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European Journal of Pharmacology 388 (2000) 57–67

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The effects of antipsychotics with 5-HT_{2C} receptor affinity in behavioral assays selective for 5-HT_{2C} receptor antagonist properties of compounds

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Received 13 September 1999; received in revised form 23 November 1999; accepted 30 November 1999

Abstract

Many antipsychotics have marked antagonist effects at 5-hydroxytryptamine (5-HT_{2C}) receptors in vitro, which, however, have been difficult to show in behavioral assays. Here, we used two assays — hypolocomotion and hypophagia induced by the 5-HT_{2C} receptor agonist 1-(3-chlorophenyl)piperazine (mCPP) — to try to characterize the 5-HT_{2C} receptor antagonist properties of antipsychotics in vivo. Clozapine, olanzapine, pipamperone, and *trans*-5-chloro-2-methyl-2,3,3*a*,12*b*-tetrahydro-1*H*-dibenz-[2,3:6,7]oxepino[4,5-*C*] pyrrolidino maleate (ORG 5222), modestly, but significantly, attenuated mCPP (10 mg/kg)-induced hypolocomotion. In contrast, risperidone and loxapine were inactive. The putative antipsychotic ORG 5222 significantly attenuated mCPP (5 mg/kg)-induced hypophagia, whereas the other antipsychotics were inactive. Selective antagonists at dopamine D_2-like receptors, α_1-adrenoceptors, α_2-adrenoceptors, or muscarinic receptors were not able to antagonize the effects of mCPP in either assay. The results suggest that mCPP-induced hypolocomotion can be used to characterize the 5-HT_{2C} receptor antagonist properties of antipsychotics, whereas mCPP-induced hypophagia appeared to be sensitive only to compounds highly selective for 5-HT_{2C} receptors. Together, these assays may help to characterize functional, in vivo, 5-HT_{2C} receptor antagonist properties of antipsychotics.

Keywords: Antipsychotic; Neuroleptic; 5-HT (5-hydroxytryptamine), serotonin; 5-HT_{2C} receptor; mCPP-(3-chlorophenyl)piperazine; Behavior; Hypolocomotion; Hypophagia; Locomotor activity; Food intake

1. Introduction

It has been suggested that 5-hydroxytryptamine (5-HT_{2C}) receptors play a role in psychiatric disorders such as anxiety and depression, as well as in sleep and feeding disorders (cf. Kennett, 1993). With respect to schizophrenia, some antipsychotic drugs have a high affinity for these receptors (Canton et al., 1990; Roth et al., 1992), and this could play a role in their effects on symptoms that often co-exist in schizophrenia, such as anxiety, depression and cognitive deficits (cf. Kennett, 1993). Furthermore, 5-HT_{2C} receptor antagonism may play a role in the adverse effects of particular antipsychotics, such as extrapyramidal side-effects (Reavill et al., 1999) and weight gain (Curzon, 1992). Nevertheless, although it seems likely that 5-HT_{2C} receptor antagonist properties play a role in the clinical effects of particular antipsychotics, clear evidence is lacking. And, in preclinical behavioral models, it has been proven difficult to demonstrate unambiguously 5-HT_{2C} receptor antagonist properties of antipsychotics, whereas a better understanding of the in vivo behavioral effects of antipsychotics mediated by 5-HT_{2C} receptors may help to determine the importance of these receptors in their clinical effects.

Various behavioral assays to detect 5-HT_{2C} receptor antagonist properties of compounds have been described (for review, see Koek et al., 1992). It has been proven difficult, however, to demonstrate effects of antipsychotics with 5-HT_{2C} receptor affinity in such assays. For example, clozapine did not antagonize the discriminative stimulus effects of the 5-HT_{2C} receptor agonist 1-(3-chlorophenyl)piperazine (mCPP), even when tested up to doses that strongly decreased rates of responding (Fiorella et al., 1996; Gommans et al., 1998). Further, clozapine and zotepine did not antagonize mCPP-induced hypolocomo-

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0014-2999/00/$ - see front matter
PII: S0014-2999(99)00859-6

tion, even though a slight attenuation was noted for clozapine (Czyrak et al., 1994). In contrast, clozapine fully blocked penile erection induced by mCPP (Protais et al., 1995), whereas the putative antipsychotic *trans*-5-chloro-2-methyl-2,3,3*a*,12*b*-tetrahydro-1*H*-dibenz-[2,3:6,7]oxepino[4,5-*C*] pyrrolidino maleate (ORG 5222), which is a potent 5-HT_{2C} receptor antagonist in vitro (Kuoppamäki et al., 1995), blocked this sign induced by the 5-HT_2 receptor agonist 6-methyl-4-(1-piperazinyl)-furo[3,2-*c*]pyridine maleate (ORG 10155; Broekkamp et al., 1990). Although the latter findings suggest that penile erection induced by 5-HT_{2C} receptor agonists is sensitive to 5-HT_{2C} receptor antagonist properties, other findings, e.g., that the antipsychotics spiperone and raclopride, which have weak affinity at 5-HT_{2C} receptors (Canton et al., 1990; Roth et al., 1992), were also active (Broekkamp et al., 1990; Protais et al., 1995), suggest that this model may have limited selectivity (cf. Berendsen et al., 1990). Thus, in general, it has been proven difficult to demonstrate unambiguously 5-HT_{2C} receptor antagonist properties of clozapine and other antipsychotics with 5-HT_{2C} receptor affinity in behavioral assays.

