British Journal of Pharmacology (1996) 117, 1443–1448

Effects of the 5-HT_{2B} receptor agonist, BW 723C86, on three rat models of anxiety

[1]G.A. Kennett, F. Bright, B. Trail, †G.S. Baxter & T.P. Blackburn

Psychiatry and †Neurology Research Departments, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW

1 BW 723C86 (3 and 10 mg kg^{-1}, s.c. 30 min pretest), a 5-HT_{2B} receptor agonist, increased total interaction, but not locomotion in a rat social interaction test, a profile consistent with anxiolysis.

2 The effect of BW 723C86 in the social interaction test is likely to be 5-HT_{2B} receptor-mediated as it was prevented by pretreatment with the 5-$HT_{2C/2B}$ receptor antagonist, SB 200646A, (1 and 2 mg kg^{-1}, p.o., 1 h pretest) which did not affect basal levels of social interaction at the doses used.

3 An anxiolytic-like action was also observed in the rat Geller-Seifter conflict test, where BW 723C86 (0.5–50 mg kg^{-1}, s.c. 30 min pretest) modestly, but significantly increased punished, but not unpublished responding.

4 In a rat 5 min elevated x-maze test, BW 723C86 (1–10 mg kg^{-1}, s.c.) had no significant effect.

5 The maximal anxiolytic-like effect of BW 723C86 approached that of the benzodiazepine anxiolytic, chloradiazepoxide (5 mg kg^{-1}, s.c. 30 min pretest) in the social interaction test, but was markedly less in the Geller-Seifter test. The effect of BW 723C86 was also clearly less than chlordiazepoxide in the elevated x-maze procedure where it had no significant effect.

6 In conclusion, BW 723C86 exerted an appreciable anxiolytic-like profile in a rat social interaction test, but had a weaker effect in the Geller-Siefter and was ineffective in the elevated x-maze test used. These effects are likely to be 5-HT_{2B} receptor-mediated.

Keywords: 5-HT_{2B} receptor; 5-HT_{2C} receptor; anxiety; BW 723C86

Introduction

The nomenclature of the 5-HT receptor subtypes has been altered to recognise the existence of an expanded 5-HT_2 receptor family sharing very similar structure and pharmacology and the use of a common secondary messenger system. This family currently consists of 3 subtypes designated 5-HT_{2A} (formerly 5-HT_2), 5-HT_{2B} (formerly the rat stomach fundus receptor) and 5-HT_{2C} (formerly 5-HT_{1C}) (Hoyer *et al.*, 1994).

Investigations of the function of the 5-HT_{2C} receptor have led to the hypothesis that it is involved in the modulation of anxiety, as *m*-chlorophenylpiperazine (mCPP), a 5-HT_{2C} receptor agonist has anxiogenic-like effects in both man and animals (see Kennett, 1993). In man, these can be blocked by ritanserin, methysergide and metergoline, non-selective antagonists of the 5-HT_{2C} receptor (see Kennett, 1993). In rats, anxiogenic-like effects of mCPP are prevented by SB 200646A (Kennett *et al.*, 1994), the first 5-$HT_{2C/2B}$ receptor antagonist with selectivity over the 5-HT_{2A} site (Forbes *et al.*, 1993). Furthermore, SB 200646A alone has anxiolytic-like properties in animal models (Kennett *et al.*, 1994; 1995, Bill *et al.*, 1995). mCPP is also a potent agonist of the 5-HT_{2B} receptor (Clineschmidt *et al.*, 1985, Baxter *et al.*, 1995) and at present, all high affinity antagonists of the 5-HT_{2C} receptor, including SB 200646A (Kennett *et al.*, 1994), are equipotent at the 5-HT_{2B} site (see Baxter *et al.*, 1995). It is therefore conceivable that the effects of mCPP and of SB 200646A are mediated by activity at the 5-HT_{2B} receptor. Until recently, this hypothesis was weakened by failure to detect 5-HT_{2B} receptor mRNA in the rat brain (Pompeiano *et al.*, 1994) and the very low levels in human brain (Schmuck *et al.*, 1994). However, both 5-HT_{2B} mRNA (Flanigan *et al.*, 1995) and receptor protein (Duxon *et al.*, 1995) have now been detected in the rat brain, particularly in areas associated with the control of anxiety, by the use of more sensitive techniques.

Recently, we have characterized an agonist of the 5-HT_{2B} receptor, BW 723C86, with some selectivity over the 5-HT_{2C}, 5-HT_{2A} and other sites, (Baxter *et al.*, 1995). In the present study, the effects of BW 723C86 on three rat models of anxiety, the social interaction (File & Hyde, 1978), elevated x-maze (Handley & Mithani, 1984) and Geller-Seifter (Geller & Seifter, 1960) tests, have been examined.

Methods

Animals

Male Sprague-Dawley (Charles River, U.K.) rats (220–250 g) were housed in groups of six under a 12 h light/dark cycle (lights on 07 h 00 min) with free access to food (CRMX, Special Diet Services) and water.

