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m-Chlorophenylpiperazine: A Central Serotonin Agonist Causing Powerful Anorexia in Rats

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Summary. Meta-chlorophenylpiperazine inhibited serotonin and noradrenaline uptake by synaptosomes to the same extent with IC_{50} of 1.3×10^{-6} M and 5.8×10^{-6} M respectively. Dopamine uptake was lesss affected by meta-chlorophenylpiperazine (IC_{50} of 2.2×10^{-5} M). Unlike *d*-amphetamine and *d*-fenfluramine, the drug did not significantly increase monoamine release in synaptosomal preparations. On the other hand, metachlorophenylpiperazine showed an IC_{50} of 620 nM in displacing ^{3}H-5HT binding to brain membranes. Meta-chlorophenylpiperazine produced a dose-dependent reduction of food intake and this effect was prevented by a pretreatment with methergoline, a serotonin antagonist. The effect of metachlorophenylpiperazine was not modified by an intraventricular injection of 6-hydroxydopamine, electrolytic lesions of nucleus medianus raphe or ventral noradrenergic bundle, nor by a pretreatment with penfluridol, propranolol or phentolamine. The data suggest that the decrease of food intake induced by metachlorophenylpiperazine depends on its ability to act as a serotonin agonist in the brain. The specificity of the effects on serotonin suggests that this compound could prove an important tool for studies aimed at elucidating the functional role of serotonin in the central nervous system.

Key words: Serotonin – Receptor stimulation – Metachlorophenylpiperazine – Anorexia – Catecholamines.

Introduction

Previous studies have shown that meta-chlorophenylpiperazine (mCPP) inhibits the uptake of serotonin 5 HT) in rat platelets (Garattini et al., 1976).

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These data, together with the fact that mCPP can completely prevent the decrease of brain 5 HT induced by fenfluramine (Garattini et al., 1976) suggest that it might inhibit serotonin uptake in the central nervous system.

Since mCPP causes powerful anorexia in rats, (Garattini, 1979) we wondered whether this effect was related to its ability to interfere with 5 HT mechanisms. Thus, the effects of mCPP on the uptake and release of labelled 5 HT in synaptosomes and its ability to displace ^{3}H-5 HT binding to rat brain membranes were studied. The effect on food intake was also evaluated in rats subjected to electrolytic lesions of the nucleus medianus raphe, which produce extensive destruction of 5 HT neurons in the brain (Samanin et al., 1972) or in rats pretreated with methergoline, a central 5 HT antagonist (Mawson and Whittington, 1970).

The specificity of the interaction with 5 HT was assessed by studying the effects of mCPP on catecholamine uptake and release and its anorectic activity in animals subjected to various procedures known to affect catecholamine (CA) mechanisms.

Materials and Methods

Female CD_1 (Charles River, Italy) rats weighing 180–220 g were used. The animals were housed in plastic cages at a constant room teperature (21 ± 1° C) and relative humidity (50%).

In vitro Studies

a) Uptake and Release by Brain Synaptosomes. Purified synaptosomes were obtained from pooled striata for dopamine (DA) uptake or whole brain exluding cerebellum and striata for noradrenaline (NA) and serotonin (5 HT) uptake as described by Gray and Whittaker (1962) and diluted with Krebs-Henseleit buffer to obtain a final protein concentration of 0.5–1 mg/ml. For uptake studies, samples of 0.6 ml were incubated at 30° C (or 4° C to assess passive diffusion) with 0.1 μM ^{14}C-monoamine (5 HT-NA-DA) (≈ 50 mCi/mmol, Radiochemical Centre, Amersham) for 5 min, and drugs

were added during a pre-incubation period of 5 min. The reaction was stopped by cooling the tubes in ice and adding 0.5 ml of ice-chilled Krebs-Henseleit buffer. Samples were then filtered through Millipore filters (0.65 μ pore size), washed with Krebs-Henseleit buffer, dissolved in Bray's solution and counted for radioactivity. 5 HT release was studied using a superfusion system (Raiteri et al., 1974) to minimize interference from the re-uptake mechanism. Portions of 1 ml of synaptosome suspension, pre-labelled by incubation with ^{14}C-5 HT 0.1 μM for 15 min, were filtered and placed at the bottom of the superfusion chambers. Drugs were dissolved in Krebs-Henseleit solution and superfused from 0–20 min, at a constant rate of 0.5 ml/min. The effluent and the filter were counted for radioactivity in a Packard Tri Carb 2425 liquid scintillation spectrometer. The counting efficiency for ^{14}C was checked for each sample using external standardization, and averaged 85% for unquenched samples.

For CA release, synaptosome suspension, prelabelled by incubation with 0.1 μM ^{14}CA for 15 min, was centrifuged at 17,000 × g for 10 min, and the pellet was gently resuspended in fresh Krebs-Henseleit buffer.