Here, we examined further the ability of antipsychotics with 5-HT_{2C} receptor affinity to antagonize the effects of mCPP in two different behavioral paradigms, i.e., mCPP-induced hypolocomotion and mCPP-induced hypophagia. These paradigms were chosen because: (1) they were thought to be very selective for 5-HT_{2C} receptor antagonist properties, even compared with closely related receptor subtypes such as 5-HT_{2B} receptors (Tecott et al., 1995; Bromidge et al. 1997; Kennett et al., 1997a); (2) clozapine is somewhat active in the hypolocomotion assay (Czyrak et al., 1994); and (3) the effects of antipsychotics on mCPP-induced hypophagia have not yet been studied. We examined six antipsychotics with high (i.e., clozapine, loxapine, olanzapine, and ORG 5222) or moderate affinity (i.e., pipamperone and risperidone) for 5-HT_{2C} receptors (Canton et al., 1990; Roth et al., 1992; Kuoppamäki et al., 1995; Schotte et al., 1996). For each of the antipsychotics, full dose–response functions were determined in combination with mCPP; to determine the highest dose of a antipsychotic that could be tested in combination with mCPP, the antipsychotics were also tested alone.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats [Ico: OFA SD (I.O.P.S. Caw) Iffa Credo, Lyon, France] weighing 170 ± 20 g on arrival, were group-housed in an environmentally controlled room (temperature, 21 ± 1°C, and relative humidity, 55 ± 5%) on a 12-h:12-h light:dark cycle (lights on at 0700 h). Food (standard rat chow; AO4, UAR, Epinay sur Orge, France) and filtered (0.22 μ) water (supplied automatically via a stainless steel sipper tube that protruded about 0.5 cm into one side of the cage) were continuously available. A 5-day acclimatisation period was allowed before animals were used in the experiments. Twenty-two hours before testing, the animals were individually housed in an environmentally controlled test room in plastic hanging cages with a grid floor (internal dimensions: $18 \times 31 \times 18$ cm, $W \times L \times H$; floor area 549 cm^2), where they had free access to water, but not food. Animals were handled and cared-for in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, US National Research Council, 1996) and the European Directive 86/609, and the protocols (No. 015 and 192) were carried out in compliance with French regulations and the local ethical committee guidelines for animal research.

2.2. Procedures

2.2.1. Drug treatment

Each animal was injected twice, first with a test compound or saline, followed 45-min later by mCPP or saline. After each injection, the animals were put back in their home cages. Animals were used for either a locomotor activity test or a food intake test, and were used only once. Compounds were tested in five animals per dose, except when antipsychotics having 5-HT_{2C} receptor affinity were combined with mCPP in the locomotor activity test ($n = 7$), because of the higher variability observed in this test. All drugs were administered subcutaneously (s.c.), except mCPP, clozapine, SB 206553 and prazosin, which were administered intraperitoneally (i.p.); an injection volume of 10 ml/kg body weight was used throughout. Because most test compounds were administered s.c., almost all control animals received a s.c. injection of saline, followed by an i.p. injection of saline or mCPP; only these animals were used as controls. Results obtained in several animals that received saline i.p. as the first injection did not differ from those in which saline was administered s.c. (data not shown).

2.2.2. Locomotor activity

Locomotor activity was assessed in a manner similar to that described in Prinssen et al. (1996). Specifically, after the second injection, the home cage was placed in an automated animal activity monitor (Multi-Varimex, Columbus Instruments, Columbus, OH, USA) and interruptions of at least 0.5 s of two parallel, horizontal infrared beams, positioned 3.5-cm above the floor of the cage and 16-cm apart, were counted for 2 h. During the experiment, water was available freely, supplied by a system identical to that described above.

2.2.3. Food intake

Ten minutes after the second injection, a weighed amount of standard rat chow (two pellets weighing about

11 g) was placed in the home cage and the amount remaining (including spillage) was weighed 60 and 120 min later. During the experiment, water was available freely.

2.3. Data analysis

The dependent variable for locomotor activity was the number of beam breaks between 5 and 25 min after mCPP. This period was chosen because it discriminated best between the locomotor activity of saline- and mCPP (10 mg/kg)-treated control animals (data not shown). The dependent variable for food intake, the amount of food consumed during the first 60 min, was chosen because the effects of 5 mg/kg mCPP were largest over this period (data not shown). In either assay, the 95th percentiles of the saline- and mCPP-treated control animals were calculated (i.e., criterion values; see Section 3). For test compounds, dose–response functions were determined from the percentage of animals showing larger effects (compared with mCPP) or smaller effects (compared with saline) than the criterion value. ED_{50} values and their confidence limits were estimated by means of the Lichfield and Wilcoxon probit analysis procedure (Tallarida and Murray, 1987) implemented using a procedure that was written using the Research Programming Language (RPL) of RS/1 (Bolt Beranek and Newman, Cambridge, MA). When less than two intermediate effects were observed, 0% and/or 100% effects were transformed by means of Berkson's adjustment (Hubert, 1984) to permit the use of the Lichfield and Wilcoxon procedure. Pretreatment-induced decreases in the effects of mCPP were considered statistically significant if the confidence intervals of the two proportions (i.e., number of animals with values larger than the criterion value/total number of animals, in drug- and saline-pretreated animals tested with mCPP) did not overlap (Wonnacott and Wonnacott, 1990). Correlations between potencies to induce hypolocomotion or hypophagia, and reported receptor occupancy potencies of the antipsychotics (all in mg/kg) were made using Pearsons product moment correlation of the log-transformed values, with a two-tailed $P < 0.05$ considered to be statistically significant. The ED_{50} of pipamperone to induce dopamine D_2 receptor occupancy was estimated (i.e., 50 mg/kg) from the data reported by Schotte et al. (1996).