Social interaction test

Rats were housed singly in a room adjacent to the testing room on day 1. On day 5, weight matched (±5 g) pairs of rats unfamiliar with each other were dosed with identical treatments and returned to their home cages. Dosing was either oral 1 h pretest (SB 200646A or vehicle) or s.c. 30 min pretest (BW 723C86 or saline). Rats were then placed in a white perspex test box (54 × 37 × 26 cm) for 15 min under bright white light (150 lux) in an adjacent darkened room containing a fan to generate white noise. Active social interaction (sniffing, following, grooming, biting, boxing and crawling over or under) was scored by a 'blind' observer by remote video monitoring and a computerised score pad. At the end of each test, the box was thoroughly wiped with moistened tissue paper.

[1] Author for correspondence.

Elevated x-maze test

The method used was based on that described by Handley & Mithani, (1984). The x-maze was raised 70 cm above the floor and consisted of two enclosed arms 45 cm long × 15 cm wide × 15 cm high and two open arms 45 cm × 10 cm × 1 cm. Both arm types were made of black perspex and marked into two equal sections by a white line. Tests were conducted under bright white light (120 lux). Group housed rats (6 per cage) were dosed s.c. and returned to their home cages. Thirty min later they were placed in the centre of the elevated x-maze. The number of entries onto and the time spent on the open arms, together with the number of section crossings, were scored for 5 min by an experimenter, 'blind' to the treatments given, via a video monitor. At the end of each individual test, the x-maze was carefully cleaned with a damp cloth.

Geller-Seifter test

Forty male Sprague-Dawley CFY rats (Interfauna 400–600 g) were housed in pairs under a 12 h light/dark cycle (lights on 07 h 00 min) and fed a restricted diet to maintain their body weight to 80% of a free-feeding animal. The rats were part of a colony and were trained initially in a typical Skinner box (Campden Instruments Ltd) to associate pressing of a lever with a food pellet reward. As training progressed, the rats were introduced to a multiple schedule of reinforcement, i.e. five 3 min variable interval components [one reinforcement every 10–50 (mean 30) s, VI30] alternating with five 3 min fixed ratio (one reinforcement every five lever presses; FR5) components. The FR component was signalled to the rat by a light above the lever and in this component reinforcement was contingent with a footshock of pulse width 15 ms at intervals of 200 ms for 1 s. The magnitude of footshock was individually titrated for each rat up to a maximum of 0.75 mA, to give a lever pressing rate of between two and seven reinforcements during each of the five, 3 min punished responding periods. Fully trained rats also had a high level of responding in the VI phases (typically 180 presses in 3 min) to detect non-specific effects such as sedation or stimulant properties. Before use, all rats had met specific performance criteria (see Kennett *et al.*, 1995) and had shown a significant positive response to a reference anxiolytic drug (e.g. chlordiazepoxide). A period of at least seven days was left between subsequent tests. No rat received two consecutive doses of the same drug or type of drug and no rat received more than five treatments.

Materials

Chlordiazepoxide and BW 723C86, 1-[5-thienylmethoxy)-1H-3-indoyl] propan-2-amine hydrochloride (Figure 1) (both synthesized by the Department of Medicinal Chemistry, SmithKline Beecham) were dissolved in 0.9% NaCl and injected s.c. in a 2 mg kg^{-1} volume at the nape of the animal's neck. BW 723C86 was maintained in solution by standing on a warm hotplate during experiments. SB 200646A, N-(1-methyl-5-indolyl)-N'-(3-pyridyl) urea hydrochloride (synthesized by the Department of Medicinal Chemistry, SmithKline Beecham), was made up as a suspension in 1% methyl cellulose containing 10 mg ml^{-1} barium sulphate (Sigma Chemical Co, Poole, Dorset, U.K.) and 10 µl + 2 ml^{-1} egg yellow food colourant and dosed orally 1 h pretest in a 2 ml kg^{-1} volume. Doses of SB 200646A are given as the salt. Drug and vehicle suspensions were independently coded prior to experiments to establish 'blind' conditions.

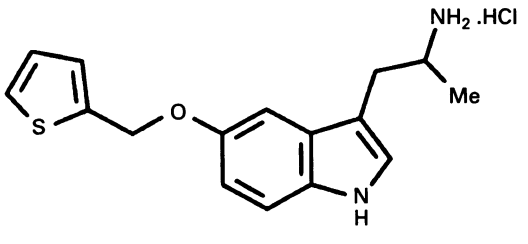

Figure 1 Structure of BW 723C86.

Data analysis and statistics

Social interaction and elevated x-maze test data were subjected to 1 way ANOVA and Dunnett's test or 2-way ANOVA and Newman-Keuls test (SB 200646A experiment. Geller-Seifter test data were analysed by 2-way ANOVA (treatment × subjects) of the number of lever presses on the 2 consecutive days before the test day (2 scores per subject), and on the test day itself (1 score per subject). Both pretest day scores were included in 1 treatment group for the purposes of this anaylsis and these were compared with the relevant test day scores for each subject. All data are cited as the mean ± s.e.mean unless otherwise stated.

