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Potencies of antagonists indicate that 5-HT_{1C} receptors mediate 1-3(chlorophenyl)piperazine-induced hypophagia

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1 1-3(Chlorophenyl)piperazine (mCPP) (5 mg kg^{-1}, i.p.) inhibited 2 h food intake in rats previously deprived of food for one day. Ten 5-hydroxytryptamine (5-HT) antagonists given s.c. opposed this hypophagic response. Calculated ID_{50} values correlated significantly with reported affinities ($r = 0.81$, $n = 10$, $P < 0.01$) for 5-HT_{1C} but not for 5-HT_2, 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1D} receptors.

2 ID_{50} values of the ten antagonists against 5-hydroxytryptophan (5-HTP) + carbidopa-induced head shakes (a 5-HT_2-mediated response) correlated significantly ($r = 0.81$, $n = 10$, $P < 0.01$) with their affinities for 5-HT_2 but not for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptors.

3 ID_{50} values for inhibition of hypophagia and head shakes did not correlate significantly with each other.

4 Ratios of ID_{50} values against hypophagia and 5-HT_2-mediated head shakes gave indices of relative *in vivo* potencies independent of differences in drug metabolism and disposition. These ratios correlated highly significantly ($r = 0.91$, $n = 10$, $P < 0.001$) with the ratios of the affinities of the drugs for 5-HT_{1C} (but not for 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1B} or 5-HT_{1D} receptors) and with their affinities for 5-HT_2 receptors. These results strongly support the hypothesis that mediation of mCPP-induced hypophagia is by stimulation of 5-HT_{1C} receptors and the mediation of 5-HTP-induced head twitches by 5-HT_2 receptors.

Keywords: 5-HT_{1C} receptors, 5-HT_2 receptors, hypophagia, 1-3(chlorophenyl)piperazine (mCPP)

Introduction

1-3(Chlorophenyl)piperazine (mCPP) and 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) cause hypophagia in food-deprived (Samanin *et al.*, 1979; Kennett & Curzon, 1988a) and freely feeding (Kennett *et al.*, 1987) rats. The drugs also cause hypolocomotion (Lucki *et al.*, 1989; Kennett & Curzon, 1988b) and anxiety-like behaviour in rat models (Kennett *et al.*, 1988). These effects are unlikely to account for the hypophagia as infusion of TFMPP into the paraventricular nucleus of the hypothalamus (PVN), a site associated with control of feeding (Shor-Posner *et al.*, 1986), causes hypophagia without hypolocomotion (Hutson *et al.*, 1988). Also, chlordiazepoxide prevented the anxiogenic effect of mCPP but neither its hypophagic nor hypolocomotor actions (Kennett *et al.*, 1988).

Since TFMPP- and mCPP-induced hypophagias are pharmacologically similar (Kennett & Curzon, 1988a) the hypophagic response to mCPP is also likely to be mediated by the PVN. Indeed, we have previously proposed that the hypophagic responses to both drugs are the result of 5-HT_{1C} receptor activation (Kennett & Curzon, 1988a). These sites appear to be located postsynaptically as mCPP-induced hypophagia is not blocked by raphe lesions (Samanin *et al.*, 1979). However, a problem in the investigation of the roles of 5-HT_{1C} receptors is that most antagonists with high affinity for them also have high affinity for 5-HT_2 receptors (Hoyer, 1988), as do many agonists such as quipazine and 1-(2,5-dimethoxy-4-iodophenyl)-L-aminopropane (DOI) (Schoeffter & Hoyer, 1989). Furthermore, both receptors share the same secondary messenger system (phosphoinositide hydrolysis) (Conn & Sanders-Bush, 1987). These similarities presumably reflect the 78% sequence homology of the two receptors (Hartig, 1989).