2.4. Drugs

The following drugs were used: clozapine, loxapine succinate, mCPP di-HCl, mianserin HCl, risperidone, and SB 206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole) HCl (Research Biochemicals, Natick, MA, USA); haloperidol and prazosin HCl (Sigma, Saint-Quentin Fallavier, France); pipamperone di-HCl (Janssen Biotech, Olen, Belgium); olanzapine (Lilly Research Laboratories, Indianapolis, IN, USA); ORG 5222 (Organon, Oss, The Netherlands); (−)-scopolamine HBr trihydrate (Fluka, Neu-Ulm, Switzerland); efaroxan HCl (synthesized by J.L. Maurel, Centre de Recherche Pierre Fabre). Drugs were dissolved in distilled water, with the exception of clozapine, haloperidol, mianserin, olanzapine, prazosin, and risperidone, which were dissolved in distilled water with a drop of lactic acid, after which the pH was adjusted to 5–7 with sodium hydroxide. Doses refer to the weight of the free base.

3. Results

3.1. Locomotor activity

3.1.1. Effects in control animals

Saline-treated control animals ($n = 22$) showed 39 ± 4.2 (mean $\pm$ SE) beam breaks during the 20-min test period. Induction of hypolocomotion in individual animals was defined as a score less than 12 beam breaks, based on the 5th percentile of the saline-treated control population. mCPP (1.25–20 mg/kg) dose-dependently reduced locomotion, and the lowest number of beam breaks (i.e.,

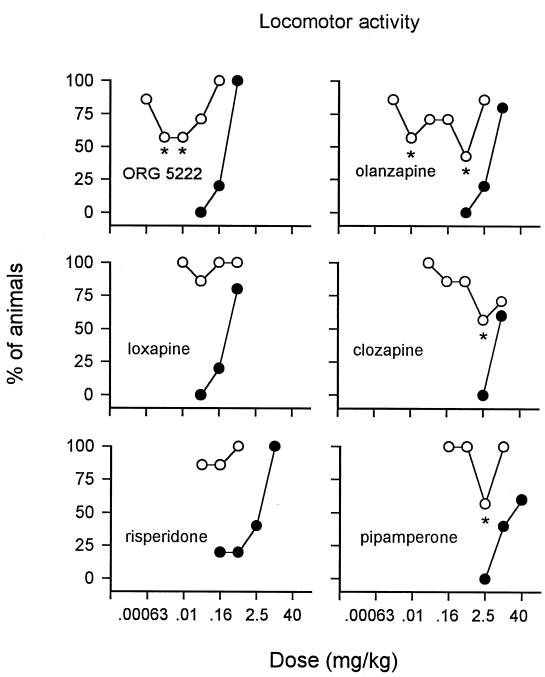

Fig. 1. The ability of antipsychotics having 5-HT_{2C} receptor affinity to antagonize mCPP-induced hypolocomotion (open symbols), and to induce hypolocomotion when given alone (filled symbols). Open symbols indicate the percentage of animals with a locomotor score similar to those induced by mCPP (i.e., within a range containing 95% of the values obtained in mCPP-treated control animals). Closed symbols indicate the percentage of animals with a locomotor score different from those induced by saline (i.e., outside a range containing 95% of the values obtained in saline-treated control animals). Antipsychotics were administered 45 min before 10 mg/kg mCPP or saline ($n = 7$/dose and $n = 5$/dose, respectively). The dependent variable was the total number of beam breaks between 5 and 25 min after the last injection. *: different from mCPP-treated controls, $P < 0.05$.

Table 1
The ability of compounds to attenuate mCPP-induced hypolocomotion and/or to induce hypolocomotion when administered alone

Drug	Attenuation of mCPP-induced hypolocomotion				Induction of hypolocomotion when administered alone			
	Dose range, mg/kg	ED_{50}, mg/kg	Maximal % animals affected[a]	Maximal beam breaks, means ± SE	Dose range, mg/kg	ED_{50} (95% CL), mg/kg	Maximal % animals affected[b]	Minimal beam breaks, means ± SE
Antipsychotics with affinity at 5-HT_{2C} receptors								
ORG 5222	0.00063–0.16	> 0.16	43 (0.0025)	16 ± 5.8	0.04–0.63	0.27 (0.12–0.63)	100 (0.63)	1 ± 1.2
Loxapine	0.01–0.63	> 0.63	14 (0.04)	11 ± 8.9	0.04–0.63	0.32 (0.16–0.63)	80 (0.63)	8 ± 1.4
Risperidone	0.04–0.63	> 0.63	14 (0.04)	13 ± 3.6	0.16–10	1.8 (0.43–7.4)	100 (10)	4 ± 2.0
Olanzapine	0.0025–2.5	0.72 (0.034–15.2)	57 (0.63)	22 ± 8.6	0.63–10	5.1 (2.6–9.9)	80 (10)	5 ± 3.0
Clozapine	0.04–10	> 10	43 (2.5)	18 ± 8.6	2.5–10	8.0[c]	60 (10)	11 ± 3.8
Pipamperone	0.16–10	> 10	43 (2.5)	17 ± 6.4	2.5–40	22 (5.6–87)	60 (40)	11 ± 3.5
5-HT_{2C} receptor antagonists								
Mianserin	0.04–10	0.38 (0.098–1.5)	100 (10)	45 ± 8.8	NT[d]			
SB 206553	0.16–10	1.8 (0.56–6.1)	80 (2.5)	49 ± 17	NT			
Other compounds								
Haloperidol	0.01–0.16	> 0.16	20 (0.16)	9 ± 2.1	0.04–0.63	0.36 (0.14–0.93)	80 (0.63)	5 ± 3.6
Prazosin	0.16–2.5	> 2.5	20 (0.63)	7 ± 5.9	2.5–10	5.8[c]	80 (10)	7 ± 1.7
Efaroxan	0.63	> 0.63	0	4 ± 2.3	0.63	> 0.63	0	52 ± 9.6
Scopolamine	0.63	> 0.63	0	1.0 ± 0.77	0.63	> 0.63	0	91 ± 15

[a] Dose at which the maximal effect (% animals having > 17 beam breaks) occurs is shown between parentheses; 5% of mCPP-treated control animals (mean 6 ± 1.1) and 82% of saline-treated control animals (mean 39 ± 4.2) had > 17 beam breaks.