Results

Effects of BW 723C86 and SB 200646A in a rat social interaction test

BW 723C86 significantly altered total interaction scores [$F(5,82) = 15.9$, $P < 0.01$]. Subsequent analysis by Dunnett's multiple comparisons procedure revealed that both 3 and 10 mg kg^{-1}, s.c. increased total interaction scores, but this effect was lost after 30 mg kg^{-1}, s.c. In the same experiment, the positive control chlordiazepoxide (5 mg kg^{-1}, s.c.) also caused a large increase in total interaction. Increased total interaction was not matched by any effect on locomotor activity. However, at 30 mg kg^{-1}, s.c. BW 723C86 significantly [$F(5,82) = 8.5$, $P < 0.01$) lowered locomotion (Figure 2). In a subsequent experiment, rats were pretreated with SB 200646A (1 or 2 mg kg^{-1}) or vehicle, p.o. 1 h pretest and BW 723C86 3 mg kg^{-1}, s.c. 30 min pretest. SB 200646A alone at 1 or 2 mg kg^{-1} had no effect on social interaction. As previously, BW 723C86 alone increased active interaction ($F(1, 42) = 6.1$, $P < 0.01$) and this was inhibited by SB 200646A ($F(2, 42) = 34.3$, $P < 0.01$). Chlordiazepoxide (5 mg kg^{-1}, s.c.), included as a positive control, also increased total interaction ($P < 0.01$ by Student's t test). No treatment significantly affected locomotion in the test (Figure 3).

Effects of BW 723C86 on rat behaviour in an elevated x-maze test

BW 723C86 caused small, non significant increases in % time spent on the open arm of the x-maxe, unlike chlordiazepoxide 5 mg kg^{-1}, s.c. which caused a pronounced increase [$F(6,75) = 5.1$, $P < 0.01$]. BW 723C86 had no effect on % entries to the open arm in contrast to the increase seen with chlordiazepoxide ($F(6,75) = 4.6$, $P < 0.01$]. Neither treatment affected total entries to open and closed arms, or total line crossings (total entries + crossing of lines bisecting open and closed arms) (Table 1).

Effects of BW 723C86 on rat behaviour in the Geller-Seifter test

Mean (± s.e.mean) total lever presses during the 5 × 3 min unpunished records was 742 ± 50.8 while during the punished periods it was 24.5 ± 0.92 after vehicle-treatment on the two preceding tests. BW 723C86 (s.c. 30 min pretest) increased punished responding after 0.5, 1, 5, and 50 mg kg^{-1} and induced an increase that did not reach significance after 10 mg kg^{-1}, s.c. BW 723C86 (0.1–50 mg kg^{-1}, s.c.) had no effect on unpunished responding. The positive control chlordiazepoxide (5 mg kg^{-1}, s.c. 30 min pretest) also increased

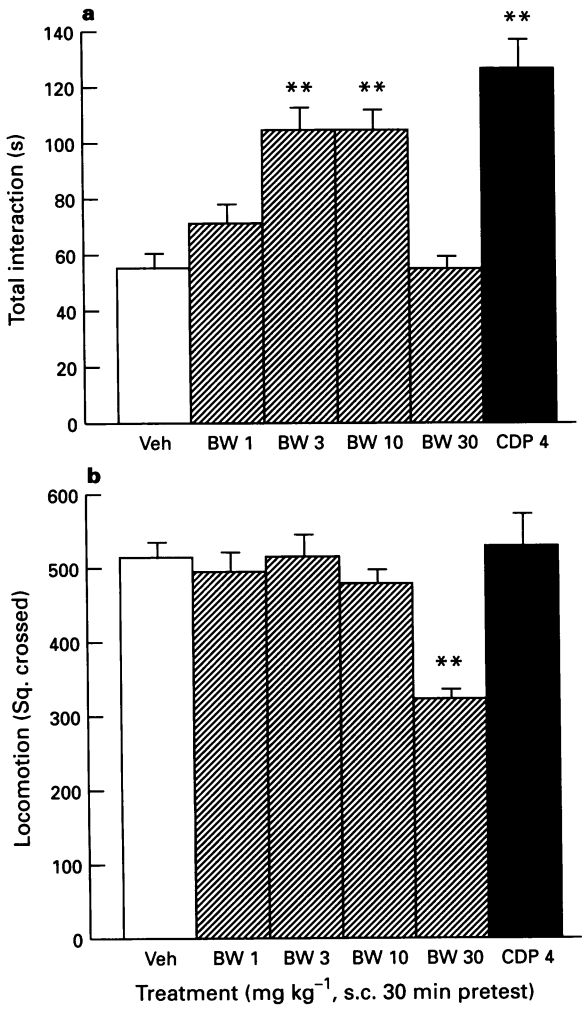

Figure 2 Effect of BW 723C86 (1–30 mg kg^{-1}, s.c. 30 min pretest) on rat behaviour in a 15 min social interaction test under high light unfamiliar conditions: (a) shows the effects on rat social behaviour in the test, (b) represents the corresponding locomotion observed during the procedure. CDP4, chlordiazepoxide 4 mg kg^{-1}, s.c. All data cited as means ± s.e.mean, $n = 12-18$ per group. Significantly different from vehicle treated group $^{**}P < 0.01$ by Dunnett's test and 1 way ANOVA.

