



## SPECIAL REPORT

# Attenuation of haloperidol-induced catalepsy by a 5-HT<sub>2C</sub> receptor antagonist

\*<sup>1</sup>C. Reavill, <sup>1</sup>A. Kettle, <sup>1</sup>V. Holland, <sup>1</sup>G. Riley & <sup>1</sup>T. P. Blackburn<sup>1</sup>Department of Neuroscience Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, England

Atypical neuroleptics produce fewer extrapyramidal side-effects (EPS) than typical neuroleptics. The pharmacological profile of atypical neuroleptics is that they have equivalent or higher antagonist affinity for 5-HT<sub>2</sub> than for dopamine D<sub>2</sub> receptors. Our aim was to identify which 5-HT<sub>2</sub> receptor contributed to the atypical profile. Catalepsy was defined as rats remaining immobile over a horizontal metal bar for at least 30 s, 90 min after dosing. Radioligand binding assays were carried out with homogenates of human recombinant 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors expressed in Human Embryo Kidney (HEK293) cells. Haloperidol (1.13 mg kg<sup>-1</sup> i.p.) induced catalepsy in all experiments. The selective 5-HT<sub>2C/2B</sub> receptor antagonist, SB-228357 (0.32–10 mg kg<sup>-1</sup> p.o.) significantly reversed haloperidol-induced catalepsy whereas the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor antagonists, MDL-100907 (0.003–0.1 mg kg<sup>-1</sup> p.o.) and SB-215505 (0.1–3.2 mg kg<sup>-1</sup> p.o.) respectively did not reverse haloperidol-induced catalepsy. The data suggest a role for 5-HT<sub>2C</sub> receptors in the anticataleptic action of SB-228357.

**Keywords:** 5HT<sub>2C</sub> and Dopamine D<sub>2</sub> receptor antagonists; catalepsy

**Abbreviations:** D<sub>2</sub>, dopamine 2; 5-HT<sub>2</sub>, serotonin 2; EPS, extrapyramidal side-effects; HEK, human embryo kidney; IC<sub>50</sub>, inhibitor constant; K<sub>D</sub>, dissociation equilibrium constant; K<sub>i</sub>, inhibitor constant; pK<sub>i</sub>, negative logarithm of inhibitor constant

**Introduction** Clozapine and the new generation of 'atypical' neuroleptics such as olanzapine are less liable than typical neuroleptics to cause extrapyramidal side-effects (EPS) when used to treat schizophrenia (Meltzer, 1996). As well as being dopamine D<sub>2</sub> receptor antagonists, the atypical neuroleptics are high affinity antagonists at 5HT<sub>2</sub> receptors. This property may be responsible for their atypical profile (Meltzer *et al.*, 1989). Therefore, we have investigated whether 5-HT<sub>2A/2B/2C</sub> receptor subtypes modulate haloperidol-induced catalepsy.

**Methods** Male Sprague Dawley rats (200–250 g; Charles River) were housed in groups of six under a 12 h light dark cycle (lights on 0700 h) with free access to food and water. 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 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'. As the raw data were derived from a non-linear quantal scoring scheme, a logistic regression analysis in SAS-RA<sup>®</sup> (SAS Institute Inc.) was used to analyse the data at the 90 min time point. MDL-100907 (R-(+)- $\alpha$ -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol) (0.003–0.1 mg kg<sup>-1</sup>), SB-215505 (6-chloro-5-methyl-1-(5-quinolylylcarbonyl) indoline) (0.1–3.2 mg kg<sup>-1</sup>) and SB-228357 (1-5[fluoro-3-(3-pyridyl)phenyl-carbamoyl]-5-methoxy-6-trifluoromethylindoline) (0.32–10 mg kg<sup>-1</sup>) were ground in one drop of BRIJ-35 and diluted in 1% methylcellulose and administered at 2 ml kg<sup>-1</sup> p.o..

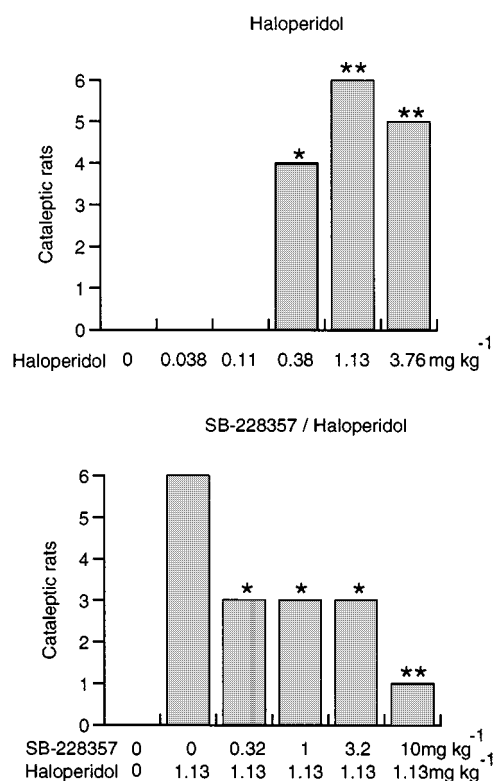
Haloperidol was dissolved with an equal weight of tartaric acid and injected at 1 ml kg<sup>-1</sup> i.p. immediately after the 5HT<sub>2</sub> receptor antagonists. In all experiments, each treatment group consisted of six rats and the assessor was blind to the treatments.