[b] Dose at which the maximal effect (% animals having < 12 beam breaks) occurs is shown between parentheses; 5% of saline-treated control animals had < 12 beam breaks (mean 39 ± 4.2).

[c] Confidence limits not determined due to insufficient data.

[d] Not tested.

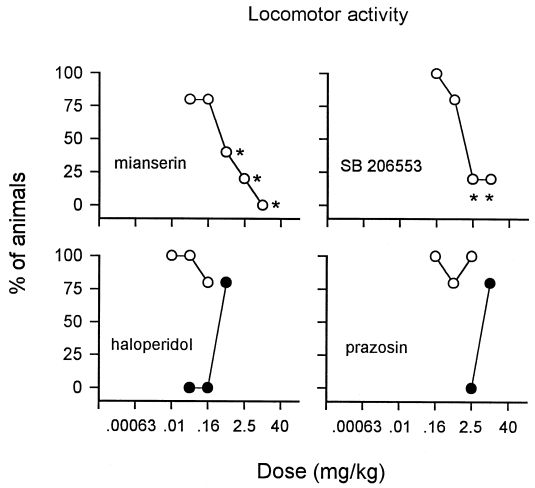

Fig. 2. The ability of mianserin, SB 206553, haloperidol, and prazosin to antagonize mCPP-induced hypolocomotion (open symbols), and of haloperidol and prazosin to induce hypolocomotion when given alone (filled symbols). Test compounds were administered 45 min before 10 mg/kg mCPP or saline ($n = 5$/dose). *: different from mCPP-treated controls, $P < 0.05$. See the legend of Fig. 1 for further details.

2 ± 1.2) was observed after administration of the highest dose. For the antagonist experiments, 10 mg/kg was chosen because it was the lowest dose to produce near-maximal hypolocomotion (6 ± 1.1). Attenuation of mCPP-induced hypolocomotion in individual animals was defined as a score higher than 17 beam breaks, based on the 95th percentile of the mCPP (10 mg/kg)-treated control population ($n = 37$). Eighty-two percent of the saline-treated controls had values meeting this criterion. Thus, in principle, complete antagonism of the effects of mCPP cannot be expected to produce values higher than criterion in more than 82% of the animals.

3.1.2. Effects of antipsychotics having 5-HT$_{2C}$ receptor affinity

Olanzapine dose-dependently attenuated mCPP-induced hypolocomotion in a biphasic manner, resulting in a U-shaped dose–response function (Fig. 1, upper right-hand panel, open symbols). The estimated ED_{50} value of olanzapine to attenuate the effects of mCPP was 0.72 mg/kg (Table 1). ORG 5222 had similar effects, but with a significant attenuation observed in a maximum of 43% of the animals at the doses 0.0025 and 0.01 mg/kg, precluding the calculation of an ED_{50} value. Clozapine and pipamperone also significantly attenuated the effects of mCPP in 43% of the animals, but only at a single dose. Loxapine and risperidone attenuated the effects of mCPP in less than 20% of the animals. When given alone, all of the antipsychotics dose-dependently attenuated locomotor activity (Fig. 1, filled symbols) with estimated ED_{50}s ranging from 0.27 (ORG 5222) to 22 mg/kg (pipamperone; Table 1). Clozapine was tested up to 10 mg/kg, because a higher dose (40 mg/kg) induced lethality.

3.1.3. Effects of other compounds

The hypolocomotor effects of mCPP were dose-dependently antagonized by the 5-HT$_{2A/2B/2C}$ receptor antagonist mianserin and by the 5-HT$_{2B/2C}$ receptor antagonist SB 206553 (Fig. 2; ED_{50} values given in Table 1). The maximal effects of SB 206553 — attenuation in 80% of animals — can be considered full antagonism of the effects of mCPP (see above). The dopamine D_2 receptor antagonist haloperidol and the α_1-adrenoceptor antagonist prazosin induced hypolocomotion when given alone, and did not attenuate mCPP-induced hypolocomotion at lower doses (Fig. 2, Table 1). Although tested only at a single dose, the α_2-adrenoceptor antagonist efaroxan and the muscarinic receptor antagonist scopolamine neither induced hypolocomotion when given alone, nor attenuated mCPP-induced hypolocomotion (scopolamine, when given alone, appeared to enhance locomotor activity, Table 1).

3.2. Food intake

3.2.1. Effects in control animals

Saline-treated control animals ($n = 66$) consumed 5.9 $\pm$ 0.17 g (mean $\pm$ SE) of food during the 60-min test