A number of publications have suggested that activation of 5-HT_2 receptors causes hypophagia. Thus the effects of antagonists on hypophagias induced by the 5-hydroxytryptamine (5-HT) releasing agent fenfluramine (Hewson *et al.*, 1988), the 5-HT_{1C}/5-HT_2 agonists DOI (Schechter & Simansky, 1988) and quipazine (Hewson *et al.*, 1989) have all been interpreted in terms of activation of 5-HT_2 receptors. Also, other reports suggest that the effect of TFMPP is 5-HT_2-mediated (Klodzinska & Chojnacka-Wojcik, 1990) and that the action of mCPP might not be 5-HT_{1C}-dependent (Aulakh *et al.*, 1989). These apparent conflicts with our findings may derive from experimental differences and from the customary use of *in vitro* affinity constants of antagonists as indices of receptor blockade *in vivo*. However, affinities *in vitro* do not necessarily closely indicate *in vivo* potencies as these could be influenced by drug absorption, metabolism and disposition.

We have now minimized the above problems by comparing the ratios of *in vivo* ID_{50} values of ten antagonists against mCPP-induced hypophagia and 5-HT_2-mediated (Bedard & Pycock, 1977; Yap & Taylor, 1983; Niemegeers *et al.*, 1983) head shakes with the corresponding ratios of *in vitro* drug affinities for various 5-HT_1 receptor subtypes and 5-HT_2 receptors. This provides indices of blockade of different 5-HT receptors which are independent of differences in drug absorption or metabolism and which can be compared with published *in vitro* affinity ratios. Results were consistent with our proposal (Kennett & Curzon, 1988a) that mCPP induces hypophagia by activating 5-HT_{1C} receptors and not 5-HT_2 receptors.

A preliminary account of this work has been presented (Kennett *et al.*, 1990).

Methods

Animals

Male Sprague Dawley rats (250–300 g, Charles River, UK) were housed individually with free access to food [Special Diet Services Ltd., Essex, England, Rm (IE) rodent diet] and water at 21 ± 2°C under a 12 h light/dark cycle (lights on 06 h 00 min) for at least 5 days prior to experimentation.

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Head shake response

Rats were placed in individual perspex cages (26 cm × 26 cm × 26 cm) at least 1 h prior to experimentation and injected with 25 mg kg^{-1} i.p. carbidopa between 13 h 00 min and 13 h 30 min. Antagonists or vehicle were injected s.c. immediately afterwards. 5-HTP 100 mg kg^{-1}, i.p., was injected 30 min later and the number of head shakes counted over 2 min periods 30, 60, 90 and 120 min later. Scores were then summed.

1-3(Chlorophenyl)piperazine-induced hypophagia

Rats were deprived of food but not water commencing between 16 h 00 min and 18 h 00 min. On the following day between 12 h 00 min and 13 h 00 min they were injected with antagonists, s.c. Twenty min later they were injected with either mCPP 5 mg kg^{-1} or saline i.p. After a further 20 min a weighed amount of food was placed in the food hoppers and food intake measured after 1 and 2 h. The data obtained at 1 h were not presented as they were largely consistent with the 2 h findings but less clearly defined.

Drugs

The 5-HT antagonists altanserin, ketanserin tartarate (Janssen, Beerse, Belgium) methysergide hydromaleate, pizotifen hydrogen maleate (Sandoz, Basle, Switzerland) metergoline (Farmitalia) and mianserin hydrochloride (Organon, Newhouse, U.K.) were dissolved in 10% acetic acid and made up to volume with 0.9% NaCl before bringing to pH 6.5 with 5 N NaOH. 1 n-Naphthyl piperazine hydrochloride (1-NP), mCPP dihydrochloride (both Research Biochemicals Inc., Wayland, U.S.A.), 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl) 4,6,6A,7,8,9,10,10A-octahydro-indolo(4,3-f,g)quinoline maleate (LY 53857, Cohen *et al.*, 1983) (Lilly, Minneapolis, U.S.A.) and (±)-propranolol hydrochloride (ICI, Macclesfield, U.K.) were dissolved in 0.9% NaCl while ritanserin (Janssen, Beerse, Belgium), carbidopa (Merck, Sharp and Dohme, Harlow) and 5-HTP (Sigma Chemical Company, Poole) were given as suspensions in 0.005% BRIJ (polyoxylethylene laurylester) in 0.9% NaCl. Antagonists were injected s.c. and other drugs i.p. (mCPP, carbidopa, 5-HTP) in a volume of 1 ml kg^{-1} body weight with the exception of 5-HTP which was given in 2 ml kg^{-1} body weight.