Radioligand binding assays were carried out with homogenates of human recombinant 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors expressed in Human Embryo Kidney (HEK293) cells (Kennett *et al.*, 1997). Washed membranes were incubated with ten concentrations (1 × 10<sup>-11</sup> M–1 × 10<sup>-5</sup> M) of test compounds and 0.5 nM [<sup>3</sup>H]-ketanserin (5-HT<sub>2A</sub>), 8 nM [<sup>3</sup>H]-5-HT (5-HT<sub>2B</sub>) or 0.6 nM [<sup>3</sup>H]-mesulergine (5-HT<sub>2C</sub>) for 30–45 min at 37°C in a pH 7.4 50 mM tris buffer. K<sub>i</sub> (dissociation equilibrium constant, mol l<sup>-1</sup> of the drug) values were calculated from the IC<sub>50</sub> (Cheng & Prusoff, 1973) using the K<sub>D</sub>: 5-HT<sub>2A</sub> 0.7 nM, 5-HT<sub>2B</sub> 11.0 nM and 5-HT<sub>2C</sub> 0.58 nM. pK<sub>i</sub> values were calculated as the negative logarithm of the molar K<sub>i</sub>.

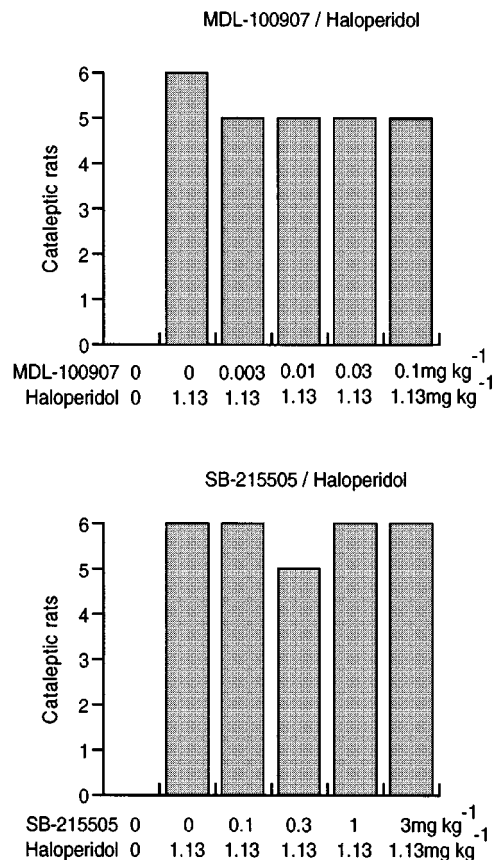
**Results** Haloperidol produced a significant cataleptic response in the range 0.38–3.76 mg kg<sup>-1</sup> (Figure 1, top panel). Maximal effect (6/6 cataleptic rats) occurred at a dose of 1.13 mg kg<sup>-1</sup> (*P* < 0.01) and this dose was used in subsequent experiments for challenge with the 5-HT<sub>2</sub> receptor antagonists. SB-228357 significantly attenuated haloperidol-induced catalepsy at 0.32–10 mg kg<sup>-1</sup>. At 10 mg kg<sup>-1</sup> catalepsy was only seen in one of the six rats (*P* < 0.01; Figure 1 bottom panel). Neither MDL-100907 nor SB-215505 reduced haloperidol-induced catalepsy (*P* > 0.05 in each case; Figure 2).

In the radioligand binding studies (Table 1), SB-228357 had a high affinity for the 5-HT<sub>2C</sub> receptor with 100 and 10 fold selectivity over the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors respectively. MDL-100907 was at least 700 and 16 fold selective for the 5-HT<sub>2A</sub> receptor over the 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptor respectively. SB-215505 was 30 fold selective for the 5-HT<sub>2B</sub>

\* Author for correspondence.



**Figure 1** Each bar shows the number of cataleptic rats, 90 min after administration of haloperidol (top) and after pretreatment with SB-228357 (bottom). Significant haloperidol-induced catalepsy (top), or significant antagonism of the haloperidol effect (bottom) denoted by \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ .



**Figure 2** Each bar shows the number of cataleptic rats 90 min after administration of haloperidol and pretreatment with MDL-100907 and SB-215505.

over the 5-HT<sub>2A</sub> receptor, and only marginally selective over the 5-HT<sub>2C</sub> receptor. Ritanserin had a high affinity for all three receptors (Table 1).

