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Modulation of 5-HT_{2A} receptor-mediated head-twitch behaviour in the rat by 5-HT_{2C} receptor agonists

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Abstract

The pharmacology of several commonly described 5-hydroxytryptamine (5-HT)$_{2C}$ receptor agonists was investigated in vivo and in vitro at rat 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. The 5-HT_{2C} receptor agonist, (*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine fumarate (Ro 60-0175), did not induce a significant head-twitch response when given alone, yet when administered to rats subsequent to an acute challenge with the selective 5-HT_{2C} receptor antagonist, 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbomyl] indoline (SB-242084), a robust head-twitch response was observed which was blocked by the selective 5-HT_{2A} receptor antagonists *R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl-ethyl)]-4-piperidine-methanol (MDL 100907) or ketanserin. The preferential 5-HT_{2C} receptor agonists Ro 60-0175, 6-chloro-2-[1-piperazinyl]-pyrazine HCl (MK-212), 1-(3-chlorophenyl)piperazine hydrochloride (*m*CPP), 1-(3-trifluoromethylphenyl)piperazine hydrochloride (TFMPP), and (*S*)-3-[(2,3-dihydro-5-methoxy-1*H*-inden-4-yl)oxy]-pyrollidine HCl (ORG-37684), the 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), the 5-HT_{2B} receptor agonist 1-[5-thienylmethoxy-1-1*H*-3-indoyl] propan-2-amine hydrochloride (BW-723C86), and nor-D-fenfluramine were administered to rats subsequent to an acute challenge of SB-242084. Under such conditions, each agonist, with the exception of BW-723C86, induced a dose-dependent increase in the incidence of head-twitches. The pharmacology of the same agonists was determined at cloned rat 5-HT_2 receptors using a fluorometric imaging plate reader (FLIPR). Both the in vivo and in vitro data suggest that for some ligands, previous reports have overestimated their in vivo selectivity for the 5-HT_{2C} receptor.

Keywords: Serotonin; 5-HT_{2C}; 5-HT_{2A}; Ro 60-0175; Nor-D-fenfluramine; *m*CPP; ORG-37684; BW-723C86; Head-twitch response; FLIPR

1. Introduction

The central actions of serotonin [5-hydroxytryptamine (5-HT)] are mediated through the activation of a number of different receptor subtypes. These subtypes have been organised into families according to various pharmacological and molecular criteria (Martin and Humphrey, 1994). One such family is the 5-HT_2 receptor family, which consists of the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor subtypes.

Both systemic and central administration of direct and indirect 5-HT receptor agonists have been reported to induce a characteristic head-twitch response in rodents (Fozard and Palfreyman, 1979; Colpaert and Janssen, 1983; Willins and Meltzer, 1997). Compounds that induce this head-twitch response include the hallucinogens 2,5-dimethoxy-4-iodoamphetamine (DOI) and 2,5-dimethoxy-4-bromoamphetamine (DOB), both of which exhibit high affinity for the 5-HT_{2A} receptor and act as agonists (Titeler et al., 1988; Pierce and Peroutka, 1989; Porter et al., 1999). Furthermore, DOI-induced head-twitches are blocked by antagonists with high affinity for the 5-HT_{2A} receptor such as ketanserin, ritanserin, mianserin, and *R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl-ethyl)]-4-piperidine-methanol (MDL 100907; Darmani et al., 1990; Pranzatelli, 1990; Schreiber et al., 1995).

5-HT_{2C} receptor activation has been linked to changes in motor behaviour (Kennett and Curzon, 1988a), increased anxiety (Kennett et al., 1989), decreased food intake (Kennett and Curzon, 1988b; Fone et al., 1998), and reduced body weight (Sargent et al., 1997; Vickers et al., 2000a). Accordingly, there are continued efforts to develop agonists and antagonists that are selective for the 5-HT_{2C} receptor subtype.

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Several preferential 5-HT_{2C} receptor agonists, for example, 1-(3-chlorophenyl)piperazine hydrochloride (*m*CPP) and 1-(3-trifluoromethylphenyl)piperazine hydrochloride (TFMPP), are described in the literature, though the selectivity of these ligands over other 5-HT_2 receptors is generally poor (Kennett, 1993). In contrast, the recently described 5-HT_{2C} receptor agonist, (*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine fumarate (Ro 60-0175), has high efficacy at the 5-HT_{2C} receptor and has 25-fold selectivity over other human 5-HT receptors, with the exception of the human 5-HT_{2B} receptor at which it also has high affinity and efficacy (Martin et al., 1998; Porter et al., 1999).

In apparent agreement with this in vitro profile at human receptors, Ro 60-0175 does not induce head-twitches in rats (Martin et al., 1998). Similarly, when administered systemically, *m*CPP and TFMPP do not cause a head-twitch response in rats (Schreiber et al., 1995); indeed, each compound dose-dependently antagonised twitches induced by DOI (Berendsen and Broekkamp, 1990; Schreiber et al., 1995) or carbidopa and 5-hydroxytryptophan (Kennett and Curzon, 1989). At least two hypotheses accommodate this finding. Firstly, a 5-HT_{2C} receptor agonist action may functionally inhibit 5-HT_{2A}-mediated responses such as the rat head-twitch (Berendsen and Broekkamp, 1990). Alternatively, the data are consistent with *m*CPP and TFMPP having a weak partial agonist or antagonist action at 5-HT_{2A} receptors (Kennett and Curzon, 1989; Schreiber et al., 1995), a hypothesis consistent with in vitro findings with these compounds (Conn and Sanders-bush, 1987; Britt et al., 1988; Porter et al., 1999).

If 5-HT_{2C} receptor activation subsequent to systemic drug administration inhibits the expression of DOI-induced head-twitches, such a hypothesis predicts that the head-twitch response would also be absent in animals treated systemically with a preferential 5-HT_{2C} receptor agonist such as Ro 60-0175, since, although the compound may exhibit marked agonist activity at 5-HT_{2A} receptors, the induction of head-twitches will be prevented through concurrent 5-HT_{2C} receptor activation. Accordingly, such experiments may provide a misleading perspective on the actions of 5-HT_{2C} receptor agonists at the closely related 5-HT_{2A} receptor in vivo. The present study directly assessed this hypothesis by administering Ro 60-0175 to rats pretreated with the selective 5-HT_{2C} receptor antagonist, 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbomyl] indoline (SB-242084) (Kennett et al., 1997b). In addition, using this approach, we assessed the ability of a number of preferential 5-HT_{2C} receptor agonists, including the 5,6-difluoro analogue of Ro 60-0175 (5,6-difluoroindolmethylethylamine), which has previously been claimed to exhibit 100-fold selectivity for 5-HT_{2C} receptors over 5-HT_{2A} receptors (Boes et al., 1997), to induce head-twitches in the rat. The 5-$HT_{2A/2C}$ receptor agonist DOI and the 5-HT_{2B} receptor agonist 1-[5-thienylmethoxy-1-1*H*-3-indoyl] propan-2-amine hydrochloride (BW-723C86) were also assessed. In a parallel study designed to complement the in vivo data generated, the effects of the agonists were characterised at recombinant rat 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (INI isoform) receptors. The current data were presented in part to the British Pharmacology Society (Vickers et al. 2000b).