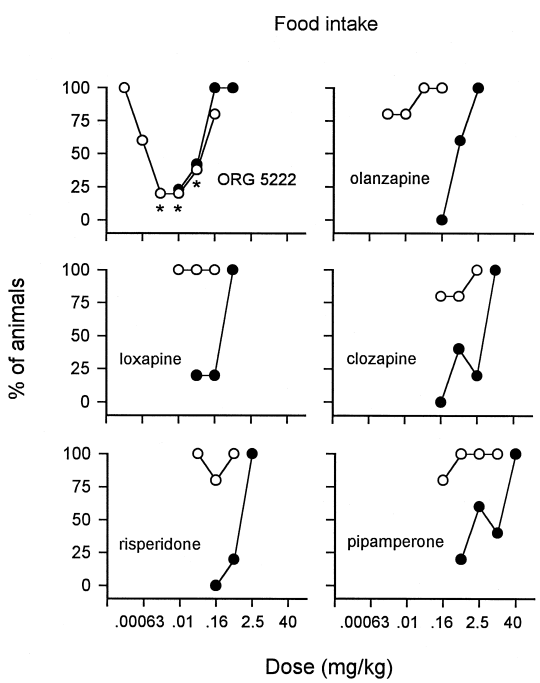

Fig. 3. The ability of antipsychotics having 5-HT$_{2C}$ receptor affinity to antagonize mCPP-induced hypophagia (open symbols) and to induce hypophagia when given alone (filled symbols). Open symbols indicate the percentage of animals with a food intake similar to those induced by mCPP (i.e., within a range containing 95% of the values obtained in mCPP-treated control animals). Closed symbols indicate the percentage of animals with a food intake different from those induced by saline (i.e., outside a range containing 95% of the values obtained in saline-treated control animals). Antipsychotics were administered 45 min before 5 mg/kg mCPP or saline ($n = 5$/dose). The dependent variable was the total food consumption during 1 h; food was made available 10 min after the last injection. *: different from mCPP-treated controls, $P < 0.05$.

Table 2
The ability of compounds to attenuate mCPP-induced hypophagia and/or to induce hypophagia when administered alone

Drug	Attenuation of mCPP-induced hypophagia				Induction of hypophagia when administered alone			
	Dose range, mg/kg	ED_{50} (95% CL), mg/kg	Maximal % animals affected[a]	Maximal food intake in g means ± SE	Dose range, mg/kg	ED_{50} (95% CL), mg/kg	Maximal % animals affected[b]	Minimal food intake in g means ± SE
Antipsychotics with affinity at 5-HT_{2C} receptors								
ORG 5222	0.00016–0.16	0.0013 (0.00028–0.0058)	80 (0.0025)	3 ± 1.0	0.01–0.63	0.039 (0.013–0.12)	100 (0.16)	0.24 ± 0.088
Loxapine	0.01–0.16	> 0.16	0	0.4 ± 0.31	0.04–0.63	0.19 (0.061–0.61)	100 (0.63)	0.7 ± 0.21
Risperidone	0.04–0.63	> 0.63	20 (0.16)	1.0 ± 0.95	0.16–2.5	1.1 (0.46–2.5)	100 (2.5)	0.5 ± 0.43
Olanzapine	0.0025–0.16	> 0.16	20 (0.0025)	0.7 ± 0.43	0.16–2.5	0.46 (0.18–1.2)	100 (2.5)	0.8 ± 0.38
Clozapine	0.16–2.5	> 2.5	20 (0.16)	0.6 ± 0.61	0.16–10	2.2 (0.53–8.9)	100 (10)	1.9 ± 0.34
Pipamperone	0.16–10	> 10	20 (0.16)	0.5 ± 0.40	0.63–40	3.8 (0.73–20.1)	100 (40)	1.5 ± 0.62
5-HT_{2C} receptor antagonists								
Mianserin	0.04–10	0.40 (0.072–2.2)	100 (2.5)	4.1 ± 0.62	NT[c]			
SB 206553	0.16–10	2.2 (0.70–7.1)	100 (10)	4.0 ± 0.59	NT			
Other compounds								
Haloperidol	0.0025–0.16	> 0.16	40 (0.01)	2 ± 1.2	0.04–0.63	0.094 (0.040–0.22)	100 (0.63)	0.06 ± 0.020
Prazosin	0.16–2.5	> 2.5	0	0.2 ± 0.16	0.63–40	3.6 (0.85–14.9)	80 (10)	2.4 ± 0.59
Efaroxan	0.63	> 0.63	0	0.5 ± 0.27	0.63	> 0.63	0	5.6 ± 0.66
Scopolamine	0.63	> 0.63	0	0.0	0.63	< 0.63	80 (0.63)	2.6 ± 0.57

[a] Dose at which the maximal effect (% animals having > 1.7 g food intake) occurs is shown between parentheses; 5% of mCPP-treated control animals (mean 0.6 ± 0.10) and 100% of saline-treated control animals (mean 5.9 ± 0.17) had > 1.7 g food intake.

[b] Dose at which the maximal effect (% animals having < 3.9 g food intake) occurs is shown between parentheses; 5% of saline-treated control animals had < 3.9 g food intake (mean 5.9 ± 0.17).

[c] Not tested.

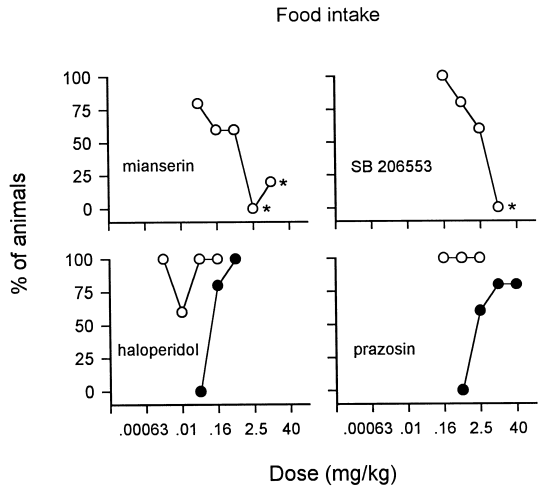

Fig. 4. The ability of mianserin, SB 206553, haloperidol, and prazosin to antagonize mCPP-induced hypophagia (open symbols), and of haloperidol and prazosin to induce hypophagia when given alone (filled symbols). Test compounds were administered 45 min before 5 mg/kg mCPP or saline ($n = 5$/dose). *: different from mCPP-treated controls, $P < 0.05$. See the legend of Fig. 3 for further details.

period. Induction of hypophagia in an individual animal was defined as consumption of less than 3.9 g of food, based on the 5th percentile of the saline-treated control population. mCPP (1.25–10 mg/kg) dose-dependently reduced food intake, with the lowest amount of food intake (0.1 ± 0.10 g) observed after administration of the highest dose. For the antagonist experiments, the 5-mg/kg dose of mCPP was chosen because it was the lowest dose that produced near-maximal hypophagia (0.6 ± 0.10). Attenuation of mCPP-induced hypophagia in individual animals was defined as consumption of more than 1.7 g of food based on the 95th percentile of the mCPP (5 mg/kg)-treated control population ($n = 47$). All of the saline-treated control animals had values meeting this criterion. Thus, complete antagonism of the effects of mCPP can be expected to produce values higher than criterion in 100% of the animals.