Statistics

The effects of antagonists and mCPP alone on food intake were analysed by Dunnett's test following significant one-way ANOVA. ID_{50} values were calculated from the ranges of antagonist doses producing 20–80% inhibition of either the head shakes or the hypophagic effect of mCPP by least squares linear regression, except in the case of ritanserin when the ID_{50} was calculated by interpolation between the two values in this range before maximum inhibition was attained (see Figure 1a). Two tailed 95% confidence limits of the values were calculated by the method of Bowman & Rand (1980). ID_{50} values were correlated with published affinities (Hoyer, 1988; Schoeffter *et al.*, 1988; Schoeffter & Hoyer, 1989; Schlicker *et al.*, 1989) following log transformation to minimize spurious significance due to the skewed distribution of values (Winer, 1971). Ratios of ID_{50} values were also log-transformed before correlation testing.

Results

Effects of 5-hydroxytryptamine antagonists on 1-3(chlorophenyl)piperazine-induced hypophagia

Groups of food-deprived rats dosed subcutaneously with 5 mg kg^{-1} (18.4 μmol kg^{-1}) mCPP 20 min before restoration of food consumed 51–79% less food over the next 2 h than control rats dosed subcutaneously with 0.9% NaCl (Table 1). 5-HT antagonists (with the exception of ritanserin) caused significant overall dose-dependent inhibition of mCPP-induced hypophagia (Figure 1a). Ritanserin had a partial effect causing about 58% inhibition at 5 mg kg^{-1} (10.4 μmol kg^{-1}). Higher doses up to 40 mg kg^{-1} (83 μmol kg^{-1}) produced no further inhibition. None of the antagonists significantly affected food intake of food-deprived rats in the absence of mCPP even at the highest dose used (Table 1). Calculated ID_{50} values (including that of ritanserin) are given in Table 2 and were in order: metergoline < pizotifen < 1-NP < mianserin < ritanserin < methysergide < LY 53587 < altanserin < ketanserin < (±)-propranolol. Figure 2a shows the significant relationship between these values and published *in vitro* affinities (pK_D) of the drugs for the 5-HT$_{1C}$ receptor subtype (given in Table 2). Corresponding correlations with affinities for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes and for the 5-HT$_2$ receptor (Table 2) were not significant.

