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SPECIAL REPORT

In vivo effects of the 5-HT_6 antagonist SB-271046 on striatal and frontal cortex extracellular concentrations of noradrenaline, dopamine, 5-HT, glutamate and aspartate

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Although the 5-HT_6 receptor subtype was identified some 5 years ago, very little is known about its function within the brain. Here we demonstrate, for the first time, the neurochemical effects of a selective 5-HT_6 receptor ligand. Using *in vivo* microdialysis in the freely moving rat, we evaluated the effects of the selective 5-HT_6 receptor antagonist SB-271046 by simultaneous measurement of 5-hydroxytryptamine (5-HT), dopamine (DA), noradrenaline (NA), glutamate and aspartate from the striatum and frontal cortex. SB-271046 did not alter basal levels of 5-HT, DA and NA in either brain region. Similarly, there was no change basal levels of either of the excitatory amino acids within the striatum. In contrast, administration of SB-271046 (10 mg kg^{-1} s.c.) produced a significant ($P<0.05$), tetrodotoxin-dependent, increase in extracellular levels of both glutamate and aspartate within the frontal cortex, reaching maximum values of 375.4 ± 82.3 and 215.3 ± 62.1% of preinjection values, respectively.
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Keywords: 5-HT_6 receptor; microdialysis; 5-HT; dopamine; noradrenaline; glutamate; aspartate; frontal cortex; striatum; SB-271046

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; NA, noradrenaline; Glu, glutamate; Asp, aspartate; TTX, tetrodotoxin

Introduction The most recently identified of the 5-hydroxytryptamine (5-HT, serotonin) receptors is the 5-HT_6 receptor. Initially cloned from rat striatum (Monsma *et al*., 1993; Ruat *et al*., 1993), the 5-HT_6, like 5-HT_4 and 5-HT_7, is a G protein-coupled receptor, which stimulates adenylate cyclase *via* G_s. *In situ* hybridization and Northern blot studies have revealed that 5-HT_6 receptor mRNA appears to be almost exclusively expressed within the brain (Monsma *et al*., 1993; Ruat *et al*., 1993). Although 5-HT_6 receptor expression is widespread the highest levels are found within the olfactory tubercle, striatum, nucleus accumbens, cerebral cortex, dentate gyrus and CA subfields of the hippocampus (Monsma *et al*., 1993; Ruat *et al*., 1993; Ward *et al*., 1995). These studies have also revealed that 5-HT_6 receptor is present within 5-HT projection fields and not in the 5-HT neurones of the raphe, indicating a probable postsynaptic role for this receptor (Ward *et al*., 1995).

The exact functional role of the 5-HT_6 receptor has yet to be ascertained however, its distribution and high affinity (nM) for many antipsychotic and antidepressant drugs, suggests a possible role in both schizophrenia and depression (Monsma *et al*., 1993; Roth *et al*., 1994). *In vivo* administration of 5-HT_6 receptor antisense oligonucleotides into the brain demonstrated a behavioural syndrome, which was blocked by the muscarinic antagonist atropine (Bourson *et al*., 1995). More recently, a number of behavioural studies (Bourson *et al*., 1998; Bentley *et al*., 1999) using the selective antagonists Ro 04-6790 have provided additional evidence to suggest a role for the 5-HT_6 receptor in the modulation of cholinergic neurotransmission. This suggests that 5-HT_6 receptor antagonists may also have therapeutic utility in the treatment of memory and cognitive dysfunction.

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Here, we demonstrate for the first time the neurochemical effects of 5-HT_6 antagonism using the potent, selective and bioavailable 5-HT_6 antagonist 5-Chloro-N-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2- benzothiophenesulfon-amide (SB-271046; Bromidge *et al*., 1999) and *in vivo* microdialysis in the freely moving rat.