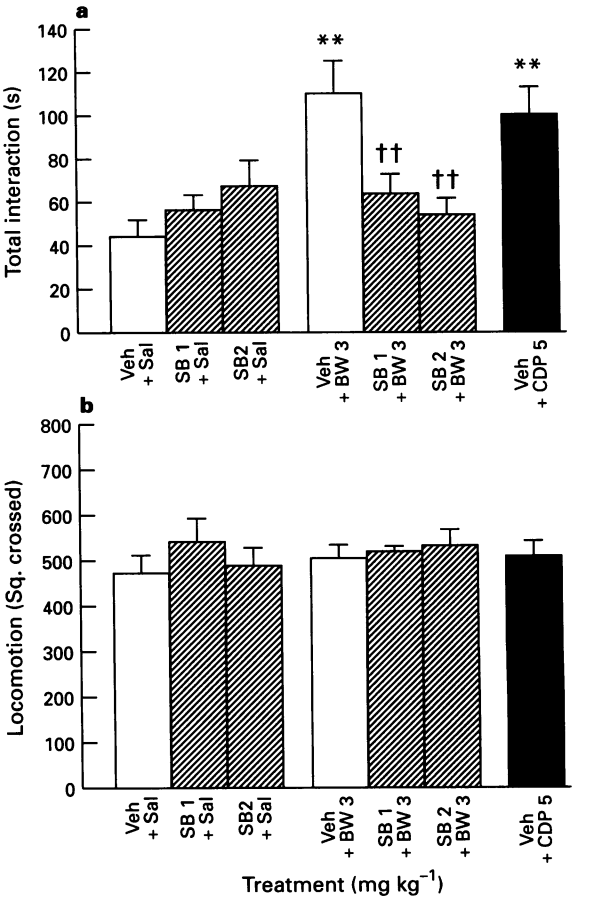

Figure 3 Effect of SB 200646A, 1 or 2 mg kg^{-1} (hatched columns) or vehicle (open columns), p.o. 1 h pretest, and chlordiazepoxide (CDP) 5 mg kg^{-1}, s.c. (solid columns) or BW 723C86, 3 mg kg^{-1}, s.c. 30 min pretest, on behaviour in a rat 15 min social interaction test under high light familiar conditions. (a) Shows the effects on rat social behaviour in the test, while (b) represents the corresponding locomotion observed during the procedure. All data cited as means ± s.e.mean, $n = 8$ per group. Significantly different from vehicle + saline treated group $^{**}P < 0.01$, from vehicle + BW 723C86 treated group $^{\dagger\dagger}P < 0.01$ by Newman-Keuls test and 2-way ANOVA or (chlordiazepoxide) by Student's t test.

punished responding, although to a much greater degree than seen after any of the doses of BW 723C86 used. Like BW 723C86, chlordiazepoxide had no effect on unpunished responding (Table 2).

Discussion

BW 723C86 increased total interaction scores at 3 and 10 mg kg^{-1} without affecting locomotor activity in the social interaction test, as did the benzodiazepine anxiolytic chlordiazepoxide, used as a positive control. This effect is consistent with anxiolysis (File & Hyde, 1978). At higher doses, BW 723C86 no longer increased total interaction and lowered locomotion suggesting the onset of sedation. BW 723C86 also exhibited an anxiolytic-like profile in the rat Geller-Seifter test (Geller *et al.*, 1962) as it increased punished responding without affecting unpublished responding. However, in this test, the minimum effective dose was lower and no loss of efficacy was seen at higher doses for reasons that are unclear at present. The benzodiazepine anxiolytic, chlordiazepoxide, also increased punished responding without affecting unpunished responding in the present procedure, as previously observed (Geller *et al.*, 1962). In a third rat test of anxiety, the elevated x-maze, BW 723C86 had no significant effect, unlike chlordiazepoxide which markedly increased % time spent on and entries to the open arm, measures of anxiolytic-like action in the paradigm (Pellow *et al.*, 1985; Dawson *et al.*, 1995).

Individual tests of anxiety can be confounded by idiosyncratic properties of the compounds tested. Body odour can affect the social interaction test for instance (Higgins *et al.*, 1991), while changes in appetite (Oxley *et al.*, 1995) or pain might affect the Geller-Seifter test and stimulant properties can interfere with the elevated x-maze (Dawson *et al.*, 1995). The significant effects of BW 723C86 in two different tests (social interaction and Geller-Seifter tests) although not in a third, the elevated x-maze, suggest that the compound does indeed possess anxiolytic-like properties. In the social interaction test, the effect of BW 723C86 is of a similar magnitude to that of chlordiazepoxide. In contrast, in the Geller-Seifter test, the efficacy of chlordiazepoxide was far greater and indeed, in the elevated x-maze, the effects of BW 723C68 did not reach statistical significance. This suggests that the overall anxiolytic-like efficacy of BW 723C86 may be modest on systemic administration. Alternatively, BW 723C86 may be more effective on types of anxiety most closely modelled by the social interaction test. What type of anxiety that might be is at present unclear. However, one could speculate that it is relevant to

Table 1 Effect of BW 723C86 and chlordiazepoxide on rat behaviour in an elevated x-maze test.