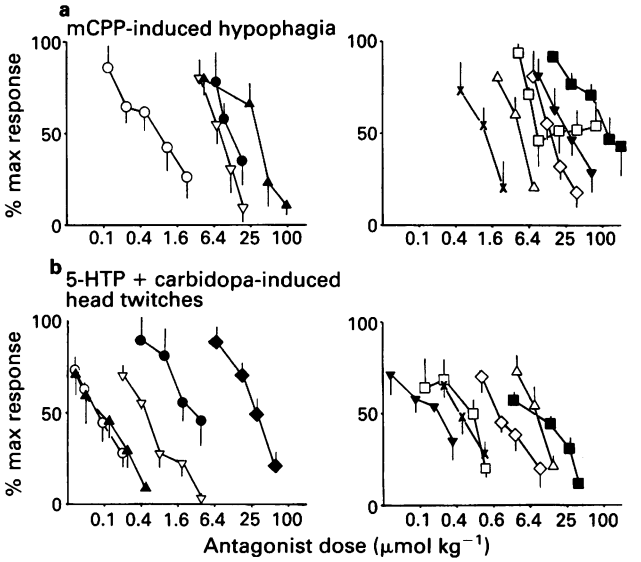

Figure 1 (a) Effects of various doses of antagonists on 1-3(chlorophenyl)piperazine (mCPP, 5 mg kg^{-1}, i.p.)-induced hypophagia in 18 h food-deprived rats. Antagonists were injected s.c. 20 min prior to mCPP and 40 min prior to food restoration. Results are shown in % form with 100% representing the decrease of food intake over 2 h in the absence of antagonists (means with s.e.mean shown by vertical lines, n = 5–7 for each drug concentration). Significance of correlations between antagonist dose and response are shown in parentheses as follows: metergoline (○) ($P < 0.01$), 1-naphthyl piperazine (△) ($P < 0.001$), mianserin (▽) ($P < 0.02$), ritanserin (□) (NS), methysergide (◇) ($P < 0.05$), LY 53857 (●) ($P < 0.05$), altanserin (▲) ($P < 0.01$), ketanserin (▼) ($P < 0.05$), (±)-propranolol (■) ($P < 0.01$), pizotifen (×) ($P < 0.05$). (b) Effects of various doses of antagonists on carbidopa (25 mg kg^{-1}, i.p.) + 5-hydroxytryptophan (5-HTP, 100 mg kg^{-1}, i.p.)-induced head shakes. The antagonists were injected s.c. immediately after carbidopa, 30 min before 5-HTP injection and 1 h before the start of the scoring period. Results are shown in % form as means with s.e.mean shown by vertical lines (n = 5–7 for each drug concentration) with 100% representing the number of head twitches in the absence of antagonists. This varied from experiment to experiment between 14 and 30. Significance of correlations between antagonist dose and response are shown in parentheses as follows: metergoline (○) ($P < 0.01$), 1-naphthyl piperazine (△) ($P < 0.001$), mianserin (▽) ($P < 0.01$), ritanserin (□) ($P < 0.05$), methysergide (◇) ($P < 0.02$), LY 53857 (●) (NS), altanserin (▲) ($P < 0.05$), ketanserin (▼) ($P < 0.05$), (±)-propranolol (■) ($P < 0.01$), pizotifen (×) ($P < 0.05$), mCPP (◆) ($P < 0.001$).

Effects of 5-hydroxytryptamine antagonists on head shakes

Results in Figure 1b show that all 10 antagonists and also mCPP inhibited head shakes induced by 25 mg kg^{-1} car-

Table 1 Effects of 1-3(chlorophenyl)piperazine (mCPP) and of 5-hydroxytryptamine (5-HT) antagonists at high dosage on 2 h food intake of 20–21 h food-deprived rats

Treatment	*Food intake* ($g\,2h^{-1}$)
0.9% NaCl (8)	8.0 ± 0.6
Altanserin 71.1 $\mu mol\,kg^{-1}$ (4)	6.5 ± 0.6
mCPP 18.4 $\mu mol\,kg^{-1}$ (8)	2.1 ± 0.5*
0.9% NaCl (8)	8.3 ± 1.8
Ketanserin 50 $\mu mol\,kg^{-1}$ (6)	7.5 ± 1.9
mCPP 18.4 $\mu mol\,kg^{-1}$ (9)	2.7 ± 0.7*
0.9% NaCl (10)	6.9 ± 0.5
LY 53857 20 $\mu mol\,kg^{-1}$ (5)	6.2 ± 0.6
mCPP 18.4 $\mu mol\,kg^{-1}$ (9)	3.4 ± 0.4*
0.9% NaCl (10)	8.3 ± 0.6
Metergoline 2.5 $\mu mol\,kg^{-1}$ (6)	7.1 ± 0.9
mCPP 18.4 $\mu mol\,kg^{-1}$ (6)	3.1 ± 0.7*
0.9% NaCl (7)	6.4 ± 0.4
Methysergide 84.6 $\mu mol\,kg^{-1}$ (4)	5.0 ± 0.6
mCPP 18.4 $\mu mol\,kg^{-1}$ (10)	3.0 ± 0.8*
0.9% NaCl (13)	5.8 ± 0.4
Mianserin 20 $\mu mol\,kg^{-1}$ (7)	6.2 ± 0.4
0.9% NaCl (10)	7.3 ± 0.7
1-NP 8.2 $\mu mol\,kg^{-1}$ (8)	6.8 ± 1.5
mCPP 18.4 $\mu mol\,kg^{-1}$ (10)	2.7 ± 1.0*
0.9% NaCl (8)	8.1 ± 1.1
(±)-Propranolol 174 $\mu mol\,kg^{-1}$ (4)	6.1 ± 1.5
mCPP 18.4 $\mu mol\,kg^{-1}$ (8)	1.7 ± 0.6*
0.9% NaCl (8)	8.2 ± 1.8
Ritanserin 80 $\mu mol\,kg^{-1}$ (5)	6.4 ± 1.3
mCPP 18.4 $\mu mol\,kg^{-1}$ (7)	2.7 ± 0.7*
0.9% NaCl (16)	8.4 ± 1.8
Pizotifen 5 $\mu mol\,kg^{-1}$ (7)	7.3 ± 2.2
mCPP 18.4 $\mu mol\,kg^{-1}$ (10)	3.5 ± 2.0*