3.2.2. Effects of antipsychotics having 5-HT_{2C} receptor affinity

ORG 5222 dose-dependently attenuated mCPP-induced hypophagia in a biphasic manner, resulting in a U-shaped dose–response function (Fig. 3). The ED_{50} value of ORG 5222 to attenuate mCPP-induced hypophagia was 0.0013 mg/kg (Table 2). ORG 5222 significantly attenuated mCPP-induced hypophagia in 80% of the animals at the doses 0.0025 and 0.01 mg/kg. All other antipsychotics were active, if at all, in at most 20% of the animals. When given alone, all of the antipsychotics dose-dependently attenuated food intake with ED_{50}s ranging from 0.039 (ORG 5222) to 3.8 mg/kg (pipamperone; Table 2).

3.2.3. Effects of other compounds

The hypophagic effects of mCPP were dose-dependently antagonized by mianserin and SB 206553 (Fig. 4). Haloperidol and prazosin induced hypophagia when given alone, and lower doses did not significantly attenuate the effects of mCPP (Fig. 4, Table 2; for haloperidol, the apparent partial effects were neither statistically significant nor dose-dependent). Although tested only at one dose, efaroxan neither induced hypophagia when given alone, nor attenuated mCPP-induced hypophagia. Scopolamine did not attenuate the effects of mCPP, and induced hypophagia when given alone (Table 2).

3.3. Relationship between the effects of the antipsychotics in the behavioral assays and their reported in vivo binding potencies

The ratios of the potencies of the antipsychotics to induce hypolocomotion or hypophagia and to induce

Table 3
The ability of antipsychotics with 5-HT_{2C} antagonist properties to alter hypolocomotion or hypophagia compared to their in vivo 5-HT_{2C} receptor occupancy

Drug[a]	Induction of hypolocomotion ED_{50} mg/kg	Induction of hypophagia ED_{50} mg/kg	In vivo occupancy of 5-HT_{2C} receptors[b] ED_{50} mg/kg	Hypolocomotion/ Occupancy ratio	Hypophagia/ Occupancy ratio	Attenuation of mCPP-induced hypolocomotion Number of significant doses	Attenuation of mCPP-induced hypophagia Number of significant doses
ORG 5222	0.27	0.039	0.020	14	2.0	2	3
Loxapine	0.32	0.19	ND[c]	–	–	0	0
Risperidone	1.8	1.1	> 10	< 0.18	< 0.11	0	0
Olanzapine	5.1	0.46	0.86	5.9	0.53	2	0
Clozapine	8.0	2.2	2.6	3.1	0.85	1	0
Pipamperone	22	3.8	14	1.6	0.27	1	0

[a] All compounds were administered s.c., except clozapine, i.p.
[b] From Schotte et al. (1996), where all compounds were administered s.c.
[c] Not determined.

Table 4
Correlation coefficients between the potency of antipsychotics to induce hypolocomotion or hypophagia and to occupy dopamine D_2, histamine H_1, and 5-HT_{2A} receptors, and α_1-adrenoceptors

	n	Hypolocomotion	Hypophagia	Dopamine D_2 receptors	α_1-adrenoceptors	Histamine H_1 receptors
Hypolocomotion	7					
Hypophagia	7	0.92[a]				
Dopamine D_2 receptors[b]	6	0.91[c]	0.94[a]			
α_1-adrenoceptors[b]	6	0.86[c]	0.81	0.78		
Histamine H_1 receptors[b]	4	−0.42	0.42	0.35	0.15	
5-HT_{2A} receptors[b]	6	0.48	0.54	0.59	0.52	0.14

[a] Significant correlation, $P < 0.01$.
[b] From Schotte et al. (1996).
[c] Significant correlation, $P < 0.05$.

5-HT_{2C} receptor occupancy are shown in Table 3. For hypolocomotion, the rank order of the ratios was: ORG 5222 > olanzapine > clozapine > pipamperone > risperidone, whereas that for the ability of the antipsychotics to antagonize mCPP (expressed as the number of significant doses) was: ORG 5222 = olanzapine > clozapine = pipamperone > risperidone. For hypophagia, the rank order of the ratio was: ORG 5222 > clozapine > olanzapine > pipamperone > risperidone. The only compound that significantly antagonized mCPP had the largest ratio, ORG 5222.

The potencies of the antipsychotics (including haloperidol) to induce hypolocomotion when administered alone were significantly correlated with their potencies to occupy dopamine D_2 receptors ($r = 0.91$, $P < 0.05$) and α_1-adrenoceptors ($r = 0.86$, $P < 0.05$), but not to occupy histamine H_1 receptors, or 5-HT_{2A} receptors (Table 4). Similarly, the potencies of the antipsychotics to induce hypophagia was significantly correlated with that for dopamine D_2 receptor occupancy ($r = 0.94$; $P < 0.01$). The potencies of the antipsychotics to induce hypophagia was not significantly correlated with their potencies to occupy α_1-adrenoceptors, histamine H_1 receptors, or 5-HT_{2A} receptors (highest r for α_1-adrenoceptors: 0.81, $P > 0.05$).