Methods *Microdialysis* Male Sprague-Dawley rats (280–350 g, Charles River) were used in all experiments. Surgical implantation was performed as previously described (Dawson and Nguyen, 1998). Coordinates for the striatum and frontal cortex were: RC +0.2, L −3.0, V −3.0 and RC +3.2, L −3.5, V −1.5 (RC and L from bregma and V coordinates from the skull), respectively. Eighteen to twenty-four hours post surgery pre-equilibrated microdialysis probes were inserted into the striatum or frontal cortex (O.D. 0.5 mm, membrane length 4 mm and 2 mm, respectively; CMA/Microdialysis, Sweden) of the unrestrained rat. Microdialysis sampling was carried out by the method of Dawson and Nguyen (1998) with a perfusion rate of 1.25 μl min^{-1}. SB-271046 or vehicle was administered *via* a surgically implanted s.c. cannula. Tetrodotoxin (TTX; 10 μM; Alamone Labs, Israel) was infused *via* the microdialysis probe for a period of 40 min ($t=180-220$).

Analysis of dialysates Dialysates (25 μl) were split and analysed for excitatory amino acids (5 μl) and monamine/catacholamines (20 μl) as follows: (1) NA, DA and 5-HT were separated by reverse phase high performance liquid chromatography (HPLC) (C18 ODS3 column, 150×3.0 mm, Metachem, Torrance, CA, U.S.A.) and detected using an ANTEC electrochemical detector (ANTEC, Netherlands) set at a potential of 0.65 V vs a Ag/AgCl reference electrode. Mobile phase was delivered by a Jasco PU980 HPLC pump (Jasco Ltd, Essex, U.K.) at

0.5 ml min^{-1} and contained 0.1 M NaH_2PO_4 buffer at pH 4.8, 0.25 mM EDTA, 0.23 mM 1-octane sodium sulphonic acid, 2% isopropanol and 7.5% methanol. (2) Analysis of glutamate and aspartate was performed using a Crystal 310 capillary electrophoresis system (Thermo BioAnalysis, MN, U.S.A.) with a Zeta laser induced fluorescence detector (ZETA Technology, Toulouse, France) by the method of Dawson *et al.* (1997). All data were acquired using the Atlas software package (Thermo Labsystems, Gulph Mills, PA, U.S.A.) for the PC.

Data analysis Results were analysed by analysis of variance with repeated measures followed by pairwise comparisons using Bonferroni adjustment for multiple comparisons using the Statview software application (Abacus Concepts Inc., Berkeley, CA, U.S.A., 1996).

Results *Effects of SB-271046 on extracellular levels of 5-HT, NA, DA, Glu and Asp in the striatum* Subcutaneous injection of 10 mg kg^{-1} SB-271046 produced no significant change in extracellular concentrations of 5-HT, DA, NA, Glu or Asp within the striatum of the freely moving rat (Table 1) for up to 240 min post-administration.

Effects of SB-271046 on extracellular levels of 5-HT, NA, DA, Glu and Asp in the frontal cortex SB-271046 (1 and 10 mg kg^{-1} s.c.) produced no significant change in basal extracellular concentrations of 5-HT or NA within the rat frontal cortex (Figure 1). A small increase in extracellular DA was observed at 10 mg kg^{-1} (Figure 1) but this failed to reach statistical significance. Conversely, SB-271046 (10 mg kg^{-1}) produced a significant ($P<0.05$) increase in extracellular Glu and Asp concentrations reaching maximum values of $375.4\pm82.3\%$ ($t=280$ min) and $215.3\pm62.1\%$ ($t=240$ min) of preinjection values, respectively (Figure 2). Infusion of TTX (10 μM) did not significantly change basal levels of either excitatory amino acid (Figure 2). In contrast, following the initial SB-271046-induced increases, infusion of TTX ($t=180-220$ min) resulted in a significant attenuation in extracellular glutamate levels reducing maximal levels to $185\pm12.4\%$ ($t=260$ min; Figure 2) of preinjection control. Concurrently, TTX infusions produced a significant ($P<0.05$) attenuation in SB-271046-induced increases in aspartate, however this effect seemed to be somewhat delayed ($t=280$; Figure 2).