Results are shown as means ± s.e.mean with numbers of rats in parentheses. * $P < 0.01$ vs appropriate 0.9% NaCl-treated group by Dunnett's test following significant 1-way ANOVA. 1-NP = 1-naphthyl piperazine.

bidopa and 100 $mg\,kg^{-1}$ 5-HTP. Significant regressions were obtained between dose of antagonist and corresponding response inhibition (Figure 1b) with the exception of LY 53857. None of the antagonists caused head shakes when given alone. Calculated ID_{50} values are given in Table 2 and were in order: metergoline = ketanserin < altanserin < ritanserin < mianserin < pizotifen < methysergide = LY 53857 < (±)-propranolol = 1-NP < mCPP. Figure 2b shows that the correlation between these values and the *in vitro* affinities (pK_D) of the drugs for the 5-HT_2 receptor (given in Table 2) was significant ($P < 0.01$) but that corresponding correlations with affinities for 5-HT_1 receptor subtypes (Table 2) were not significant.

Comparison of effects of antagonists on 1-3(chlorophenyl)piperazine-induced hypophagia and head shakes

ID_{50} values against mCPP-induced hypophagia did not correlate significantly with ID_{50} values against head shakes ($r = 0.40$, d.f. 8, NS).

Inspection of Figure 2a and b reveals that ID_{50}s for inhibition of hypophagia and head shakes correlated significantly with affinities (pK_D) for 5-HT_{1C} and 5-HT_2 sites respectively. However, ID_{50} values for methysergide and ritanserin were rather larger than predictable from the overall relationships between ID_{50} and affinity (approximately 3 fold for hypophagia in both cases and 6 fold (methysergide) and 3 fold (ritanserin) for head shakes). These common discrepancies from linearity largely cancel each other out when ratios of the two ID_{50} values are plotted against the corresponding ratio of affinities (Figure 2c) so that the correlation between the ratios is more significant than that between ID_{50} values against hypophagia and affinities for 5-HT_{1C} sites. Significant correlations were not obtained when the ratios of the ID_{50}s were plotted against the ratios of the affinities for the 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1D} sites to the affinities for 5-HT_2 sites.

Discussion

The present findings strengthen previous evidence (Kennett & Curzon, 1988a) that mCPP causes hypophagia by activating 5-HT_{1C} receptors. Thus, ID_{50} values for inhibition of the hypophagia by 5-HT antagonists correlated significantly with published *in vitro* 5-HT_{1C} receptor affinities but not with affinities for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} or 5-HT_2 receptors. Affinities for 5-HT_2 receptors correlated significantly with ID_{50}s for inhibition of 5-HTP-induced head shakes, which depend on activation of 5-HT_2 sites (Bedard & Pycock, 1977; Yap & Taylor, 1983; Niemegeers *et al.*, 1983).