2. Methods

2.1. Animals

All work reported in this manuscript was performed in accordance with Home Office regulations as detailed in the Animals (Scientific Procedures) Act 1986. Male Lister hooded rats (Charles River, Margate, UK), weighing between 200 and 250 g upon arrival at the laboratory, were group-housed in an air-conditioned room maintained under a 12 h light/dark cycle (lights on at 08:00 h). Ambient temperature was 21 ± 1°C. A red light was the sole source of illumination during the dark period. Except where stated, animals had continuous access to standard rodent diet (Bantin & Kingman UK, Hull) and tap water. Each animal was used once.

2.2. Procedure

On the morning of testing, animals were transferred to an experimental room. All agonists were administered 30 min prior to the test period. Where employed, SB-242084, MDL 100907, and ketanserin were given 10 min prior to agonist administration. Immediately prior to the test, animals were placed individually into clean, transparent, polycarbonate cages (42.5 × 26.6 × 18.5 cm; Techniplast UK). The floor of each cage was covered with a light sprinkling of sawdust such that animals could not bury their heads in the shavings. The number of head-twitches exhibited over the subsequent 20 min period was recorded by an observer blind to the drug treatment. Neither food nor water was available for the 20 min test session.

2.3. Molecular biology and cell culture

Rat 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor cDNAs were obtained from either whole rat cortex or rat stomach fundus (5-HT_{2B}) cloned and subcloned into pCDNA3.1 (accession numbers EMBL: X13971, X66842, and GB: M21410, respectively). CHO-K1 (ECACC) cells were stably transfected with each receptor transcript using the calcium phosphate method. Stably transfected cell lines were selected using G-418 and clonal cell lines developed by limit dilution. Clonal cell lines were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% heat inactivated dialysed foetal bovine serum (FBS), 1% penicillin–streptomycin, 1% L-glutamine, and 1% nonessential amino acids.

2.4. Assay preparation

Assay procedures were as described previously (Porter et al., 1999). Briefly, cells were plated at a density of

30,000 cells/well into black 96-well clear-bottomed plates the day before. Hank's balanced salt solution without phenol red containing 20 mM HEPES and 2.5 mM probenecid was prepared fresh on the day of assay and used as the assay buffer. Dye loading was performed in serum-free media containing a final concentration of 4 μM Fluo-3-AM (dissolved in DMSO and pluronic acid) and 20 mM HEPES and 2.5 mM probenecid for approximately 90 min at 37°C in a 5% CO_2 incubator at 95% humidity. The cells were then washed thoroughly on a Denley cell washer with the assay buffer to remove any unincorporated dye. Exactly 100 μl of assay buffer was left in each well.

2.5. Assay conditions

The dye-loaded plates were placed into the fluorometric imaging plate reader (FLIPR; Molecular Devices; Sunnyvale, CA) drawer and the laser intensity altered to obtain basal values of approximately 10,000 fluorescence units (ca. 0.4–0.7 W). Agonist additions (50 μl) were made 10 s after assay initiation by an integrated 96-head pipettor using black tips at a rate of 70 μl/s to ensure rapid equilibration. Fluorescence readings (recorded every second for the first 60 s and every 5 s for the next 30 s) were captured by a cooled CCD camera and integrated to an on-line PC. The maximum fluorescent signal obtained was recorded and was typically in the range of 10,000–20,000 fluorescent units and obtained within 10–15 s of drug addition.

2.6. Determination of agonist potencies and relative efficacies

Each 96-well plate contained four wells dedicated to a positive control defined as 10 μM 5-HT and four wells as a negative control defined as assay buffer alone. For pharmacological characterisation, all data were normalised to the positive control wells, which were expressed as 100% signal. Each agonist dose–response curve was constructed using a four-parameter logistic equation from GraphPad Prism as follows: $Y = \text{bottom} + (\text{top} - \text{bottom})/1 + 10^{(\log \text{EC50} - X)\text{nH}}$. The efficacy of the compound was determined from the Top value, which is the maximum value of the Y plateau. The concentration of agonist that produced a half-maximal response is represented by the EC_{50} value.

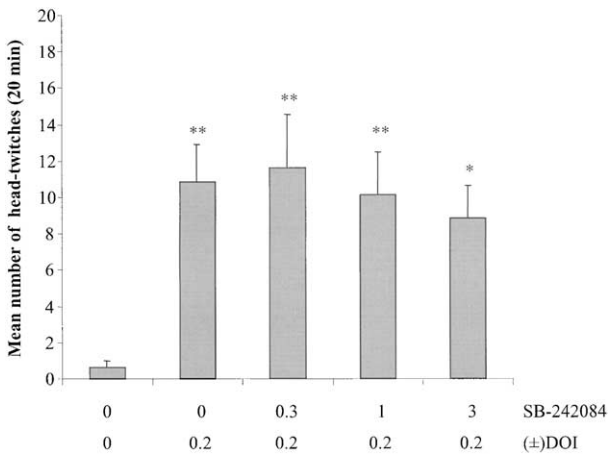


Fig. 1. Effect of the selective 5-HT_{2C} receptor antagonist SB-242084 (0.3–3 mg/kg) on (±)DOI-induced head-twitches (0.2 mg/kg) over a 20 min test session ($n = 6$). Results are treatment group means and vertical lines represent S.E.M. Significant differences from vehicle-treated animals are denoted by ** $P < .01$. Differences were assessed for significance by Dunnett's test subsequent to significant one-way ANOVA.