Discussion These data demonstrate for the first time the *in vivo* neurochemical effects of 5-HT_6 receptor antagonism on basal extracellular concentrations of neurotransmitters. SB-271046 produced no significant change in extracellular concentrations of any of the neurotransmitters measured in the striatum. Similarly, SB-271046 (1 and 10 mg kg^{-1}) produced no significant change in basal levels of NA, DA or

Table 1 Effects of SB-271046 (10 mg kg^{-1} s.c.) on striatal neurotransmitter levels

	Maximum % of preinjection control levels	
	Vehicle treated	*SB-271046 treated*
NA	111.0 ± 24.5	129.0 ± 24.1
DA	96.2 ± 18.8	125.4 ± 19.2
5-HT	102.4 ± 14.9	78.5 ± 16.9
Glu	115.5 ± 11.5	104.3 ± 25.8
Asp	113.3 ± 28.9	111.5 ± 29.7

Data expressed as mean$\pm$s.e.mean ($n=8$ per study group). Basal preinjection levels of neurotransmitter within the striatum were: 5-HT $-8.8+0.2$, NA -37 ± 1.3, DA -38.2 ± 0.8 fmol per 20 μl microdialysate and Glu -1.09 ± 0.03, Asp -0.38 ± 0.01 μM ($n=44$). None of these changes attained statistical significance.