Portions of 0.6 ml were preincubated for 5 min at 37° C to allow equilibration. At the end of this period (0 time) drugs were added and the mixtures were then incubated for 20 min. Samples were filtered and counted as described for uptake studies. % release was calculated as follows:

$$\frac{\text{CPM control 20 min} - \text{CPM drug 20 min}}{\text{CPM control 0 min}} \times 100$$

More detailed information about the methodology of the studies on uptake and release of monoamines is given elsewhere (Mennini et al., 1978).

b) Binding of ^{3}H-Serotonin. Binding of ^{3}H 5 HT was studied in telencephalon (striata excluded) membrane preparations as described by Bennett and Snyder (1976). Proteins were adjusted to 1 mg/ml using a Bio Rad Protein assay. The stock membrane preparation was maintained at −20° C until assay. After brief homogenization in a glass-teflon homogenizer 2 ml portions were preincubated at 37° C for 10 min, with or without 10 μM unlabelled 5 HT (to determine non specific binding which, in our conditions, was about 40% of total ^{3}H 5 HT binding). 7 nM ^{3}H-5 HT (12 Ci/mmol, Radiochemical Centre, Amersham) and the test drug were then simultaneously added and the mixture was incubated for 15 min. After addition of 5 ml of 0,05 M Tris HCl buffer pH 7.4, the samples were rapidly vacuum filtered through Whatman GF/B filters. Each filter was then rinsed twice with 5 ml of Tris buffer and put in counting vials containing 10 ml of Bray's solution. The counting efficiency for ^{3}H was 50%.

c) IC_{50} were calculated as the molar concentration of drug causing 50% uptake inhibition or 50% displacement of specific ^{3}H serotonin binding, from log-dose effect plots, based on 4 points at least Data given in the text are mean values of repeated assay, whose results varied less than 25%.

In vivo Studies

a) Electrolytic Lesions and Microinjections. Electrolytic lesions were made to some rats by applying a 1.5 mA (VNB lesions) or 2.5 mA (MR lesions) anodal current for 15 s through a stainless steel electrode insulated except for the tip (tip 0.5 mm, 0.3 mm in diameter). The lesions were made according to the following coordinates given in the König and Klippel rat brain atlas (1963): Ventral noradrenergic bundle (VNB bilateral): A 1, L 1.3, H −1.9 nucleus medianus raphe (MR): A 0.4, L 0, H-2.6. Control rats were sham-operated. Another group of animals received 200 μg of 6-hydroxydopamine (6-OHDA) intraventricularly as described by Noble et al. (1967), dissolved in 20μl of saline containing ascorbic acid (1 mg/ml). Thirty minutes before the 6-OHDA injection the animals were injected with 50 mg/kg i.p. of pargyline since this procedure increases the effect of 6-OHDA on dopamine (Breese and Traylor, 1971). Control animals received pargyline and were injected with an equal volume of the vehicle. All the animals were used 12–15 days later for food intake testing. Animals taken randomly were used for assay of catecholamines (Chang, 1964; Laverty and Taylor, 1968) and serotonin (Curzon and Green, 1970) in the forebrain. The lower part of the brainstem (midbrain + pons + medulla oblongata) of all the animals with electrolytic lesions was used for histological examination of the lesions. The tissue was kept in 10% formalin and after fixation, sections were cut at 40 μ and stained with cresyl violet.

b) Food Intake. The animals were caged singly and trained to take their daily food (Altromin MT pellets for rats) during 4 out of 24 h (from 10.00 a.m. to 2.00 p.m.). They developed a consistent food intake habit within 10 days. mCPP hydrochloride was administered intraperitoneally as a solution prepared immediately before the experiments. 30 min after drug administration, the animals were placed in a cage containing a weighed amount of food. One hour later they were removed and food was re-weighed to the nearest 0.1 g. The difference between the original amount and food left constituted the intake.

One group of intact animals was injected intraperitoneally with methergoline maleate (1 mg/kg) or with an equal volume of vehicle (ascorbic acid 2.5%). Three hours later, they received intraperitoneally one of 3 doses of mCPP (1.25, 2.5 and 5 mg/kg) or saline and 30 min later were tested for food intake. In another experiment, intact animals received penfluridol (2.5 mg/kg p.o.), phentolamine methane sulphonate (5 mg/kg i.p.) or propranolol hydrochloride (5 mg/kg i.p.) respectively 18 h, 1 h and 30 min before injection of 2.5 mg/kg i.p. of mCPP. mCPP was also administered at a dose of 2.5 mg/kg i.p. to the animals lesioned in VNB or MR or treated with 6-OHDA 30 min before food intake testing. The data were analyzed statistically by ANOVA (2 × 2 or 2 × 3) factorial analysis followed by Tukey's test (Kirk, 1968).

c) Drugs. mCPP HCl was kindly supplied by Dr. B. Silvestrini (F. Angelini, Roma). 6-OHDA (Roche, Milan), methergoline maleate (Farmitalia, Milan), penfluridol (Janssen, Beerse, Belgium), phentolamine methane sulphonate (Ciba-Geigy, Milan), propranolol HCl (Icpharma, Milan), *d*-LSD (Sandoz, Basel), cyproheptadine (Merck Sharph and Dohme, Pavia), *d*-fenfluramine (Servier, Suresnes), *d*-amphetamine (Recordati, Milan).