Treatment (s.c. 30 min pre-test)	*% time on, or entries to, the open arm*		*Total line crossings*	*Total entries*
	Entries	Time		
Vehicle	41.1±3.2	23.7±3.4	32.2±2.5	15.1±1.5
BW 1 mg kg^{-1}	39.7±3.3	29.0±3.2	30.7±1.2	15.1±0.7
BW 2 mg kg^{-1}	42.5±3.4	31.4±3.7	27.2±1.8	13.3±0.8
BW 5 mg kg^{-1}	33.8±2.9	23.4±3.6	27.7±2.0	12.8±1.0
BW 10 mg kg^{-1}	41.5±2.5	29.9±2.8	32.0±2.4	15.3±1.3
BW 20 mg kg^{-1}	41.8±2.0	28.2±3.7	26.8±2.7	13.3±1.4
CDP 5 mg kg^{-1}	54.7±3.2*	45.5±2.5**	35.7±1.7	19.2±1.3

$n=12$ per group. Significantly different from vehicle pretreated group; *$P<0.05$; **$P<0.01$ by Dunnett's test and 1-way ANOVA.

Table 2 Effect of BW 723C86 and chlordiazepoxide on behaviour in the rat Geller-Seifter test

Treatment	*Dose* (mg kg^{-1}, s.c. 30 min pretest)	*% change in lever presses compared with mean score on 2 preceding days after vehicle treatment*	
		Unpunished	*Punished*
BW 723C86	0.1	0.0±4.0	+12.2±12.7
	0.5	+6.8±4.7	+39.0±14.3**
	1.0	−3.6±7.6	+33.8±18.0*
	5.0	−1.5±4.3	+49.9±29.1*
	10.0	+4.4±7.0	+32.4±26.9
	50.0	−1.7±3.1	+66.1±19.7**
Chlordiazepoxide	5.0	+8.7±5.8	+234.6±97.0**

All data cited as means±s.e.mean, $n=6-8$ per group. Significantly different from mean level of responding on two preceding vehicle-treated tests: *$P<0.05$, **$P<0.01$ by 2-way ANOVA (treatment x subjects). Significant F values for punished responding; BW 0.5 mg kg^{-1} F (1, 11)=11.4, $P<0.01$, BW 1 mg kg^{-1}, F (1, 11)=6.0, $P<0.05$, BW 5 mg kg^{-1}, F (1, 11)=5.7, $P<0.05$, BW 50 mg kg^{-1}, F (1, 11)=15.4, $P<0.01$, chlordiazepoxide 5 mg kg^{-1}, F (1, 13)=13.1, $P<0.01$.

Table 3 Profile of BW 723C86, a 5-HT_{2B} receptor agonist

Receptor	*pEC_{50}* (intrinsic activity) or *pK_i	*Receptor*	*pEC_{50}* (intrinsic activity) or *pK_i
5-HT_{2B} (human)	7.1*	5-HT_7 (human)	5.5*
(rat)	7.9 (0.8)	(rat)	<6.0
5-HT_{2C} (human)	6.3 (1.0)	Dopamine D_1	<5.0*
(rat)	6.9*		
5-HT_{2A} (human)	7.0 (0.4)	Dopamine D_2	<5.0*
(rat)	6.6		
5-HT_{1A} (rat)	<5.9*	Dopamine D_4	<5.0*
5-$HT_{1D\alpha}$ (human)	6.9*	Adrenoceptor α_1	<5.0
5-$HT_{1D\beta}$ (human)	6.2*	Adrenoceptor α_2	<5.0
5-HT_{1E} (human)	5.5*	Adrenoceptor β_1	<5.0
5-HT_{1F} (human)	5.1*	Adrenoceptor β_2	<5.0
5-HT_3 (rat)	6.5*	Histamine H_1	<5.0
5-HT_4 (rat)	<5.0	Histamine H_2	<5.0

Data largely as reported by Baxter (1995) and Baxter *et al.* (1995), except binding to the cloned human 5-HT_{2B} receptor expressed in HEK 293 cells by the method of Kursar *et al.* (1992) to the cloned human 5-HT_7 receptor according to To *et al.* (1995), to the cloned 5-HT_{1F} receptor according to Adham *et al.* (1993) and to the cloned human D_4 receptor by the method of Van Tol *et al.* (1991). Intrinsic activity values represent the maximal response to BW 723C86 in a functional assay expressed as a ratio of the maximal response to 5-HT in the same system. Rat 5-HT_{2B} receptor function activity was derived from the rat stomach fundus preparation, 5-HT_{2C} function was derived from phosphoinositide hydrolysis responses in HEK 293 cells expressing the human cloned 5-HT_{2C} receptor, while human 2-HT_{2A}-receptor function studies were carried out on phosphoinositide hydrolysis responses in HEK 293 cells expressing cloned human 5-HT_{2A} receptors.

social phobia, particularly as the 5-HT_3 receptor antagonist, ondansetron, which has anxiolytic-like effects in the test (Jones *et al.*, 1988; Blackburn *et al.*, 1993), but not in the Geller-Seifter procedure (Tyers *et al.*, 1987; Piper *et al.*, 1988), has recently been reported to be effective in clinical trials (Bell & DeVeaugh-Geiss, 1994).