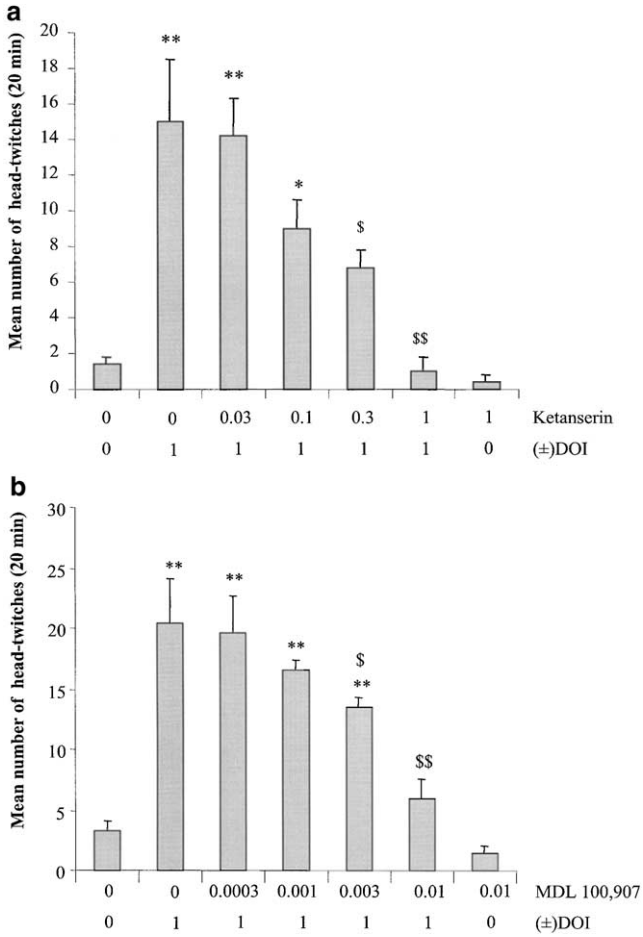


Fig. 2. (a) Effect of the 5-HT_{2A} receptor antagonist ketanserin (0.03–1 mg/kg) on (±)DOI-induced head-twitches (1 mg/kg) over a 20 min test session ($n = 5$). Results are treatment group means and vertical lines represent S.E.M. Significant differences from vehicle/vehicle-treated animals are denoted by * $P < .05$ and ** $P < .01$. Differences were assessed for significance by Dunnett's test subsequent to significant one-way ANOVA. Significant differences to vehicle/(±)DOI-treated animals are denoted by $^{\$}P < .05$ and $^{\$\$}P < .01$ (Tukey's HSD post hoc test). (b) Effect of the 5-HT_{2A} receptor antagonist MDL 100907 (0.0003–0.01 mg/kg) on (±)DOI-induced head-twitches (1 mg/kg) over a 20 min test session ($n = 5$). Results are treatment group means and vertical lines represent S.E.M. Significant differences from vehicle/vehicle-treated animals are denoted by * $P < .05$ and ** $P < .01$. Differences were assessed for significance by Dunnett's test subsequent to significant one-way ANOVA. Significant differences to vehicle/(±)DOI-treated animals are denoted by $^{\$}P < .05$ and $^{\$\$}P < .01$ (Tukey's HSD post hoc test).

2.7. Drugs

SB-242084 was administered intraperitoneally in the volume 2 ml/kg. MDL 100907 and ketanserin were administered subcutaneously in the volume 1 ml/kg. All agonists were administered subcutaneously in the volume 1 ml/kg. With one exception, all drugs were dissolved in saline. SB-242084 was dissolved by sonicating in PEG400 (20% of the final volume) and then adding 10% cyclodextrin with 25 mM citric acid to the required volume. Drops of 1.0 N NaOH (Sigma) were added until pH 6 was reached.

SB-242084, Ro 60-0175, 5,6-difluoroindolmethylethylamine, (*S*)-3-[(2,3-dihydro-5-methoxy-1*H*-inden-4-yl)oxy]-pyrollidine HCl (ORG-37684), BW-723C86, MDL 100907, and nor-D-fenfluramine were all synthesised in the Department of Chemistry, Vernalis Research. 6-chloro-2-[1-piperazinyl]-pyrazine HCl (MK-212), *m*CPP, and TFMPP were purchased from Tocris Cookson (Bristol, UK). Ketanserin, (±)-DOI, and (±)-DOB were purchased from Sigma–Aldrich (Poole, Dorset, UK). All drug doses are expressed as free base.

2.8. Statistical analysis

All behavioural data are presented as mean ± S.E.M. Head-twitch data were analysed by one-way ANOVA where drug treatment was a between-subjects factor. Significant differences from vehicle-treated animals were assessed using Dunnett's test. Correlations were assessed using the Pearson product–moment correlation coefficient (*r*). Additional post hoc comparisons were made using Tukey's HSD Test. All data analysis was performed using Statistica V5 (Statsoft).

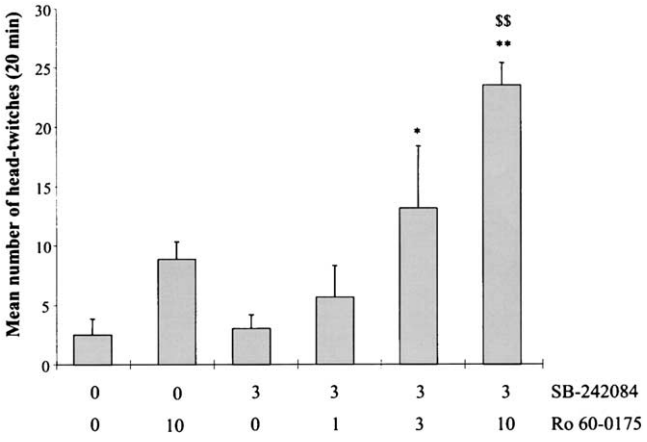


Fig. 3. The number of rat head-twitches subsequent to administration of the preferential 5-HT_{2C} agonist Ro 60-0175 (1–10 mg/kg) in the presence of a selective dose of the 5-HT_{2C} receptor antagonist SB-242084 (3 mg/kg). Results are treatment group means ($n=6$) and vertical lines represent S.E.M. Significant differences from vehicle/vehicle-treated animals are denoted by $*P<.05$ and $**P<.01$. Differences were assessed for significance by Dunnett's test subsequent to significant one-way ANOVA. Significant differences to vehicle/Ro 60-0175-treated animals are denoted by $^{\$\$}P<.01$ (Tukey's HSD post hoc test).