4. Discussion

Many antipsychotics have demonstrable antagonist effects at 5-HT_{2C} receptors in vitro. It has been difficult, however, to show these effects in vivo, especially in behavioral assays. The most important finding in the present study is that several antipsychotics (i.e., clozapine, olanzapine, and pipamperone) significantly attenuated mCPP-induced hypolocomotion, and that this was related to their 5-HT_{2C} receptor antagonist properties. Another interesting finding was that only the putative antipsychotic ORG 5222 attenuated mCPP-induced hypophagia, suggesting that it is a relative selective 5-HT_{2C} receptor antagonist.

mCPP has complex pharmacological activities: it acts as a prominent agonist at 5-HT_{2C} receptors, and to a lesser extent at 5-HT_{1B}, and 5-HT_{2B} receptors (Conn and Sanders-Bush, 1987; Maj et al., 1988; Baxter et al., 1994), it acts as an antagonist at 5-HT_{2A} and 5-HT_3 receptors (Conn and Sanders-Bush, 1987; Robertson et al., 1992), and is a 5-HT releaser (Baumann et al., 1993; Eriksson et al., 1999). In addition, mCPP has relatively weak affinity for a number of catecholaminergic receptors and among those, its affinity at α_2-adrenoceptors is the highest (cf. Kennett, 1993). Nevertheless, there is growing evidence that mCPP induces hypolocomotion and hypophagia primarily via its stimulation of 5-HT_{2C} receptors. Hypophagia has been studied most extensively, and it has been shown that the potency of 5-HT receptor antagonists to attenuate mCPP-induced hypophagia correlates with their affinities for 5-HT_{2C} receptors (Kennett and Curzon, 1991). Several recently developed, selective 5-$HT_{2B/2C}$ receptor antagonists were equally effective against mCPP-induced hypophagia (Forbes et al., 1993, 1995; Kennett et al., 1994; Nozulak et al., 1995). Similarly, hypolocomotion induced by mCPP was antagonized by non-selective 5-HT_2 receptor antagonists (Kennett and Curzon, 1988b; Lucki et al., 1989) and by selective 5-$HT_{2B/2C}$ receptor antagonists (Kennett et al., 1994, 1996; Forbes et al., 1995; Bromidge et al., 1997). These earlier studies could not exclude the involvement of the closely related 5-HT_{2B} receptors. Recent findings, however, indicate the unique involvement of 5-HT_{2C} receptors in these assays: (1) the selective 5-HT_{2B} receptor agonist 1-[5-(2-thienylmethoxy)-1*H*-3-indoyl]propan-2-amine (BW 723C86) induces neither hypolocomotion nor hypophagia (Kennett et al., 1997a); (2) the 5-HT_{2C} receptor antagonist 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB 242084), which has 100-fold selectivity over 5-HT_{2B} receptors, potently antagonizes hypolocomotion and hypophagia induced by mCPP (Kennett et al., 1997b); and (3) mutant mice lacking 5-HT_{2C} receptors are obese and are insensitive to the hypophagic effects of mCPP (Tecott et al., 1995). In the present study, the dose-related and complete antagonism of mCPP-induced hypolocomotion and hy-

pophagia by the 5-$HT_{2A/2B/2C}$ receptor antagonist mianserin and the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 is in agreement with a unique role for 5-HT_{2C} receptors in these assays.

The assays used in the present study are selective for 5-HT_{2C} receptors compared with other 5-HT receptors. However, antipsychotics with 5-HT_{2C} receptor affinity also have antagonist activity at several other non-serotonergic receptors, and to demonstrate their 5-HT_{2C} receptor antagonist properties, it is necessary to consider the involvement of these other activities. The most relevant other antagonist activity of the antipsychotics is at dopamine D_2-like receptors, α_1- and α_2-adrenoceptors, muscarinic receptors, and histamine H_1 receptors (Leysen et al., 1993; Schotte et al., 1996). The findings that haloperidol, prazosin, efaroxan, and scopolamine were unable to alter the effects of mCPP indicated that antagonism at dopamine D_2-like receptors, α_1-adrenoceptors, α_2-adrenoceptors, or muscarinic receptors, respectively, was not sufficient to antagonize mCPP. These results confirm and extend earlier findings (Kennett and Curzon, 1988a,b; Klodzinska et al., 1989). Finally, a role for histamine H_1 receptor antagonism is unlikely because of the well-known sedative effects of this class, and because olanzapine, but not ORG 5222, has some histamine H_1 receptor selectivity in vivo (Schotte et al., 1996), whereas, ORG 5222, but not olanzapine, antagonized mCPP-induced hypophagia. Altogether, attenuation of the effects of mCPP by the antipsychotics is most likely due to their 5-HT_{2C} receptor antagonist properties.