Current knowledge of the pharmacology of BW 723C86 suggests that its actions are likely to be mediated by the 5-HT_{2B} receptor for which it has a relatively high affinity and some selectivity as indexed by its pEC_{50} on the rat stomach fundus (Table 3). This argument is supported by the observed antagonism of BW 723C86-induced anxiolytic-like actions in the social interaction test by SB 200646A. This compound is a mixed 5-$HT_{2C/2B}$ receptor antagonist with at least 50 fold se-

lectivity over 5-HT_{2A} receptors and all other sites tested (Kennett *et al.*, 1994). The antagonist potency of SB 200646A in the present experiments is also consistent with its ability to block 5-HT_{2C} receptor-mediated behaviours and with its slightly higher affinity for the rat 5-HT_{2B} site (Kennett *et al.*, 1994). At the doses used, SB 200646A had little effect alone on social interaction in the present study, although in previous studies 2 mg kg^{-1} and above have exerted an anxiolytic-like profile in both this and other tests (Kennett *et al.*, 1994; 1995). Thus the SB 200646A-induced antagonism observed in this study, cannot be interpreted as response competition. Furthermore, the low potency agonist properties of BW 723C86 at the 5-HT_{2C} receptor cannot explain its effects in the social interaction test as they would be expected to cause anxiogenic-like and not anxiolytic-like activity (Kennett *et al.*, 1989; 1994). Indeed, if these properties are present at the doses studied, they may have limited the efficacy of BW 723C86 in the anxiety paradigms.

The above arguments in favour of central 5-HT_{2B} receptor mediation of the effects of BW 723C86 in the rat is tempered by the failure of some groups to detect 5-HT_{2B} receptor mRNA in the rat brain (Foquet *et al.*, 1992; Pompeiano *et al.*, 1994) while only very low levels had been detected in the mouse (Loric *et al.*, 1992) and human (Schmuck *et al.*, 1994) brains. However, recently, Flanigan *et al.* (1995) using a more sensitive assay procedure, have reported that 5-HT_{2B} receptor mRNA is concentrated in the rat hippocampus as well as the cortex, midbrain and hypothalamus while Duxon *et al.* (1995) have located 5-HT_{2B} receptor protein principally in the rat medial amygdala and lateral septum using receptor specific antibodies. All three areas are associated with the control of anxiety (Kuhar, 1986; Higgins *et al.*, 1991; Yadin *et al.*, 1993). The distribution of the 5-HT_{2B} receptor is therefore consistent with 5-HT_{2B} receptor involvement in the anxiolytic-like actions of BW 723C86.

It is of some interest that the mixed 5-$HT_{2C/2B}$ receptor agonist, mCPP, has axiogenic effects in man (see Kennett, 1993) and anxiogenic-like effects in the rat social interaction (Kennett *et al.*, 1989), elevated x-maze (Blackburn *et al.*, 1993; Gibson *et al.*, 1994) and Vogel conflict (Cronin *et al.*, 1992) tests. The anxiogenic-like effects of mCPP in the social interaction test are prevented by the 5-$HT_{2C/2B}$ receptor antagonist, SB 200646A (Kennett *et al.*, 1994) and by less selective antagonists of these sites (Kennett *et al.*, 1989). The effects of mCPP in the elevated x-maze have at the present time only been reversed by less selective antagonists of the 5-$HT_{2C/2B}$ receptors (Gibson *et al.*, 1994). One site of action of mCPP is thought to be hippocampus, as direct infusion into this site has anxiogenic-like effects, while no effect was seen after infusion into the medial amygdala (Whitton & Curzon, 1990). The present results with BW 723C86, if mediated via the 5-HT_{2B} receptor, imply that the anxiogenic-like effects of mCPP and the anxiolytic-like actions of SB 200646A are indeed likely to be accounted for by their activity at the 5-HT_{2C} receptor. They also point to an opposing role for the 5-HT_{2C} and 5-HT_{2B} receptors in these models. It is therefore conceivable that the anxiolytic-like properties of a selective 5-HT_{2C} receptor antagonist could be enhanced by co-administration of a selective 5-HT_{2B} receptor agonist. In the present study, this effect would not have been apparent due to the antagonist properties of SB 200646A at the 5-HT_{2B} site.

