SB-272183 (1 μM) also inhibited 5-HT-induced stimulation of binding in the DRN but did not show evidence of intrinsic activity up to 100 μM (Figures 8 and 9a).

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

Electrically-stimulated [^{3}H]-5-HT release was measured in rat and guinea-pig cortex previously shown to be mediated *via* inhibitory 5-HT$_{1B}$ autoreceptors (Roberts *et al*., 1996). SB-272183 significantly potentiated [^{3}H]-5-HT release at 100 and 1000 nM in guinea-pig cortex and at 1000 nM in rat cortex

suggesting that SB-272183 blocks the inhibitory effects of endogenous 5-HT at these autoreceptors (Figure 10). The magnitude of this potentiation is comparable to the potentiation of [^{3}H]-5-HT release produced by the selective 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor antagonist GR127935, as previously reported (Roberts *et al*., 1996).

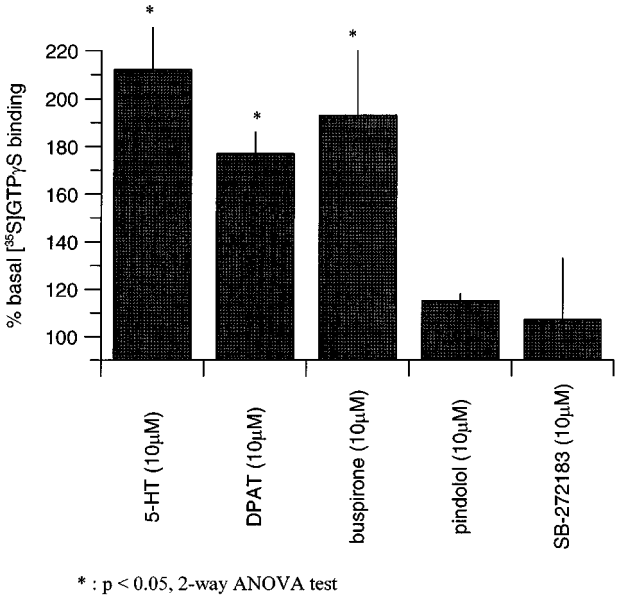


Figure 6 Quantitative data from [^{35}S]-GTPγS autoradiography in rat mid-brain sections cut at the level of the DRN. Data are expressed as per cent of basal [^{35}S]-GTPγS binding and are the mean ± s.e.mean of duplicate sections from three individual rats.

Cell firing studies

Bath applied (+)8-OH-DPAT elicited a reversible and concentration-dependent inhibition of cell firing, in 19 out of 25 neurones, with an $IC_{50} = 12 \pm 2$ nM (Figure 11a). The remaining six cells did not respond to (+)8-OH-DPAT up to 1 μM. WAY100635 (300 nM) reversed the (+)8-OH-DPAT-induced inhibition of firing to give an apparent $pK_b = 8.6 \pm 0.1$ (Figure 11a) which is comparable to its affinity at 5-HT$_{1A}$ receptors (Fletcher *et al*., 1996). Superfusion with WAY100635 alone (300 nM for 20 min) had no effect on cell firing (data not shown).

SB-272183, up to 1 μM, did not significantly effect the frequency of action potential firing in neurones (Figure 11b) but was able to reverse (+)8-OH-DPAT-induced inhibition of firing (Figure 11c) in a competative manner to give an apparent pK_b of 7.1.

Fast cyclic voltammetry in rat DRN

Release of 5-HT in the somatodentritic region of the DRN is attenuated by 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. SB-272183 (1 μM) did not inhibit basal 5-HT efflux from rat DRN suggesting lack of intrinsic activity at 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors receptors (Figure 12). However, SB-272183 (1 μM) did attenuate sumatriptan-induced inhibition

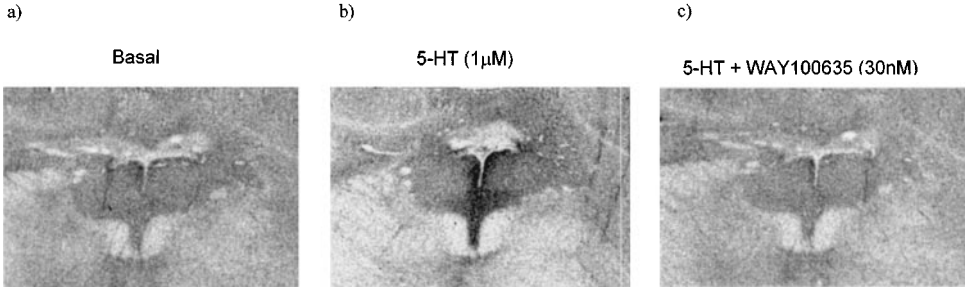


Figure 7 (a) [^{35}S]-GTPγS autoradiography in human mid-brain sections cut at the level of the DRN. (b) shows 5-HT-induced stimulation of [^{35}S]-GTPγS binding to the DRN and (c) shows blockade of stimulated binding by WAY100635. Autoradiograms are representative of duplicate sections from three individual donors.