Several antipsychotics partially, but significantly, antagonized mCPP-induced hypolocomotion. Because this assay is selective for 5-HT_{2C} receptor antagonist properties, and because we observed a relationship between the behavioral effects of the antipsychotics and their reported in vivo 5-HT_{2C} receptor occupancy (Schotte et al., 1996), this implies that these antipsychotics antagonized mCPP via their 5-HT_{2C} receptor antagonist properties. Although none of the antipsychotics fully antagonized mCPP-induced hypolocomotion, it should be noted that the maximum percentages of animals affected as shown in Fig. 1 and Table 1 are somewhat underestimated. Because the saline- and mCPP-treated control populations overlap (only 82% of saline-treated animals have scores higher than the criterion used to define antagonism of mCPP), compounds that would completely antagonize the decrease in mean beam breaks would still not affect all of the animals. When we express the effects as a percentage of the maximal attainable effects, we obtained maximal values ranging from 50% to 70% of animals affected for the four antipsychotics, compared with 100% for the selective 5-HT_2 receptor antagonists. Thus, the antipsychotics affected an important percentage of animals, even though none of them produced full antagonism. It seems unlikely that these partial effects are due to weak 5-HT_{2C} receptor agonist activity of the antipsychotics (Canton et al. 1994; Kuoppamäki et al., 1995). Instead, it seems more likely that their ability to induce hypolocomotion prevented the use of doses high enough to fully antagonize mCPP. This is suggested by: (1) the relatively small separation between the ascending part of the dose-response functions of a antipsychotic combined with mCPP and that of the same antipsychotic combined with saline; (2) the apparent relationship between the ability of antipsychotics to attenuate mCPP and the ratios of their potencies to induce hypolocomotion and to occupy 5-HT_{2C} receptors in vivo (Schotte et al., 1996); and (3) the finding that risperidone, which was inactive in the present study, did show apparent 5-HT_{2C} receptor antagonist properties in an electrophysiology study at higher doses (Bergqvist et al., 1999). Thus, the maximal effects of the antipsychotics having 5-HT_{2C} receptor affinity to antagonize mCPP appear to be limited by their own hypolocomotor effects. Nonetheless, the findings that we were able to detect 5-HT_{2C} receptor antagonist properties of antipsychotics are important, because previous behavioral studies have failed to demonstrate this (Lucki et al., 1989; Czyrak et al., 1994; Fiorella et al., 1996; Gommans et al., 1998).

With respect to hypophagia, only ORG 5222 significantly antagonized mCPP-induced hypophagia, whereas the other five antipsychotics were inactive. That some of the compounds antagonized the hypolocomotor but not the hypophagic effects of mCPP supports other findings that the hypophagic effects of mCPP are independent of its effects on general activity (Hutson et al., 1988; Kennett and Curzon, 1988b; Aulakh et al., 1989). The finding that mCPP-induced hypophagia is relatively insensitive to antipsychotics suggests that ORG 5222 is a relatively selective 5-HT_{2C} receptor antagonist. Indeed, a comparison of the in vivo occupancy of 5-HT_{2C} receptors with dopamine D_2 receptors, showed that ORG 5222 had the highest separation in favor of 5-HT_{2C} receptors (Schotte et al., 1996). Following the same reasoning as that mentioned above for hypolocomotion, the inability to detect antagonism of the hypophagic effects of mCPP by most of the antipsychotics is most likely due to their hypophagic effects, in particular because: (1) the ascending part of the dose–response functions of ORG 5222 combined with mCPP and that of ORG 5222 combined with saline overlapped; and (2) only the compound with the highest ratio between the potency to induce hypolocomotion and to occupy 5-HT_{2C} receptors in vivo (Schotte et al., 1996), ORG 5222, attenuated mCPP-induced hypophagia. The apparent greater potency of the antipsychotics to induce hypophagia compared with hypophagia (Table 3) would explain why they were less able to antagonize mCPP-induced hypophagia compared with mCPP-induced hypolocomotion. However, it should be noted that a direct comparison of the two assays is complicated by the finding that the distributions of the control populations differed between the assays. Nevertheless, the present data suggest that hypolocomotion and hypophagia induced by mCPP

can be used to demonstrate 5-HT_{2C} receptor antagonist properties of antipsychotics having 5-HT_{2C} receptor affinity. Hypolocomotion induced by mCPP is somewhat more sensitive to antipsychotics having 5-HT_{2C} receptor affinity than mCPP-induced hypophagia, which appears to be sensitive only to relatively selective 5-HT_{2C} receptor antagonists.

The pharmacological mechanism underlying the effects of the antipsychotics when administered alone is most likely dopamine D_2 receptor blockade. That is, the potencies of the antipsychotics to reduce food intake correlated significantly with their reported in vivo occupancies at dopamine D_2 receptors, but not at α_1-adrenoceptors, or histamine H_1 and 5-HT_{2A} receptors (Schotte et al., 1996; Table 4). Similarly, the potencies of the antipsychotics to reduce locomotor activity correlated significantly with their reported in vivo occupancies at dopamine D_2 receptors, even though in this case, a significant correlation was also observed with α_1-adrenoceptor occupancy. Because these correlations could be confounded by the relatively high correlation between dopamine D_2 receptor and α_1-adrenoceptor occupancies ($r = 0.78$), we examined the partial correlations between these variables and the results suggested that dopamine D_2 receptors (partial $r = 0.76$; $P < 0.05$) play a more important role in the ability of antipsychotics to reduce locomotor activity than α_1-adrenoceptors (partial $r = 0.56$; $P > 0.1$). Furthermore, a role for dopamine D_2 receptors in these assays is supported also by studies showing that dopamine D_2 receptor blockade attenuates spontaneous locomotor activity in a novel environment (e.g., Waters et al., 1994) and food intake in food-deprived animals (Schneider et al., 1986; Salamone et al., 1990). Thus, although the involvement of other receptors cannot be excluded, the most likely mechanism by which the antipsychotics induced hypolocomotion and hypophagia was their blockade of dopamine D_2 receptors.

In conclusion, the assays used in the present study — hypolocomotion and hypophagia induced by mCPP — can be used to characterize the 5-HT_{2C} receptor antagonist properties of antipsychotics having 5-HT_{2C} receptor affinity. Hypolocomotion induced by mCPP is most sensitive to these mixed compounds, and hypophagia appears to be sensitive only to relatively selective 5-HT_{2C} receptor antagonists, such as the putative antipsychotic ORG 5222. The assays described here may help to determine 5-HT_{2C} receptor antagonist properties of antipsychotics in vivo, and, consequently, the involvement of these properties in their clinical effects.

Acknowledgements

The authors thank Christelle Marc and Martine Alquier for their technical assistance and Joël Besnard for assistance with data management and analysis. We thank Lilly Research Laboratories and Organon for their generous gifts of compounds.

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