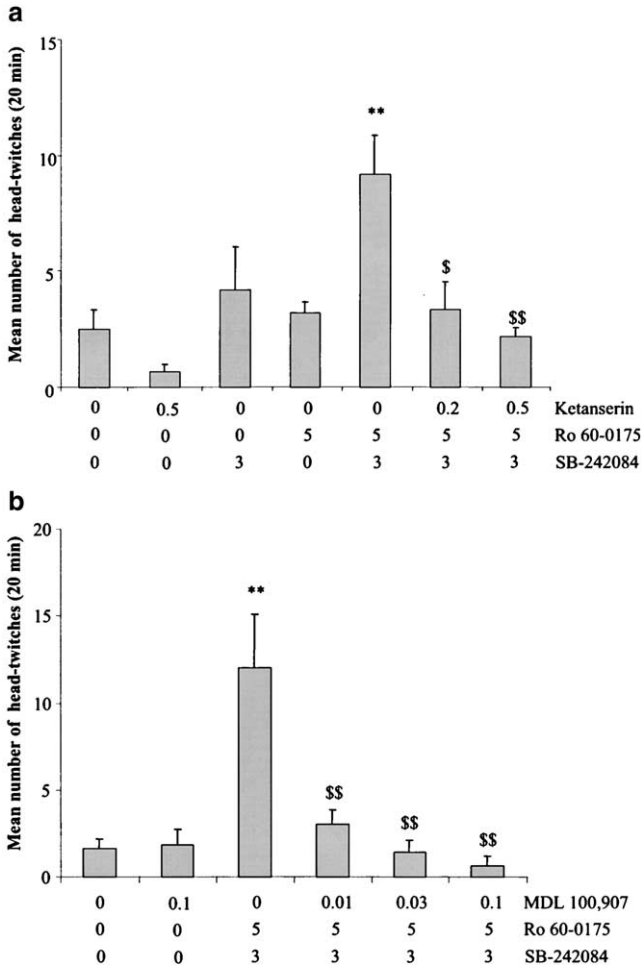


Fig. 4. (a) Effect of the 5-HT_{2A} receptor antagonist ketanserin (0.2–0.5 mg/kg) on the head-twitch response observed after administration of Ro 60-0175 (5 mg/kg) in the presence of SB-242084 (3 mg/kg; $n=5$). Results are treatment group means and vertical lines represent S.E.M. Significant differences from vehicle/vehicle/vehicle-treated animals are denoted by ** $P<.01$. Differences were assessed for significance by Dunnett's test subsequent to significant one-way ANOVA. Significant differences to vehicle/Ro 60-0175/SB-242084-treated animals are denoted by $^{\$}P<.05$ and $^{\$\$}P<.01$ (Tukey's HSD post hoc test). (b) Effect of the 5-HT_{2A} receptor antagonist MDL 100907 (0.01–0.1 mg/kg) on the head-twitch response observed after administration of Ro 60-0175 (5 mg/kg) in the presence of SB-242084 (3 mg/kg; $n=5$). Results are treatment group means and vertical lines represent S.E.M. Significant differences from vehicle/vehicle/vehicle-treated animals are denoted by ** $P<.01$. Differences were assessed for significance by Dunnett's test subsequent to significant one-way ANOVA. Significant differences to vehicle/Ro 60-0175/SB-242084-treated animals are denoted by $^{\$\$}P<.01$ (Tukey's HSD post hoc test).

3. Results

3.1. Experiment 1: effect of SB-242084, MDL 100907, and ketanserin on the (±)DOI-induced head-twitch response

The dose and route (subcutaneous) of administration of DOI were selected on the basis of pilot data obtained at Vernalis (data not shown). DOI (0.2 mg/kg) administration led to a statistically significant head-twitch response. Pre-

Table 1
The induction of head-twitches by a number of 5-HT_2 receptor agonists in the presence of SB-242084 (3 mg/kg)

	Dose (mg/kg)							
	0	0.1	0.3	1	3	10	20	30
TFMPP	1.16 (0.60)	–	–	1.83 (0.60)	2.50 (0.92)	1.16 (0.31)	–	–
*m*CPP	1.82 (0.82)	–	–	2.83 (0.60)	4.83 (0.60)*	5.50 (1.84)*	1.33 (0.33)	0.50 (0.34)
(±) DOI	0.33 (0.21)	2.66 (0.66)	5.16 (0.60)*	23.50 (2.07)**	–	–	–	–
MK-212	5.00 (1.13)	–	–	8.00 (1.48)	20.25 (4.47)**	14.88 (1.20)**	–	–
Ro 60-0175	4.30 (1.68)	2.83 (0.65)	8.67 (3.08)	7.00 (1.06)	17.83 (4.47)**	21.50 (2.40)**	–	–
5,6-difluorindol methylethylamine	3.71 (1.30)	–	5.12 (1.71)	8.38 (1.94)	14.88 (2.66)**	20.50 (1.32)**	–	–
ORG-37684	2.25 (0.49)	–	–	8.50 (2.70)	13.88 (2.39)**	15.88 (3.14)**	–	–
BW-723C86	0.33 (0.33)	–	–	0.83 (0.83)	0.50 (0.34)	0 (0)	–	–
nor-D-fenfluramine	4.25 (1.57)	3.14 (0.98)	6.63 (1.39)	12.88 (2.53)*	10.50 (3.35)	–	–	–

Data are mean (±S.E.M.) number of head-twitches observed over a 20 min period ($n = 6–8$). All animals received the 5-HT_{2C} receptor antagonist SB-242084 (3 mg/kg). Dunnett's test from vehicle-treated group following significant ANOVA.

* $P < .05$.

** $P < .01$.

treatment with SB-242084 (0.3–3 mg/kg) had no significant effect on the head-twitch response induced by a low, submaximal dose (0.2 mg/kg) of DOI (Fig. 1). There was a trend whereby the top dose of SB-242084 (3 mg/kg) tended to inhibit DOI-induced head-twitches, suggesting that doses of SB-242084 in excess of this may begin to block 5-HT_{2A} receptors in vivo. In contrast to SB-242084, ketanserin (Fig. 2a) and MDL 100907 (Fig. 2b) dose-dependently blocked head-twitches observed subsequent to a higher DOI dose (1 mg/kg). The minimum effective dose in blocking DOI-induced head-twitches was 0.01 mg/kg for MDL 100907 and 0.3 mg/kg for ketanserin.

3.2. Experiment 2: effect of SB-242084 on the head-twitch response subsequent to Ro 60-0175 administration

Neither SB-242084 (3 mg/kg) nor Ro 60-0175 (10 mg/kg) significantly induced head-twitches when given alone. However, where SB-242084 treatment was succeeded by Ro 60-0175 (1–10 mg/kg) administration, a dose-dependent increase in the incidence of head-twitches was observed [main effect of treatment from ANOVA, $F(5,30) = 15.11$, $P < .01$; Fig. 3]. Although not quantified, it was observed that in the presence of SB-242084, Ro 60-0175 also induced skin jerks (paraspinal muscle contractions; Pranzatelli, 1990). Ro 60-0175-induced head-twitches in the presence of SB-242084 were abolished by the 5-HT_{2A} receptor antagonists ketanserin and MDL 100907 (Fig. 4a and b).