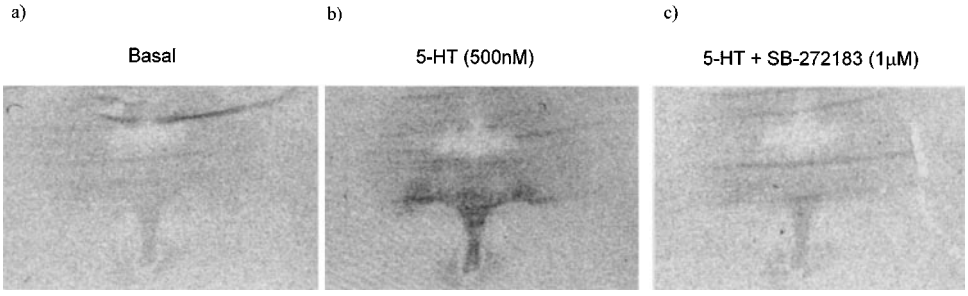


Figure 8 (a) [^{35}S]-GTPγS autoradiography in human mid-brain sections cut at the level of the DRN. (b) shows 5-HT-induced stimulation of [^{35}S]-GTPγS binding to the DRN and (c) shows blockade of stimulated binding by SB-272183. Autoradiograms are representative of duplicate sections from three individual donors.

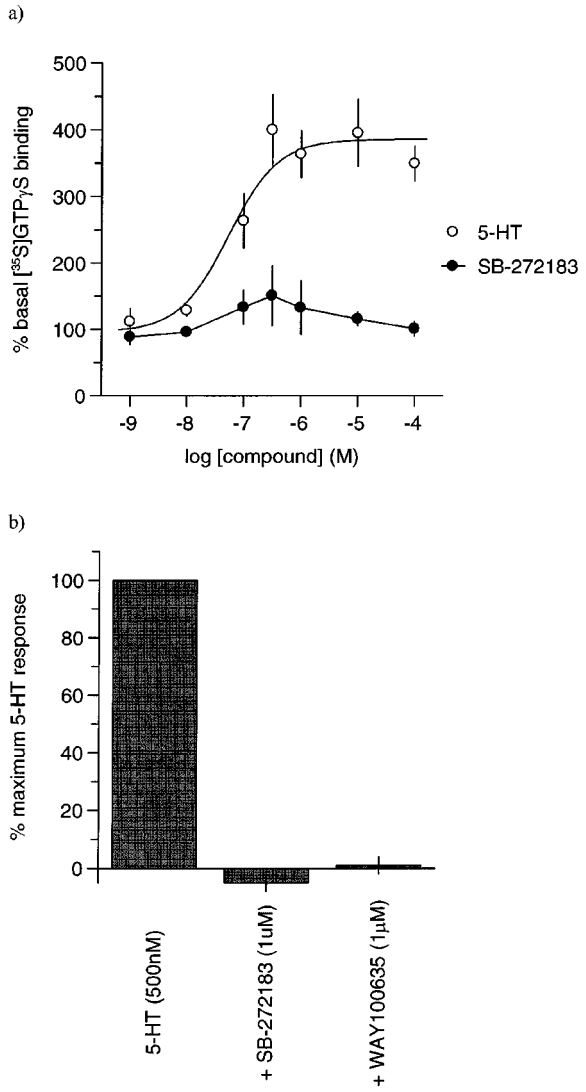


Figure 9 Quantitative data from [^{35}S]-GTPγS autoradiography in human mid-brain sections cut at the level of the DRN. (a) shows concentration-related curves to 5-HT and SB-272183. (b) shows inhibition of 5-HT-induced [^{35}S]-GTPγS binding by WAY100635 and SB-2721783. Data are the mean $\pm$ s.e.mean of duplicate sections from three individual donors.