When we began this study we expected that some antagonists would show a relatively week relationship between ID_{50} against mCPP-induced hypophagia and affinity for 5-HT_{1C}

Table 2 ID_{50} values for inhibition of 1-3(chlorophenyl)piperazine (mCPP)-induced hypophagia and carbidopa and 5-hydroxytryptophan (5-HTP)-induced head shakes and corresponding affinities for 5-HT receptor subtypes

Drug	ID_{50} ($\mu mol\,kg^{-1}$) *vs hypophagia*	ID_{50} ($\mu mol\,kg^{-1}$) *vs head shakes*	$\frac{ID_{50}\ (hypophagia)}{ID_{50}\ (head\ shakes)}$	*Affinity* ($-\log M^{-1}$) 5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}	5-HT_2
Metergoline	0.50 (0.30–0.94)	0.084 (0.025–2.5)	5.9	8.10	7.39	9.19	9.09	9.03
Pizotifen	1.11 (0.45–2.65)	1.25 (0.52–2.71)	0.89	6.10	5.50	8.1	5.65	7.8
1-NP	4.1 (2.5–5.7)	7.06 (6.4–7.9)	0.57	7.18	6.56	8.24	7.83	7.24
Mianserin	8.4 (4.8–13.6)	0.48 (0.4–0.6)	17.5	6.03	5.21	8.00	6.37	8.08
Ritanserin	9.6 (3.8–24.4)	0.40 (0.19–0.79)	24.4	5.37	4.00	8.64		9.25
Methysergide	11.0 (7.4–16.0)	2.30 (1.2–4.5)	4.82	7.63	5.82	8.61	8.42	8.57
LY 53857	12.8 (6.6–24.6)	2.40 (1.2–4.5)	5.42	6.41	5.53	8.08		7.34
Altanserin	24.9 (18.5–31.6)	0.144 (0.075–0.28)	173	5.55	5.98	6.93		8.58
Ketanserin	32.2 (22.2–47.2)	0.09 (0.045–0.18)	358	5.86	5.72	7.01	6.00	8.86
(±)-Propranolol	142.0 (101.0–181.7)	6.7 (4.8–9.4)	21.2	6.48	7.07	6.23	5.39	6.46

95% confidence limits of ID_{50} values are shown in parentheses. Affinity data from Hoyer (1988), Schoeffter & Hoyer (1989), Schoeffter *et al.* (1988) and Schlicker *et al.* (1989) using membranes from pig brain cortex (5-HT_{1A}), rat brain cortex (5-HT_{1B}), pig choroid plexus (5-HT_{1C}), pig caudate nucleus (5-HT_{1D}) and rat brain cortex (5-HT_2).
The ID_{50} values can be converted from $\mu mol\,kg^{-1}$ to $mg\,kg^{-1}$ by multiplying by the following: metergoline 0.403, pizotifen 0.395, 1-NP 0.249, mianserin 0.251, ritanserin 0.478, methysergide 0.469, LY 53857 0.501, altanserin 0.562, ketanserin 0.409, (±)-propranolol 0.296, mCPP 0.270.