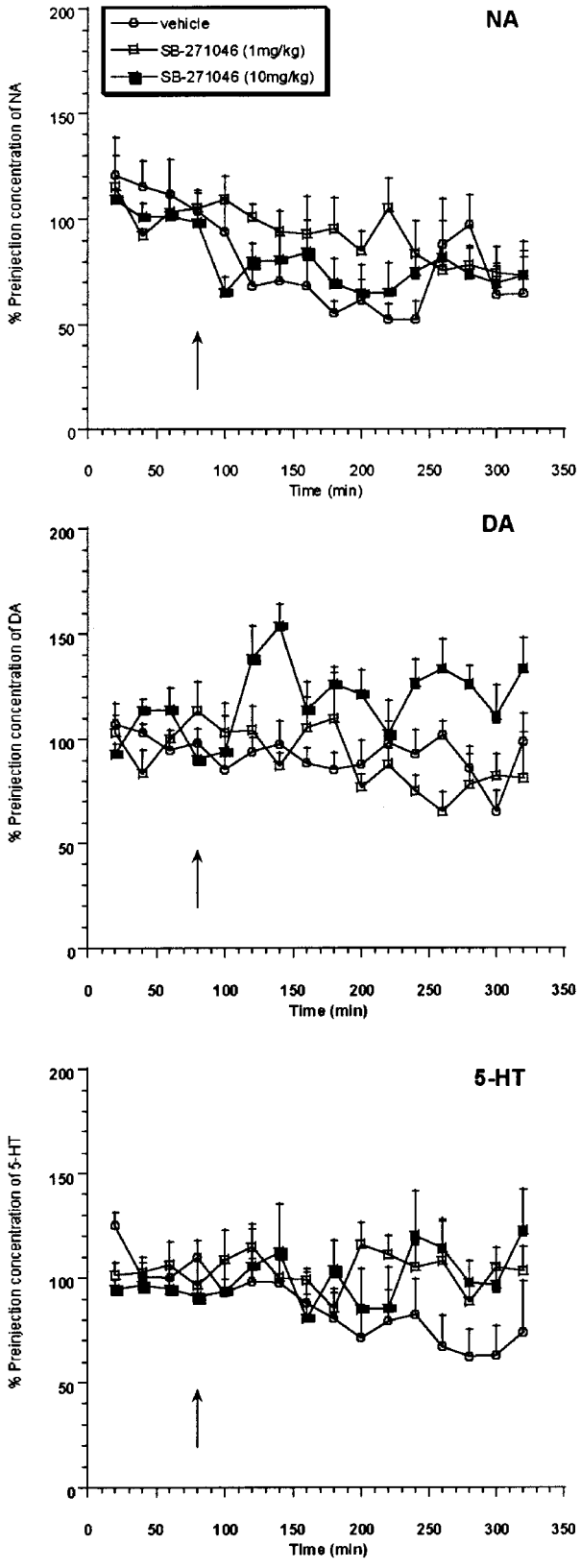

Figure 1 Effects of SB-271046 (1 and 10 mg kg^{-1} s.c.) on frontal cortex extracellular levels of NA, DA and 5-HT. Data expressed as mean$\pm$s.e.mean, ($n=7-12$ per study group). Arrow denotes subcutaneous drug or vehicle injection points. Basal preinjection levels of neurotransmitter within the frontal cortex were: 5-HT -9.7 ± 0.2, NA -11.1 ± 0.2, DA -14.1 ± 0.25 fmol per 20 l microdialysate ($n=42$).

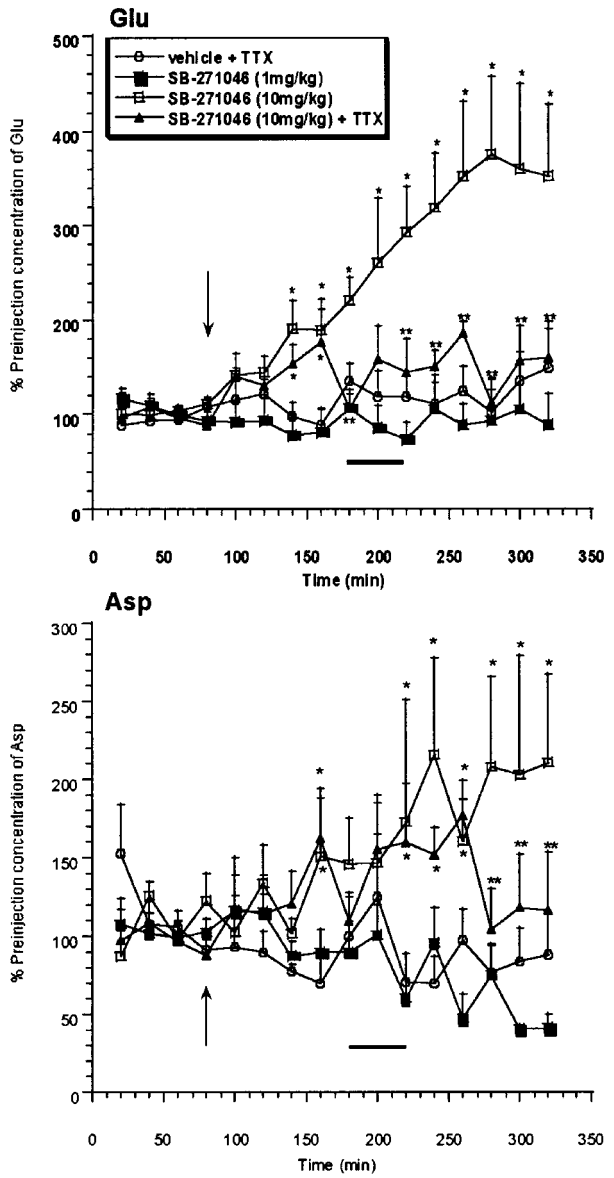

Figure 2 Effects of SB-271046 (1 and 10 mg kg^{-1} s.c.) on frontal cortex extracellular levels of Glu and Asp. Data expressed as mean ± s.e.mean, ($n = 7-12$ per study group). Arrow denotes subcutaneous drug or vehicle injection points; solid bar denotes TTX or vehicle infusion. Basal preinjection levels of neurotransmitter within the frontal cortex were: and Glu − 1.08 ± 0.02, Asp − 0.17 ± 0.01 μM ($n = 42$).*Denotes statistical significance ($P < 0.05$) from vehicle treated animals. **Denotes statistical significance ($P < 0.05$) SB-271046 alone vs SB-271046 + TTX treated animals.

5-HT within the frontal cortex. However, 10 mg kg^{-1} of SB-271046 induced a prolonged and robust 3 and 2 fold increase in frontal cortex glutamate and aspartate, respectively. These increases were attenuated *via* the infusion of the voltage dependent Na^+ channel blocker TTX, indicating that these amino acids are released from glutamatergic neurones. Given that the 5-HT_6 receptor is highly expressed in the cortex and basal ganglia structures (Ward *et al*., 1995), it is likely that the observed enhancement of excitatory transmission originates from a blockade of tonic serotonergic inhibition of intrinsic cortical projections or basal ganglia/thalamocortical connections.