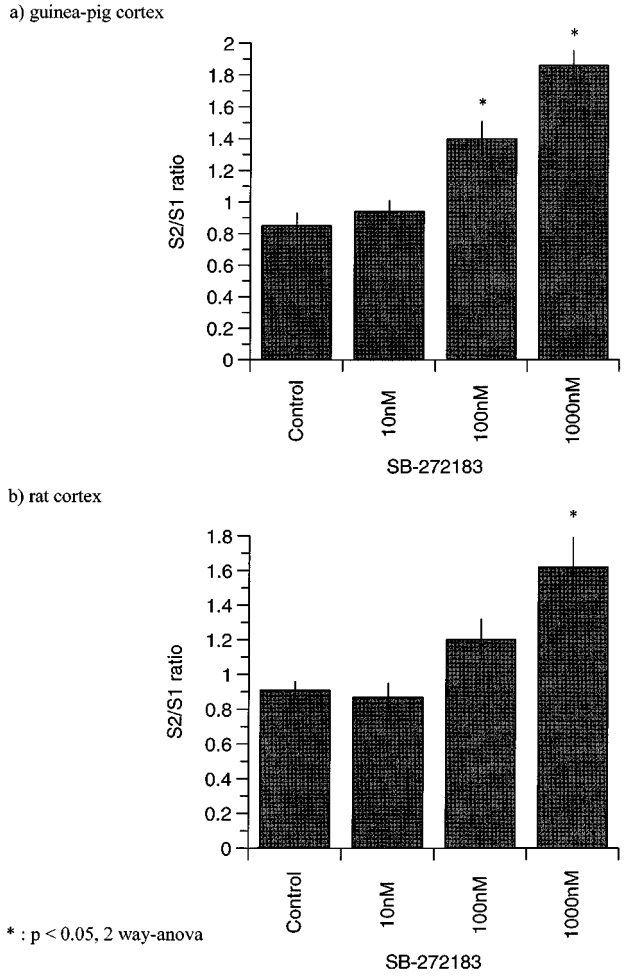


Figure 10 Electrically-stimulated [^{3}H]-5-HT release from native tissue cortical slices. The data shows the potentiation of [^{3}H]-5-HT release, by increasing concentrations of SB-272183, from guinea-pig cortex (a) and rat cortex (b). Results are expressed as S2/S1 ratio and are the mean of three individual experiments, each performed in duplicate.

of 5-HT efflux to give an apparent pK_b of 7.2, indicating blockade of 5-HT_{1B} and/or 5-HT_{1D} receptor agonist activity.

Discussion

In this study we have described the *in vitro* pharmacological profile of SB-272183 at human recombinant and native tissue 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. Radioligand binding studies showed that SB-272183 has high affinity for human recombinant 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors and is at least 30 fold selective over a range of other 5-HT, dopamine and adrenergic receptors. From native tissue binding studies, SB-272183 has high affinity for rodent 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors, the pK_i values being close to, but slightly lower than, those at the human receptors.

Functional studies using [^{35}S]-GTPγS binding showed that SB-272183 is a potent, partial agonist at human recombinant 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors with intrinsic activities ranging from 0.4 to 0.8. This partial agonism may be due to the presence of receptor reserve, a common phenomena that occurs in high receptor expression systems. In cells expressing h5-HT_{1A} receptors the beta adrenergic receptor ligand, pindolol, also acted as a partial agonist with intrinsic activity similar to that of SB-272183. However, from *in vitro* electrophysiological studies performed in rat DRN, pindolol is an antagonist at 5-HT_{1A} receptors (Corradetti *et al*., 1998; Watson *et al*., 2001). This suggests that, similarily, SB-272183 would not display intrinsic activity at native tissue 5-HT_{1A} receptors. Indeed in [^{35}S]-GTPγS autoradiographic binding studies, in both rat and human DRN, the binding of which was shown to be mediated *via* 5-HT_{1A} receptors, SB-272183 and pindolol, up to 10 μM, did not stimulate binding but were able to inhibit agonist-induced stimulation of [^{35}S]-GTPγS binding at these presynaptic 5-HT_{1A} autoreceptors. These studies indicate that, at least in the human and rat