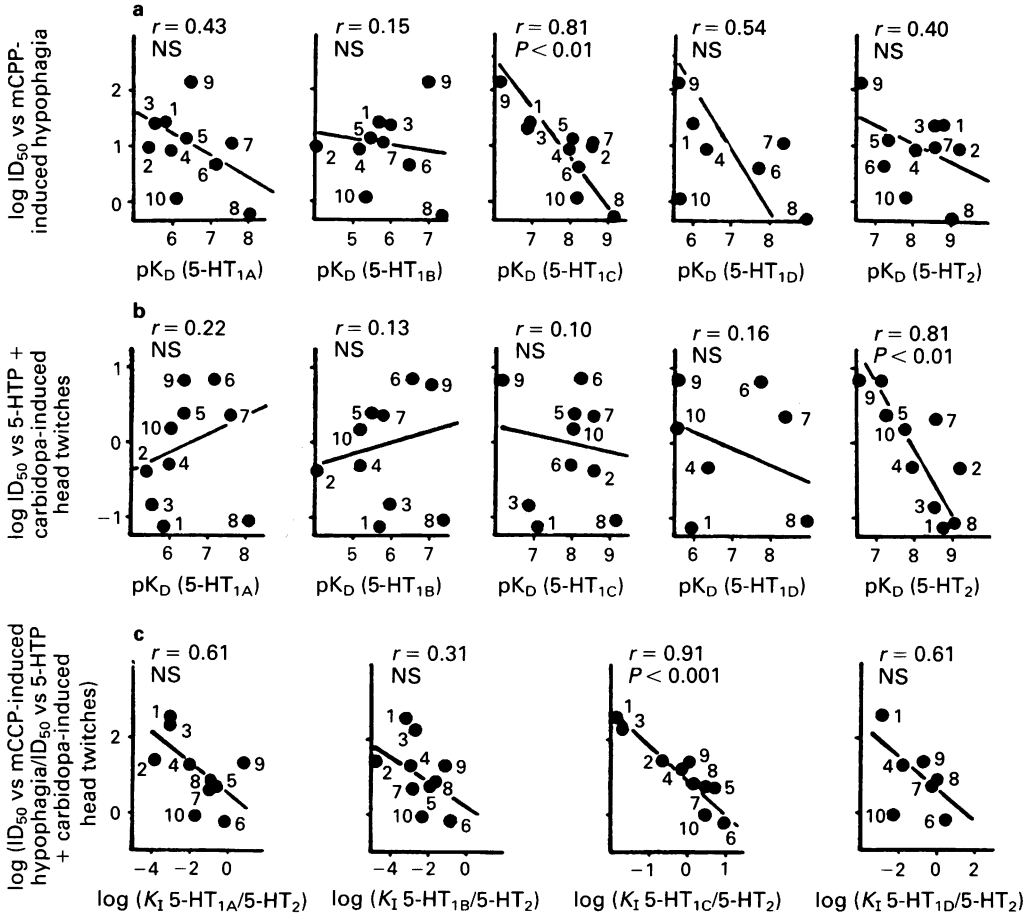

Figure 2 (a) Correlations between log [ID_{50} (μmol kg^{-1}) vs 1-3(chlorophenyl)piperazine (mCPP)-induced hypophagia] and $-$log affinity (pK_D) for 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$ or 5-HT$_2$ receptors (data from Table 2). The following 10 antagonists were used: (1) ketanserin, (2) ritanserin, (3) altanserin, (4) mianserin, (5) LY 53857, (6) 1-naphthyl piperazine, (7) methysergide, (8) metergoline, (9) ($\pm$)-propranolol and (10) pizotifen. Correlation coefficients (r) and statistical significances of correlations are given. NS = not significant. (b) Correlations between log [ID_{50} (μmol kg^{-1}) vs 5-hydroxytryptophan (5-HTP) + carbidopa-induced head shakes] and $-$log affinity (pK_D) for 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$ and 5-HT$_2$ receptors for each of 10 antagonists (key as in (a)). (c) Correlations between log [ID_{50} vs mCPP-induced hypophagia/ID_{50} 5-HTP + carbidopa-induced head twitches] and logs of the following ratios of affinities (K_I) for receptors: 5-HT$_{1A}$/5-HT$_2$, 5-HT$_{1B}$/5-HT$_2$, 5-HT$_{1C}$/5-HT$_2$ or 5-HT$_{1D}$/5-HT$_2$. Values for each of the 10 antagonists in (a) and (b). (Key as in (a)).

sites. This was found for ritanserin, methysergide and pizotifen. Rather similar deviations were found for the first two drugs in the relationship between ID_{50} against 5-HTP-induced head shakes and affinity for 5-HT$_2$ sites. These common deviations presumably reflect aspects of the metabolism or disposition of the drugs which comparably affect their availability to both central 5-HT$_{1C}$ and 5-HT$_2$ sites *in vivo* but not *in vitro*. They therefore tend to cancel out when the ratios of ID_{50} values for inhibition of the two behavioural effects are calculated. Thus, the ratios correlate more significantly with the corresponding ratios of 5-HT$_2$ to 5-HT$_{1C}$ receptor affinities than do the respective ID_{50} values with the affinities for the individual sites. This further strengthens the hypothesis that mCPP-induced hypophagia is mediated by 5-HT$_{1C}$ receptor activation. A somewhat similar procedure has been used by Leysen *et al.* (1978) when studying the binding of neuroleptics at 5-HT receptors.