**Discussion** Serotonergic mechanisms are known to influence neuroleptic-induced catalepsy (Balsara *et al.*, 1979), and 5-HT<sub>2</sub> 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<sub>2A/2C</sub> receptor antagonist, mianserin has been shown to reduce neuroleptic-induced akathisia (Poyurovsky & Weizman, 1997).

Other workers have attempted to discover if 5-HT<sub>2</sub> receptor antagonists modulate catalepsy. Kalkman *et al.* (1998) used SB-200646 and 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<sub>2C</sub> 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<sup>-1</sup>) of haloperidol but not high doses (0.75 mg kg<sup>-1</sup>). However while ritanserin had a high affinity for 5-HT<sub>2</sub> receptors (Table 1) it is a non-selective compound. The recent discovery of high affinity and more selective 5-HT<sub>2</sub> receptor antagonists has enabled further investigation of the role of these receptors in catalepsy.

Further evidence has recently been provided that 5-HT<sub>2C</sub> receptor antagonism may produce a favourable outcome in states of motor disturbance. Thus, oro-facial dyskinesias elicited by stimulation of subthalamic 5-HT<sub>2C</sub> receptors are blocked by the 5-HT<sub>2C</sub> receptor antagonist, SDZ SER 082 (Eberle-Wang *et al.*, 1996), and injection of the 5-HT<sub>2C</sub> receptor antagonist, SB-206553 (5-methyl-1-(3-pyridylcarbamoyl)-2,3-dihydropyrrolo[2,3-f]indole), into the substantia nigra zona reticulata produces an antiparkinsonian effect in the rat (Fox *et al.*, 1998). Furthermore, 60% of striatal 5-HT-mediated phosphoinositide hydrolysis is accounted for by 5-HT<sub>2C</sub> receptors despite there being 3 fold fewer striatal 5-HT<sub>2C</sub> than 5-HT<sub>2A</sub> receptors (Wolf & Schutz, 1997).

MDL-100907, has been reported to have 100 fold selectivity for the 5HT<sub>2A</sub> over the 5-HT<sub>2C</sub> receptor (5-HT<sub>2A</sub>  $K_i = 0.85$  nM; 5-HT<sub>2C</sub>  $K_i = 87$  nM, Kehne *et al.*, 1996), although our data (Table 1) show this compound to have less than 100 fold selectivity. MDL-100907 is in clinical development for schizophrenia, following an 'atypical' profile in preclinical tests (Kehne *et al.*, 1996). In these experiments, antagonism of apomorphine-induced stereotypy was used as a method of predicting EPS liability. In our study, MDL-100907 failed to attenuate haloperidol-induced catalepsy at doses that were active in the rat 5-HTP head twitch model (Kehne *et al.*, 1996). Kalkman *et al.* (1998) have shown that MDL-100151 (R-(±)- $\alpha$ -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperi-

**Table 1** Receptor radioligand binding profile of 5-HT<sub>2</sub> receptor antagonists [ $pK_i \pm$  s.e.mean ( $n$ )]

	$pK_i$ 5-HT <sub>2A</sub>	$pK_i$ 5-HT <sub>2B</sub>	$pK_i$ 5-HT <sub>2C</sub>
MDL-100907	8.86 ± 0.08 (4)	6.02 ± 0.03 (4)	7.66 ± 0.09 (3)
Ritanserin	9.42 ± 0.05 (5)	9.25 ± 0.06 (3)	9.65 ± 0.07 (5)
SB-215505	6.77 ± 0.23 (3)	8.3 ± 0.05 (6)	7.66 ± 0.09 (4)
SB-228357	6.97 ± 0.06 (16)	8.14 ± 0.08 (14)	9.14 ± 0.07 (16)

dinemethanol), the racemate of MDL-100907, is cataleptogenic at a dose of  $0.3 \text{ mg kg}^{-1}$ . This could explain why MDL-100907 failed to attenuate catalepsy. In contrast, SB-228357 had a pronounced anticataleptic profile. This compound had a high affinity for the 5-HT<sub>2C</sub> receptor and is 100 and 10 fold selective over the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor respectively (Table 1). This suggests that 5-HT<sub>2C</sub> receptor antagonism, or possibly mixed 5-HT<sub>2C/2B</sub> receptor antagonism is the optimum profile for antagonising haloperidol-induced catalepsy. How-

ever, there was no evidence that SB-215505 blocked the cataleptic response. This compound has a high affinity for the 5-HT<sub>2B</sub> receptor and is moderately selective over both 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors (Table 1).

By deduction, our data suggest that 5-HT<sub>2C</sub> receptor antagonism, and not 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub> receptor antagonism, is likely to be the mechanism by which 'atypical' antipsychotic drugs lack EPS.

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