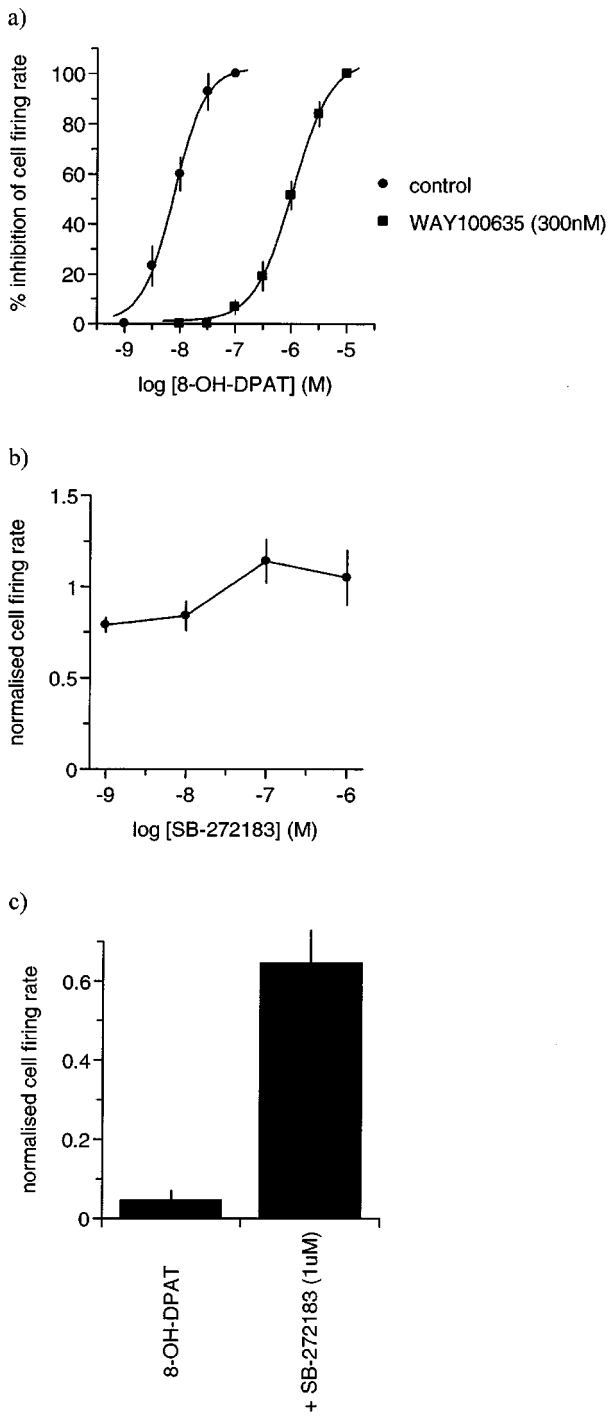


Figure 11 Measurement of cell firing in rat mid-brain slices containing the DRN. (a) shows a concentration-related inhibition of cell firing rate by (+)8-OH-DPAT and its blockade by WAY100635. (b) shows the effect of SB-272183, up to 1 μM, on normalized cell firing rate and (c) shows attenuation of (+)8-OH-DPAT-induced inhibition of cell firing by SB-272183. Data are mean$\pm$s.e.mean from three or more slices.

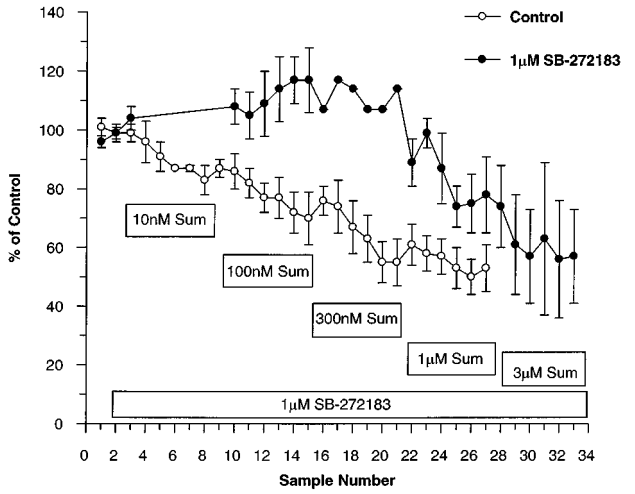


Figure 12 Measurement of 5-HT efflux from rat brain slices, containing the DRN, using fast cyclic voltammetry. Data show attenuation of sumatriptan-induced inhibition of 5-HT efflux by SB-272183. Results are expressed as a per cent of control 5-HT efflux and are the mean$\pm$s.e.mean from at least three individual slices.