In conclusion, systemic administration of BW 723C86, an agonist at the 5-HT_{2B} receptor with some selectivity over other sites tested, has anxiolytic-like effects in a rat social interaction and Geller-Seifter tests (albeit weak in the latter) but had no significant action in the elevated x-maze procedure. In the rat social interaction test, these effects were antagonized by SB 200646A, a 5-HT_{2B} receptor antagonist. These effects are consistent with the location of the 5-HT_{2B} receptor in areas of the brain associated with the control of anxiety and are largely opposite to the effects of the mixed 5-$HT_{2C/2B}$ agonist, mCPP (see above). Further proof of the role of 5-HT_{2B} receptors in the effects of BW 723C86 awaits the development of selective ligands for this site.

References

ADHAM, N., KAO, H.T., SCHECHTER, L.E., BARD, J., OLSEN, M., URQUHART, D., DURKIN, M., HARTIG, P.R., WEINSHANK, R.L. & BRANCHEK, A. (1993). Cloning of another human serotonin receptor (5-HT_{1F}): A fifth 5-HT_1 receptor subtype coupled to the inhibition of adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 408–412.

BAXTER, G.S. (1995). Novel discriminatory ligands for 5-HT_{2B} receptors. *Behav. Brain Res.*, (in press).

BAXTER, G.S., KENNETT, G.A., BLACKBURN, T.P. & BLANEY, F. (1995). 5-HT_2 receptor subtypes, a family reunited? *Trends Pharmacol. Sci*, **16,** 105–110.

BELL, J. & DE VEAUGH-GEISS, J. (1994). Multicentre trial of 5-HT_3 antagonist, ondansetron in social phobia. *ACNP Meeting abstract.*

BILL, D.J., FORSTER, E.A., GREWAL, S.S. & FLETCHER, A. (1995). Functional antagonistic and anxiolytic effects of the 5-HT2C/2B receptor antagonist, SB 200646A, in rodents. *Br. J. Pharmacol.*, **116,** 217P.

BLACKBURN, T.P., BAXTER, G.S., KENNETT, G.A., KING, F.D., PIPER, D.C., SANGER, G.J., THOMAS, D.R., UPTON, N. & WOOD, M.D. (1993). BRL 46470A: a highly potent, selective and long acting 5-HT_3 receptor antagonist with anxiolytic-like properties. *Psychopharmacology*, **110,** 257–264.

CLINESCHMIDT, B.V., REISS, D.R., PETTIBONE, D.J. & ROBINSON, J.L. (1985). Characterization of 5-hydroxytryptamine receptors in rat stomach fundus. *J. Pharmacol. Exp. Ther.*, **235,** 696–708.

CRONIN, S.M., BILL, D.J. & FLETCHER, A. (1992). Evidence for the involvement of 5-HT_{1C} receptors in the anxiogenic-like effects of fenfluramine in a modified Vogel conflict test. *Br. J. Pharmacol.*, **106,** 128P.

DAWSON, G.R., CRAWFORD, S.P., COLLINSON, N., IVERSEN, S.D. & TRICKLEBANK, M.D. (1995). Evidence that the anxiolytic-like effects of chlordiazepoxide on the elevated plus maze are confounded by increases in locomotor activity. *Psychopharmacology*, **118,** 316–323.

DUXON, M.S., REAVELEY, A.C., FLANIGAN, T.P., BLACKBURN, T.P. & FONE, K.C.F. (1995). Expression of 5-HT_{2B} receptor protein in the rat brain. *Br. J. Pharmacol.*, **115,** 105P.

FILE, S.E. & HYDE, J.R.G. (1978). Can social interaction be used to measure anxiety? *Br. J. Pharmacol.*, **62,** 19–24.

FLANIGAN, T.P., REAVELEY, A.C., CAREY, J.E. & LESLIE, R.A. (1995). Evidence for expression of the 5-HT_{2B} receptor mRNA in rat brain. *Br. J. Pharmacol.*, **114,** 369P.

FOQUET, M., HOYER, D., PARDOE, L.A., PAREKH, A., KLUXEN, F.W., KALKMAN, H.O., STUHMER, W. & LUBBERT, H. (1992). Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J.*, **11,** 3481–3487.

FORBES, I.T., KENNETT, G.A., GADRE, A., HAM, P., HAYWARD, C.J., MARTIN, R.T., THOMPSON, M., WOOD, M.D., BAXTER, G.S., GLEN, A., MURPHY, O.E., STEWART, B. & BLACKBURN, T.P. (1993). N-(1-methyl-5-indolyl)-N'-(3-pyridyl)urea hydrochloride: The first selective 5-HT_{1C} receptor antagonist. *J. Med. Chem.*, **36,** 1104–1107.

GELLER, I., KULAK, J.T. & SEIFTER, J. (1962). The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. *Psychopharmacologia*, **3,** 374–385.

GELLER, I. & SEIFTER, J. (1960). The effects of meprobamate, barbituates, d-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia*, **1,** 482–492.