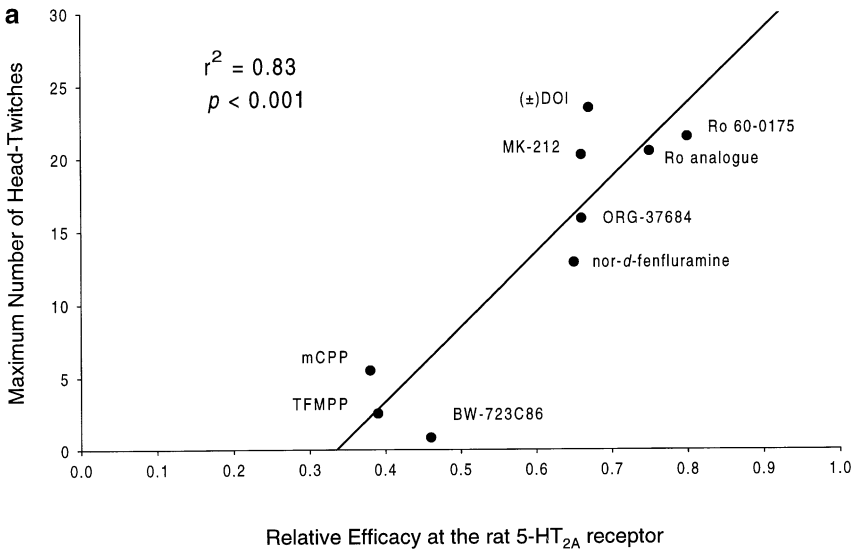
3.3. Experiment 3: effect of SB-242084 on the head-twitch response subsequent to a range of 5-HT_2 receptor agonists

With the exception of BW-723C86 and TFMPP, all agonists led to a significant and dose-dependent increase in the number of head-twitches exhibited (Table 1). The minimum effective dose required to induce a significant number of head-twitches and the maximum number of head-twitches observed differed for the agonists (Table 1). The rank order of the maximum number of head-twitches observed in the presence of SB-242084 was DOI > Ro 60-

Table 2
Functional characterisation of compounds at cloned rat 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors expressed in CHO-K1 cells

	5-HT_{2A}		5-HT_{2B}		5-HT_{2C}	
	pEC_{50}	Rel. Eff.	pEC_{50}	Rel. Eff.	pEC_{50}	Rel. Eff.
5-HT	7.51 ± 0.08	0.96 ± 0.01	9.00 ± 0.06	0.96 ± 0.02	8.64 ± 0.11	0.96 ± 0.02
5-CT	5.80 ± 0.08	0.79 ± 0.05	8.28 ± 0.06	1.02 ± 0.03	6.86 ± 0.11	0.96 ± 0.03
α-methyl-5-HT	7.29 ± 0.02	0.96 ± 0.02	8.59 ± 0.12	0.85 ± 0.02	8.19 ± 0.07	0.98 ± 0.01
*m*CPP	7.12 ± 0.10	0.38 ± 0.04	7.55 ± 0.10	0.38 ± 0.05	7.58 ± 0.09	0.85 ± 0.01
TFMPP	6.96 ± 0.16	0.39 ± 0.03	7.49 ± 0.12	0.48 ± 0.07	7.33 ± 0.15	0.80 ± 0.03
Ro 60-0175	6.78 ± 0.11	0.80 ± 0.03	8.60 ± 0.13	0.85 ± 0.04	7.92 ± 0.11	0.90 ± 0.01
ORG-37684	7.91 ± 0.13	0.66 ± 0.03	8.16 ± 0.11	0.35 ± 0.06	8.37 ± 0.07	0.76 ± 0.02
5,6-difluoroindolmethylethylamine	6.67 ± 0.16	0.75 ± 0.05	8.67 ± 0.24	0.85 ± 0.04	7.61 ± 0.27	0.94 ± 0.01
MK-212	5.89 ± 0.10	0.66 ± 0.07	6.89 ± 0.13	0.83 ± 0.07	6.95 ± 0.10	0.90 ± 0.01
nor-D-fenfluramine	6.49 ± 0.13	0.65 ± 0.03	7.74 ± 0.22	0.84 ± 0.03	7.05 ± 0.17	0.91 ± 0.03
BW-723C86	6.77 ± 0.10	0.46 ± 0.01	9.42 ± 0.21	0.88 ± 0.03	7.01 ± 0.09	0.77 ± 0.02
(±)DOI	8.86 ± 0.27	0.67 ± 0.06	8.67 ± 0.26	0.75 ± 0.06	7.88 ± 0.21	0.69 ± 0.06
(±)DOB	8.61 ± 0.21	0.75 ± 0.05	8.57 ± 0.32	0.81 ± 0.05	7.89 ± 0.23	0.79 ± 0.06

pEC_{50} values are the mean ± S.E.M. of four independent experiments. All compounds were examined on the same day. Relative efficacy (Rel. Eff.) values are the corresponding fraction of the response elicited by the compounds compared to the 10 μM 5-HT positive control.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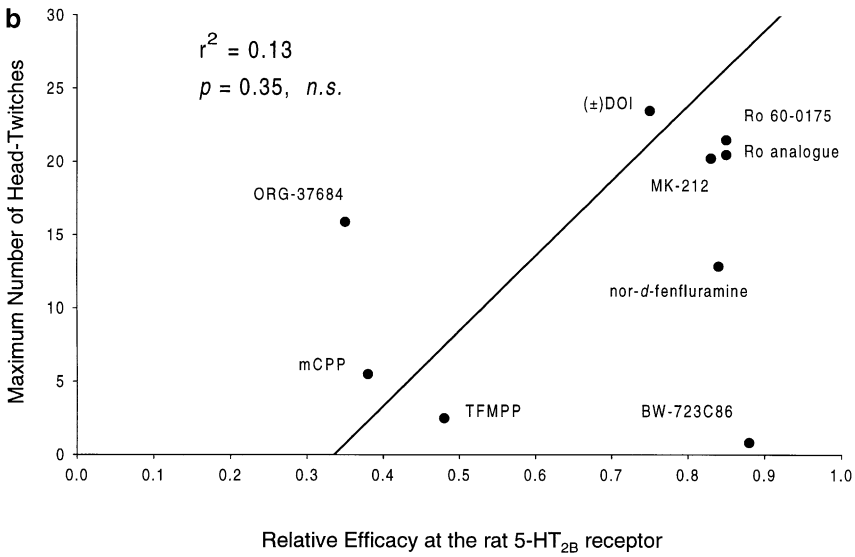
BW-723C86
Maximum Number of Head-Twitches
Relative Efficacy at the rat 5-HT2A receptor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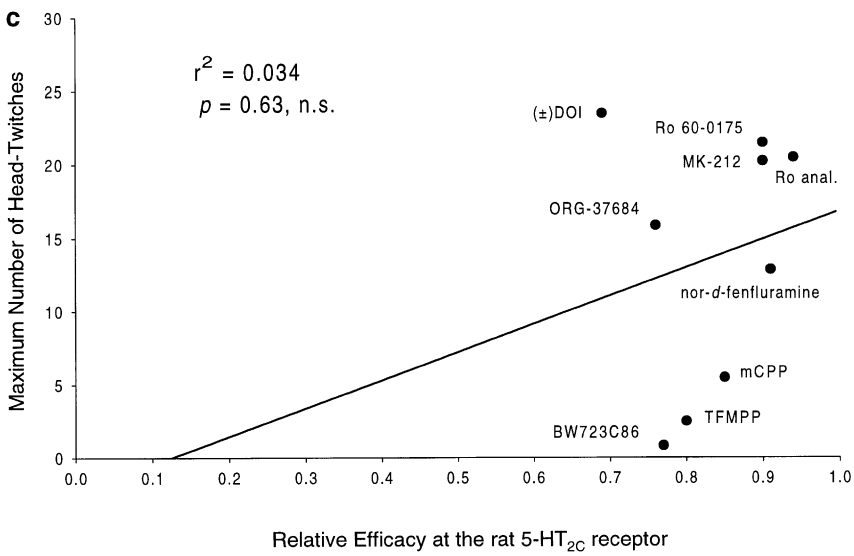
nor-d-fenfluramine
mCPP
TFMPP
BW-723C86
Maximum Number of Head-Twitches
Relative Efficacy at the rat 5-HT2B receptor