DRN, SB-272183 does not act as an agonist at 5-HT_{1A} receptors. Additional studies in our group have suggested that SB-272183 also blocks post-synaptic 5-HT_{1A} receptors in the hippocampus (data not shown).

Measurement of cell firing, using single extracellular recording electrodes placed in rat DRN, also supports the autoradiographic studies showing lack of intrinsic activity of SB-272183 at native tissue 5-HT_{1A} receptors. WAY100635 blocked (+)8-OH-DPAT-induced inhibition of cell firing rate with a potency comparable to that of its affinity at 5-HT_{1A} receptors (Fletcher *et al*., 1996). This and other reports (Corradetti *et al*., 1996; 1998) suggest that the response to (+)8-OH-DPAT is mediated *via* 5-HT_{1A} receptors. In these studies, SB-272183 did not affect cell firing rate up to 1 μM, showing the lack of intrinsic activity of this compound at 5-HT_{1A} receptors, but did attenuate (+)8-OH-DPAT-induced inhibition of cell firing. The potency of SB-272183 measured in these studies was approximately an order of magnitude lower than its radioligand binding affinity for 5-HT_{1A} receptors. The reason for this discrepancy between recombinant and native tissue 5-HT_{1A} receptors is not clear but may be due to differences in agonist affinity states of 5-HT_{1A} receptors in this preparation, a phenomenon that has been reported to occur for 5-HT_{1A} receptors (Clawges *et al*., 1997; Watson *et al*., 2000). Preliminary studies have shown that SB-272183 has high affinity at h5-HT_{1A} receptors, when these receptors are labelled in their high agonist affinity state i.e. when using [^{3}H]-8-OH-DPAT. However when both human recombinant and guinea-pig and rat native tissue 5-HT_{1A} receptors are labelled in their low affinity state i.e. when using [^{3}H]-WAY100635 in the presence of GppNHp, SB-272183 displays 30 fold lower affinity. Therefore despite SB-272183 displaying no intrinsic activity in native tissues the compound discriminates between agonist states.

[^{35}S]-GTPγS binding studies in recombinant cells showed that SB-272183 also acts as a potent, partial agonist at h5-HT_{1B} and h5-HT_{1D} receptors, with similar efficacy to that of the 5-HT_{1B} and 5-HT_{1D} receptor antagonist GR127935 (Skingle *et al*., 1993). Previous studies have shown that GR127935 acts as an antagonist in *in vitro* [^{3}H]-5-HT release and *in vivo* microdialysis studies (Roberts *et al*., 1996; Skingle

et al., 1995), thus suggesting that a compound with comparable or lower intrinsic activity than GR127935 at h5-HT_{1B} receptors would also act as an antagonist at native tissue 5-HT_{1B} receptors. In agreement with this prediction, SB-272183 potentiated electrically-stimulated [^{3}H]-5-HT release from rat and guinea-pig cortical slices suggesting that it acts as an antagonist at native tissue 5-HT_{1B} receptors. The fact that SB-272183 was significantly more potent at enhancing [^{3}H]-5-HT release from guinea-pig cortex may be due to the presence of more endogenous 5-HT tone in this species compared to rat. The antagonist activity of SB-272183 at native tissue 5-HT_{1B} receptors is also supported by studies measuring 5-HT release from rat DRN using fast cyclic voltammetry. SB-272183 (1 μM) attenuated the inhibition of release caused by the 5-HT_{1B} and 5-HT_{1D} receptor agonist, sumatriptan, but had no effect on basal 5-HT release alone. As seen in cell firing studies, the potency of SB-272183 was an order of magnitude lower than its affinity for human recombinant 5-HT_{1B} and 5-HT_{1D} receptors which, again, may be due to the reason discussed for 5-HT_{1A} receptors. Thus, in conclusion, SB-272183 has been shown to be a potent and selective ligand at human recombinant and native tissue 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. SB-272183 is also the first compound with simultaneous antagonist activity at all three presynaptic autoreceptors. The current therapy for antidepressant treatment relies largely on the use of SSRIs but delayed onset of action is one of the major drawbacks of this class of drug. A novel way to overcome this delayed onset of action may be to block both cell body and terminal 5-HT autoreceptors since such blockade may result in an increase in extracellular 5-HT levels which is not limited by autoreceptor stimulation. Whether or not this class of compound will posses antidepressant properties remains to be seen.

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