GIBSON, E.L., BARNFIELD, A.M.C. & CURZON, G. (1994). Evidence that mCPP-induced anxiety in the plus-maze is mediated by postsynaptic 5-HT_{2C} receptors but not by sympathomimetic effects. *Neuropharmacology*, **33,** 457–465.

HANDLEY, S.L. & MITHANI, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behaviour. *Naunyn-Schmied. Arch. Pharmacol.*, **327,** 1–5.

HIGGINS, G.A., JONES, B.J., OAKLEY, N.R. & TYERS, M.B. (1991). Evidence that the amygdala is involved in the disinhibitory effects of 5-HT_3 receptor antagonists. *Psychopharmacology*, **104,** 545–551.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). VII. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46,** 157–203.

JONES, B.J., COSTALL, B., DOMENEY, A.M., KELLY, M.E., NAYLOR, R.J., OAKLEY, N.R. & TYERS, M.B. (1988). The potential anxiolytic activity of GR 38032F, a 5-HT_3 receptor antagonist. *Br. J. Pharmacol.*, **93,** 985–993.

KENNETT, G.A. (1993). 5-HT_{1C} receptors and their therapeutic relevance. *Curr. Opin. Invest. Drugs*, **2,** 317–362.

KENNETT, G.A., BAILEY, F., PIPER, D.C. & BLACKBURN, T.P. (1995). Effect of SB 200646A, a 5-HT_{2C}/5-HT_{2B} receptor antagonist in two conflict models of anxiety. *Psychopharmacology*, **118,** 178–182.

KENNETT, G.A., WHITTON, P., SHAH, K. & CURZON, G. (1989). Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. *Eur. J. Pharmacol.*, **164,** 445–454.

KENNETT, G.A., WOOD, M.D., GLEN, A., GREWAL, S., FORBES, I.T., GADRE, A. & BLACKBURN, T.P. (1994). *In vivo* properties of SB 200646A, a 5-$HT_{2C/2B}$ receptor antagonist. *Br. J. Pharmacol.*, **111,** 797–802.

KUHAR, M.J. (1986). Neuroanatomical substrates of anxiety: a brief survey. *Trends Neurosci.*, **9,** 307–311.

KURSAR, J.D., NELSON, D.L., WAINSCOTT, D.B., COHEN, M.L. & BAEZ M. (1992). Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine$_{2F}$) from rat stomach fundus. *Mol. Pharmacol.*, **42,** 549–557.

LORIC, S., LAUNAY, J.M., COLAS, J.-F. & MAROTEAUX, L. (1992). New mouse 5-HT_2-like receptor. Expression in brain, heart and intestine. *FEBS Lett.*, **312,** 203–207.

OXLEY, K., STANHOPE, K.J., SHEPHERD, J.K., CRONIN, S. & DOURISH, C.T. (1995). Absence of an anxiolytic effect of the 5-$HT_{2C/2B}$ antagonist SB 200646A in the conditioned emotional response model of anxiety in the rat. *Br. J. Pharmacol.*, **116,** 215P.

PELLOW, S., CHOPIN, P., FILE, S.E. & BRILEY, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods*, **14,** 149–167.

PIPER, D., UPTON, N., THOMAS, D.R. & NICHOLASS, J. (1988). The effects of 5-HT_3 receptor antagonists BRL 43694 and GR 38032F in animal models of anxiety. *Br. J. Pharmacol.*, **94,** 314P.

POMPEIANO, M., PALACIOS, J.M. & MENGOD, G. (1994). Distribution of the serotonin 5-HT_2 receptor family mRNAs:comparisons between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.*, **23,** 163–178.

SCHMUCK, K., ULLMER, C., ENGELS, P. & LUBBERT, H. (1994). Cloning and functional characterization of the human 5-HT_{2B} serotonin receptor. *FEBS Lett.*, **342,** 85–90.

TO, Z.P., BONHAUS, D.W., EGLEN, R.M. & JAKEMAN, L.B. (1995). Characterization and distribution of putative 5-HT_7 receptors in guinea-pig brain. *Br. J. Pharmacol.*, **115,** 107–116.

TYERS, M.B., COSTALL, B., DOMENEY, A., JONES, B.J., KELLY, M.E., NAYLOR, R.J. & OAKLEY, N.R. (1987). The anxiolytic activities of 5-HT_3 antagonists in laboratory animals. *Neurosci. Lett.*, **29** (suppl. 1), 68–77.

VAN TOL, H.H.M., BUNZOW, J.R., GUAN, H.C., SUNAHARA, R.K., SEEMAN, P., NIZNIK, H.B. & CIVELLI, O. (1991). Cloning of the gene for a human D_4 receptor with high affinity for the antipsychotic clozapine. *Nature*, **350,** 610–614.

WHITTON, P. & CURZON, G. (1990). Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus. *Psychopharmacology*, **100,** 138–140.

YADIN, E., THOMAS, E., GRISHKAT, H.L. & STRICKLAND, C.E. (1993). The role of the lateral septum in anxiolysis. *Physiol. Behav.*, **53,** 1077–1083.

(Received August 11, 1995
Revised November 6, 1995
Accepted December 8, 1995)