The inability of ritanserin to antagonize completely mCPP-induced hypophagia was striking; rather similar but less detailed findings are reported for the inhibition of quipazine-induced hypophagia by both ritanserin and ketanserin (Hewson *et al.*, 1989) and for the inhibition of fenfluramine-induced hypophagia by ritanserin (Neill & Cooper, 1989). Partial inhibition could conceivably be due to the ritanserin being a partial agonist but this seems unlikely as it did not cause hypophagia when given alone. Another possibility which cannot be excluded is that (uniquely among the antagonists tested) it discriminates between two sites which are simultaneously stimulated by mCPP to induce hypophagia.

In agreement with Simansky & Schechter (1987), mCPP blocked 5-HT-induced head shakes. It therefore appears to be not an agonist but an antagonist at 5-HT$_2$ sites. This is consistent with its inhibitory effect on the stimulation of cortical phosphoinositide hydrolysis by 5-HT (Conn & Sanders Bush, 1987).

In the present study, ketanserin and ritanserin inhibited mCPP-induced hypophagia with ID_{50} values of 32.2 and 9.6 μmol kg^{-1}. The failure of Aulakh *et al.* (1989) to antagonize the hypophagia with 10.4 μmol kg^{-1} ritanserin (albeit under unspecified conditions) is therefore surprising. The finding of Klodzinska & Chojnacka-Wojcik (1990) that TFMPP-induced hypophagia was opposed by 6.2 and 1.05 μmol kg^{-1} respectively of ketanserin and ritanserin may reflect their use of freely feeding rats as, under these conditions, feeding is significantly more sensitive to the hypophagic effects of mCPP (and RU 24969) and hence possibly also to their antagonists, than when food-deprived animals are used as in the present study (Table 3). A similar argument may also explain why doses of ketanserin and ritanserin needed to antagonize hypophagias induced by fenfluramine (Hewson *et al.*, 1988), quipazine (Hewson *et al.*, 1989) and DOI (Schechter & Simansky, 1988) were less than those we found to inhibit

Table 3 Calculated ED_{50} values (and 95% confidence limits) for the hypophagic response to 1-3(chlorophenyl)piperazine (mCPP) and RU 24969 in freely feeding and food-deprived rats

	ED_{50} (μmol kg^{-1})	
Drug	*Freely feeding*	*Food-derived*
mCPP	2.56 (1.04–5.9)	10.3 (8.4–12.5)*
RU24969	2.37 (1.1–5.2)	16.3 (9.2–32.3)*

Significant difference * $P < 0.001$ by t test following comparison of significant dose-response regression lines using data from Kennett *et al.* (1987) and Kennett & Curzon (1988a) expressed as percentages and Arcsin-transformed (Winer, 1971) as outlined by Bowman & Rand (1980).

mCPP-induced hypophagia as these groups used fed rats exposed to a palatable diet.

Most of these findings by other groups were taken to suggest that the hypophagic effects reported resulted from activation of 5-HT_2 receptors. However, the above comments and the present findings point strongly to the involvement of 5-HT_{1C} sites. It is also relevant that the doses of ketanserin and ritanserin required for inhibition of hypophagia were much greater than those we found necessary for inhibition of 5-HT_2 receptor-mediated head shakes. Furthermore, Neill & Cooper (1989) did not confirm the inhibitory effect of ketanserin on fenfluramine-induced hypophagia reported by Hewson *et al.* (1988).

The present findings strengthen our previous evidence (Kennett & Curzon, 1988a,b) that mCPP-induced hypophagia occurs through the activation of 5-HT_{1C} receptors. Our work also suggested that 5-HT_{1B} receptors were needed for the response to occur in the rat (Kennett & Curzon, 1988a). As 5-HT_{1D} receptors have a similar distribution to rat 5-HT_{1B} receptors in species in which these are absent (man, pig, calf) (Waeber *et al.*, 1988) a similar requirement for 5-HT_{1D} sites may occur in the latter species.

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