c
r² = 0.034
p = 0.63, n.s.
(±)DOI
Ro 60-0175
MK-212
Ro anal.
ORG-37684
nor-d-fenfluramine
mCPP
TFMPP
BW723C86
Maximum Number of Head-Twitches
Relative Efficacy at the rat 5-HT2C receptor

0175 > 5,6-difluoroindolmethylethylamine > MK-212 > ORG-37684 > nor-D-fenfluramine > *m*CPP > TFMPP > BW-723C86.

3.4. Experiment 4: functional characterisation of agonists at cloned rat 5-HT$_{2a}$, 5-HT$_{2b}$, and 5-HT$_{2C}$ receptors: in vitro and in vivo correlations

The agonist potencies and relative efficacies are detailed in Table 2. 5-HT, 5-CT, and α-methyl-5-HT were included for comparison. The commonly described 5-HT$_{2C}$ receptor agonists were all found to be high-efficacy agonists, which exhibited reasonable potency for the rat 5-HT$_{2C}$ receptor. The rank order of potency at the 5-HT$_{2C}$ receptor was 5-HT > ORG-37684 > Ro 60-0175 > DOI > 5,6-difluouroindolmethylethylamine > *m*CPP > TFMPP > nor-D-fenfluramine > BW-723C86 > MK-212. The rank order of relative efficacy at this receptor was 5-HT > 5,6-difluouroindolmethylethylamine > nor-D-fenfluramine > Ro 60-0175/MK-212 > *m*CPP > TFMPP > BW-723C86 > ORG-37684 > DOI (Table 2). The differences in potency or efficacy between some compounds at the 5-HT$_{2C}$ receptor, such as the potencies of Ro 60-0175 and DOI or the efficacies of BW-723C86 and ORG-37684, are very small and may be negligible (Table 2).

In addition, all the agonists tested were potent at the rat 5-HT$_{2B}$ receptor; indeed, Ro 60-0175 was found to be a high-efficacy agonist almost 5-fold selective for this receptor compared to the 5-HT$_{2C}$ receptor. Under the same conditions, the previously described 5-HT$_{2B}$ receptor agonist, BW-723C86, was over 200-fold selective for this receptor subtype over the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes. The rank order of potency at the rat 5-HT$_{2B}$ receptor was BW-723C86 > 5-HT > DOI/5,6-difluoroindolmethylethylamine > Ro 60-0175 > ORG-37684 > nor-D-fenfluramine > *m*CPP > TFMPP > MK-212. The rank order of relative efficacy at this receptor was 5-HT > BW-723C86 > Ro 60-0175/5,6-difluoroindolmethylethylamine > nor-D-fenfluramine > MK-212 > DOI > TFMPP > *m*CPP > ORG-37684 (Table 2). As observed at the 5-HT$_{2C}$ receptor, the differences in potency or efficacy between some compounds at the 5-HT$_{2B}$ receptor, such as the potencies of Ro 60-0175 and 5,6-difluoroindolmethylethylamine or the efficacies of nor-D-fenfluramine and MK-212 for example, are very small and may be negligible (Table 2).

Consistent with the behavioural data, the compounds exhibited differential potencies and efficacies at the rat 5-HT$_{2A}$ receptor. Thus, the rank order of potency at the 5-HT$_{2A}$ receptor was DOI > ORG-37684 > 5-HT > *m*CPP > TFMPP > Ro 60-0175 > BW-723C86 > 5,6-difluoroindolmethylethylamine > nor-D-fenfluramine > MK-212. DOI and DOB were most potent at the 5-HT$_{2A}$ receptor, though they were also found to be potent and high-efficacy 5-HT$_{2B}$ receptor agonists. These compounds exhibited approximately 10- and 5-fold selectivity, respectively, for the rat 5-HT$_{2A}$ receptor over the rat 5-HT$_{2C}$ receptor. None of the compounds tested had greater than 14-fold selectivity (Ro 60-0175) for the 5-HT$_{2C}$ over the 5-HT$_{2A}$ receptor and this selectivity was equivalent to that observed with 5-HT. The rank order of relative efficacy at the 5-HT$_{2A}$ receptor was 5-HT > Ro 60-0175 > 5,6-difluoroindolmethylethylamine > DOI > ORG-37684/MK-212 > nor-D-fenfluramine > BW-723C86 > TFMPP > *m*CPP (Table 2). As observed at the 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors, the differences in potency or efficacy between some compounds at the 5-HT$_{2A}$ receptor, such as the potencies of Ro 60-0175 and BW-723C86 or the efficacies of nor-D-fenfluramine and MK-212, for example, are very small and may be negligible (Table 2).