Since the discovery of the 5-HT_6 receptor, evidence has been accumulating to suggest a role for this receptor in the modulation of cholinergic function. Early experiments showed that administration of 5-HT_6 receptor antisense oligonucleotides into the brain induced a behavioural syndrome, which could be blocked by the muscarinic antagonist atropine (Bourson *et al*., 1995). More recently, Bourson *et al*. (1998) and Bentley *et al*. (1999) have demonstrated that the selective 5-HT_6 receptor antagonist Ro 04-6790 can induce stretching and inhibit 6-OHDA lesion-induced rotational behaviours. Again, both of these behaviours can be blocked by the application of muscarinic antagonists. Moreover, a recent report actually demonstrated increases in extracellular levels of acetylcholine within both the cortex and hippocampus with a similar selective antagonist Ro 65-7199, at doses shown to be effective in behavioural models of memory deficit (Sleight *et al*., 1999). Routledge *et al*. (1999) showed that SB-271046 could potentiate physostigmine induced chewing behaviours and the same compound has been shown to be effective in enhancing cognitive function in models of learning and memory (Rogers *et al*., 1999). It therefore seems clear from these data that the 5-HT_6 receptor is playing some role in cholinergic transmission. A direct link between acetylcholine and cortical glutamate has been demonstrated (Consolo *et al*., 1996; Sanz *et al*., 1997), therefore, the enhanced cholinergic effects and the observations presented here may be connected but the exact mechanism cannot be fully elucidated at this time.

Interestingly, Stean *et al*. (1999) has reported anticonvulsant properties of SB-271046 at doses much lower than those shown to be effective here or within cognitive studies (Rogers *et al*., 1999). The mechanism of this anticonvulsant action is not clear, particularly given the enhancement of glutamatergic function. There does seem to be some differential between effective doses in these models (0.1 and 1 mg kg^{-1} minimum effective dose for the electroconvulsive shock and cognitive models, respectively) and the effects observed here. The reason for this maybe due to a difference in the tone experienced by the 5-HT_6 receptor in the various models. Non selective, as would occur in electroconvulsive shock and more selective stimulation, as occurs in models of learning and memory, is likely to bring about elevations in serotonergic tone (although this is not necessarily selective for 5-HT). In a higher tonic environment the effective doses of antagonist required to block the receptor will be somewhat less than in a non-stimulated basal situation when tone is likely to be much lower.

Given this neurochemical profile of a 5-HT_6 antagonist one can speculate on the therapeutic utility of such a compound. Previous findings (Bourson *et al*., 1995) have suggested a therapeutic role for 5-HT_6 antagonists in the treatment of memory and cognitive dysfunction and more recent data has demonstrated enhanced cognitive function in various preclinical models (Sleight *et al*., 1999; Rogers *et al*., 1999). The role of the prefrontal cortex (Koechlin *et al*., 1999) and more specifically glutamate (Dudkin *et al.,* 1996) in cognition has become evident. Enhancement of excitatory neurotransmission within the frontal cortex may therefore have some utility in the treatment of memory and/or cognitive dysfunction. Atypical antipsychotics such as clozapine, which posses nM affinity for the 5-HT_6 receptor, have also been shown to be somewhat effective in the treatment of the cognitive deficits associated with schizophrenia (Tollefson, 1996). Interestingly, clozapine has also been shown to selectively enhance excitatory transmission within the frontal cortex (Daly & Moghaddam, 1993).

In summary, we demonstrate here, for the first time, the enhancement of excitatory neurotransmission by a 5-HT_6 antagonist. Selective augmentation of extracellular concentrations of both glutamate and aspartate within the frontal cortex by SB-271406 suggests that 5-HT_6 antagonists may have some therapeutic utility in the treatment of cognitive dysfunction.

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