The maximum number of head-twitches observed after administration of each of the test compounds was obtained from Table 1. Plots of the maximum number of head-twitches vs. relative efficacy at the 5-HT$_{2A}$ receptor showed a strong correlation between the variables where the maximum number of head-twitches observed was positively linked to the efficacy of the test compound at the 5-HT$_{2A}$ receptor (r^2=.83, P<.001). In contrast, similar plots for the relative efficacy of compounds at 5-HT$_{2B}$ (r^2=.13, P=.34) or 5-HT$_{2C}$ (r^2=.034, P=.64) receptor showed no correlation with the behavioural data (Fig. 5a–c).

4. Discussion

The 5-HT$_{2C}$ receptor agonist, Ro 60-0175, when administered alone did not lead to a significant head-twitch response in rats; however, in the presence of a high dose of the 5-HT$_{2C}$ receptor antagonist, SB-242084, which did not, on its own, affect DOI-induced head-twitches, Ro 60-0175 induced a dose-dependent increase in the number of head-twitches exhibited. This head-twitch response observed subsequent to Ro 60-0175 administration was attributable to 5-HT$_{2A}$ receptor activation since it could be blocked by low, selective, doses of the 5-HT$_{2A}$ receptor antagonists ketanserin or MDL 100907. These data suggest that, in the rat, 5-HT$_{2C}$ receptor activation inhibits the expression of 5-HT$_{2A}$ recep-

Fig. 5. (a) Scatterplot, with regression line of the maximum number of head-twitches (obtained from Table 1) as a function of the relative efficacy at the rat 5-HT$_{2A}$ receptor (obtained from Table 2). Each point represents a compound. Ro analogue is the close structural analogue of Ro 60-0175, 5,6-difluoroindolmethylethylamine. Pearson product–moment correlation r^2=.83, P<.001. (b) Scatterplot, with regression line, of the maximum number of head-twitches (obtained from Table 1) as a function of the relative efficacy at the rat 5-HT$_{2B}$ receptor (obtained from Table 2). Each point represents a compound. Ro analogue is the close structural analogue of Ro 60-0175, 5,6-difluoroindolmethylethylamine. Pearson product–moment correlation r^2=.13, P=.34, n.s. (c) Scatterplot, with regression line, of the maximum number of head-twitches (obtained from Table 1) as a function of the relative efficacy at the rat 5-HT$_{2C}$ receptor (obtained from Table 2). Each point represents a compound. Ro analogue is the close structural analogue of Ro 60-0175, 5,6-difluoroindolmethylethylamine. Pearson product–moment correlation r^2=.034, P=.64, n.s.

tor-mediated head-twitches and perhaps, therefore, other behaviours exhibited after 5-HT_{2A} receptor activation. Accordingly, such data explain why the head-twitch response is not observed after Ro 60-0175 administration (Martin et al., 1998) and, in addition, provide a new model for accurately determining in vivo 5-HT_{2A} receptor agonist properties of compounds that also possess agonist activity at 5-HT_{2C} receptors.

The present data are also consistent with initial reports demonstrating that some 5-HT_{2C} receptor agonists inhibit DOI-induced head-twitches in rats (Berendsen and Broekkamp, 1990; Schreiber et al., 1995). Interestingly, both of these studies utilised the 5-HT_{2C} receptor agonists *m*CPP and TFMPP, which, in the present study, were found to be low-efficacy 5-HT_{2A} receptor partial agonists and which may be expected to antagonise the response induced by a 5-HT_{2A} receptor agonist. However, the study of Berendsen and Broekkamp (1990) also demonstrated the inhibition of DOI-induced head-twitches with MK-212, which, in the present study, though not in others (Willins and Meltzer, 1997), exhibited an in vivo profile consistent with it possessing reasonable potency and efficacy at the 5-HT_{2A} receptor. With the use of 5-HT_2 receptor agonists with high efficacy at the rat 5-HT_{2A} receptor (Ro 60-0175 for example), the present study provides strong evidence that 5-HT_{2C} receptor activation inhibits the expression of the 5-HT_{2A} receptor-mediated head-twitch response in the rat.

FLIPR allows G-protein-coupled receptor-induced calcium responses to be accurately and reliably quantified (Porter et al., 1999). In the present study, FLIPR has been used to measure the intracellular calcium response subsequent to the administration of a range of doses of 5-HT_2 receptor agonists to CHO-K1 cells expressing either rat 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors. The response obtained in assays that measure the functional consequences of receptor activation is dependent upon receptor expression level (Lucaites et al., 1996). The cell lines used were selected on the basis that they expressed the receptors of interest at relatively low levels (148, 528, and 197 fmol/mg at the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, respectively) and best fitted the limited data reported from studies with physiological tissue.

In the present functional assay, the 5-HT_{2B} receptor agonist, BW-723C86, was found to be 200-fold selective for the 5-HT_{2B} receptor where it was a high-efficacy agonist. Furthermore, in agreement with other studies (Titeler et al., 1988; Marek and Aghajanian, 1996), the hallucinogens DOI and DOB were found to be most potent at the rat 5-HT_{2A} receptor. Such findings with a number of standards endorse the methods used to characterise the current selection of ligands in the present functional assay.

In contrast to Ro 60-0175 treatment (Martin et al., 1998), administration of the hallucinogens DOI and DOB leads to a robust head-twitch response in rats in the absence of SB-242084 (Pranzatelli, 1990; Schreiber et al., 1995). This finding was replicated in the present study where it was observed that DOI and DOB were the only compounds to be more potent at the 5-HT_{2A} receptor than at the 5-HT_{2C} receptor, though both were more efficacious at the 5-HT_{2C} receptor. Accordingly, the extent of 5-HT_{2C} receptor agonist inhibition of 5-HT_{2A} receptor-mediated head-twitches appears to be dependent upon the potency of the compound at both the 5-HT_{2A} and 5-HT_{2C} receptors. Specifically, preferential 5-HT_{2A} receptor agonists would be expected to induce a head-twitch response.

The head-twitch response characterised with Ro 60-0175 in the presence of SB-242084 was also evident with a range of 5-HT_{2C} receptor agonists, including ORG-37684, MK-212, and *m*CPP. The relative efficacy obtained for each test compound from our experiments using FLIPR is defined as the maximal response (rise in intracellular calcium) expressed as a fraction of that of 10 μM 5-HT. Accordingly, the maximum number of head-twitches observed may be a measure of the efficacy of the compound in vivo. Indeed, it was found that there was a significant positive correlation between the relative efficacy of a compound at the rat 5-HT_{2A} receptor and the maximum head-twitch score in vivo. Thus, animals treated with high-efficacy 5-HT_{2A} receptor agonists such as Ro 60-0175 showed, in the presence of SB-242084, a greater mean maximum number of head-twitches than low-efficacy partial agonists such as *m*CPP and TFMPP. No such correlation was observed at the 5-HT_{2B} and 5-HT_{2C} receptors, however. Not only does this finding provide additional evidence for the role of the 5-HT_{2A} receptor in mediating the head-twitch response but it also demonstrates that the in vitro and in vivo approaches used complemented one another to a high degree.

For some agonists tested in the presence of SB-242084, an inverted U-shaped dose–response curve was seen. Such a profile was evident for MK-212, nor-D-fenfluramine, and to a lesser extent, *m*CPP. One explanation may be that as the dose of the agonist was increased, the effects of the compound at the 5-HT_{2C} receptor may have surmounted the antagonism obtained with the dose of SB-242084 employed. Accordingly, as 5-HT_{2C} receptor activation inhibits the head-twitch response, a reduced incidence of head-twitches is observed at high-agonist doses. If true, such a phenomenon would mean that the present paradigm might underestimate the maximum number of head-twitches obtained for such compounds. Solving this problem is complicated by the finding that the dose of SB-242084 would need to be increased, yet the dose chosen was selected on the basis that a higher dose may block 5-HT_{2A} receptors in vivo. An alternative hypothesis, however, is that at higher doses, such compounds exhibit activity at other receptors. Candidate receptors may include the 5-HT_{1A} receptor, for which *m*CPP has affinity (Kennett, 1993), since compounds selective for this receptor have been reported to modulate the incidence of DOI-induced head-twitches (Berendsen and Broekkamp, 1990; Darmani et al., 1990; Schreiber et al., 1995; Willins and Meltzer, 1997). Whatever the explanation, the highly significant

correlation seen when the maximum head-twitch score (regardless of dose) was plotted against relative efficacy of the compound at the rat 5-HT_{2A} receptor suggests that the current paradigm enabled a good estimate of the "true" maximum number of head-twitches to be determined.

The present behavioural and functional studies suggest that *m*CPP is a low-efficacy agonist at the 5-HT_{2A} receptor. Such findings are consistent with other reports reporting that *m*CPP and TFMPP may behave as antagonists at rat 5-HT_{2A} receptors (Conn and Sanders-bush, 1987; Schreiber et al., 1995). Our finding is also consistent with the report that central administration of *m*CPP into the medial prefrontal cortex induces a head-twitch response that is abolished by pretreatment with 5-HT_{2A} receptor antagonists (Willins and Meltzer, 1997). Head-twitches may be present when *m*CPP is administered centrally into specific neural substrates rather than when given systemically since, upon such central infusion, the 5-HT_{2C} receptor population that inhibits 5-HT_{2A} receptor-mediated behaviours may not be activated.

In addition to having poor selectivity for the rat 5-HT_{2C} receptor over the rat 5-HT_{2A} receptor, both Ro 60-0175 and its homologue 5,6-difluouroindolmethylethylamine were found to be most potent at rat 5-HT_{2B} receptors where they exhibited high efficacy; indeed, the compounds were found to be 5- and 11-fold selective, respectively, for this receptor over the 5-HT_{2C} receptor. These data are similar to the findings of a study characterising these ligands at human 5-HT_2 receptors (Porter et al., 1999) and to a study showing that Ro 60-0175 potently stimulates contraction of the rat stomach fundus, a model of 5-HT_{2B} receptor activation, with a relative efficacy of 70% (Martin et al., 1998). Since the 5-HT_{2B} receptor agonist, BW-723C86, is found to be anxiolytic (Kennett et al., 1998) and increases feeding under conditions of low baseline intake (Kennett et al., 1997a), it would be interesting to assess whether low doses of Ro 60-0175 exhibit a similar profile in rats.

In comparing the human receptor data of Porter et al. (1999), which were generated using almost identical assay conditions to those used in the present study, to the current rat receptor data, there seem to be no major species differences for the compounds at rat and human 5-HT_2 receptors when assessed under similar conditions. Ro 60-0175 was slightly less potent at rat 5-HT_{2B} and 5-HT_{2C} receptors though more potent and efficacious at the rat 5-HT_{2A} receptor compared to the human clones. Accordingly, the compound may possess greater selectivity over 5-HT_{2A} receptors in man. *m*CPP and TFMPP showed little selectivity for either the rat or the human 5-HT_{2C} receptor though, in contrast to the findings at human receptors (Porter et al., 1999), both compounds exhibited markedly higher efficacy for this receptor than either the 5-HT_{2A} or 5-HT_{2B} receptors; indeed, in the rat, the efficacy of *m*CPP was only slightly less than that of Ro 60-0175 at the 5-HT_{2C} receptor. Such data suggest that, drug-safety issues aside, *m*CPP may not be an ideal drug choice for probing the role of the 5-HT_{2C} receptor in humans.

Nor-D-fenfluramine, the major metabolite of the clinically effective antiobesity agent D-fenfluramine, was found to exhibit moderate potency at rat 5-HT_{2B} and 5-HT_{2C} receptors where it was a high-efficacy agonist. The modest affinity observed with nor-D-fenfluramine at 5-HT_{2C} receptors is in agreement with similar in vitro studies using rat, mouse, and guinea pig brain tissue (Mennini et al., 1991) or cloned human receptors (Porter et al., 1999). In addition, the data are in agreement with in vivo studies in the mouse (Vickers et al., 1999), rat (Neill and Cooper, 1989), and man (Goodall et al., 1993), suggesting that D-fenfluramine-induced hypophagia is mediated, at least in part, through activation of the 5-HT_{2C} receptor. Although, nor-D-fenfluramine possessed reduced potency and efficacy at the 5-HT_{2A} receptor, in the present study, this compound was found to induce head-twitches in rats pretreated with SB-242084. Accordingly, it is possible that when blocking the effects of D-fenfluramine with the 5-$HT_{2A/2C}$ receptor antagonist ritanserin in human volunteers (Goodall et al., 1993), some of the effects observed may be differentially mediated through either the 5-HT_{2A} or 5-HT_{2C} receptors.

In conclusion, the current data demonstrate that 5-HT_{2C} receptor activation inhibits the expression of 5-HT_{2A} receptor-mediated head-twitches in the rat. Furthermore, the data provide a novel paradigm in the rat for assessing the agonist activity of preferential 5-HT_{2C} receptor agonists at 5-HT_{2A} receptors. Finally, in the present study, Ro 60-0175 was found to be a highly potent and efficacious 5-HT_{2B} receptor agonist, which, in addition, possessed marked agonist activity at 5-HT_{2A} receptors both in vivo and in vitro. Accordingly, this may compromise the utility of Ro 60-0175 in probing the role of the 5-HT_{2C} receptor in vivo.

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