Results

In vitro Studies

mCPP inhibited 5 HT and NA uptake by synaptosomes to the same extent, with IC_{50} of 1.3×10^{-6} M and 5.8×10^{-6} M respectively. mCPP inhibited the DA uptake by 50% at a concentration of 2.2×10^{-5} M.

The data on monoamine release are shown in Fig. 1. The effects of mCPP were compared with those of *d*-amphetamine (for dopamine) and *d*-fenfluramine (for noradrenaline and serotonin). These drugs are known to be active in releasing monoamines in various preparations (Ziance and Rutledge, 1972; Fuxe et al., 1975; Garattini et al., 1975; Glowinski, 1970; Heikkila et al., 1975).

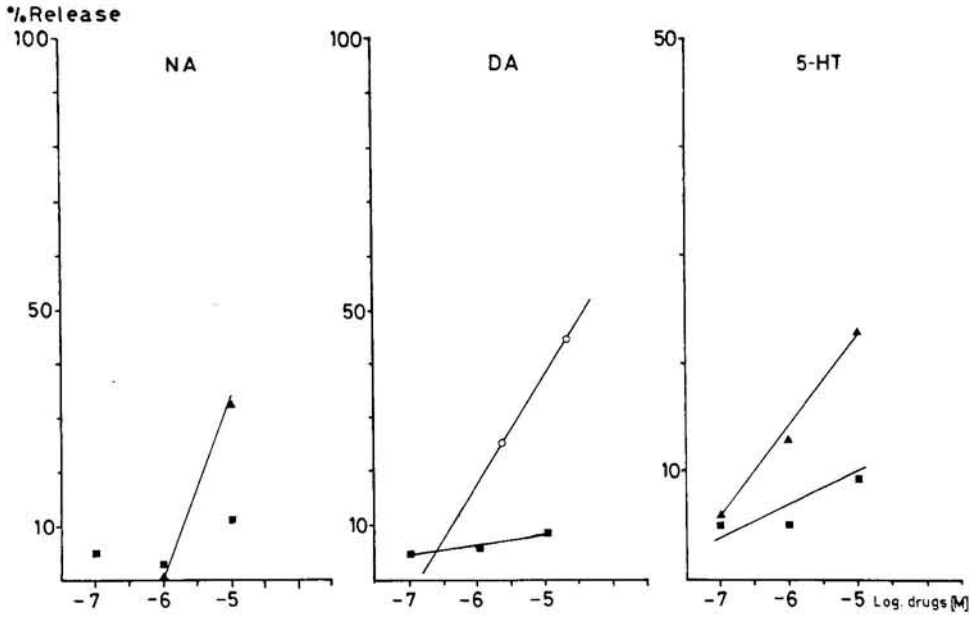

Fig. 1. Effect of mCPP, *d*-Fenfluramine and *d*-Amphetamine on ^{14}C-amine release from brain synaptosomes. (▲) *d*-Fenfluramine; (○) *d*-Amphetamine; (■) mCPP. Percentage of amine released during 20 min is plotted versus logarithm of the molar concentration of drugs. % of catecholamine release was calculated as described in the text. The release from controls during 20 min was negligible (less than 10%). % of *5 HT* release was calculated as the net increase over the control value, which was about 50%. Figures are the mean value of repeated assays, whose results varied less 25%

In view of the fact that, under the present experimental conditions, significant differences are found only with values 10% higher than those of controls, mCPP had little noteworthy effects on monoamine release. At concentrations ranging between 10^{-6} and 10^{-5} M, *d*-amphetamine and *d*-fenfluramine markedly increased the release of DA and that of NA and 5 HT respectively. mCPP potently displaced ^{3}H-5 HT binding to brain membranes, showing an IC_{50} of 620 nM. The IC_{50} values obtained in the present study for *d*-LSD and cyproheptadine (18 nM and 1.3 μM) are in the same range as those obtained by Bennett and Snyder (1976) indicating the comparability of results obtained in the two systems.

Table 1. Effect of mCPP and methergoline pretreatment on food intake of rats

Treatment	mg/kg i.p.	Food intake (g/rat/h)	
		Control	Pretreatment[a]
Saline	–	8.4 ± 0.8	8.4 ± 1.0
mCPP	1.25	4.3 ± 0.6*	6.9 ± 0.4
mCPP	2.50	1.9 ± 0.4*	4.9 ± 0.8**
mCPP	5.00	0.9 ± 0.6*	4.1 ± 0.3**

Each value represents the mean ± S.E. of 1 h food intake for 6 animals

[a] = the animals received methergoline (1 mg/kg i.p.) 3 h before mCPP

* $P < 0.01$ compared with saline

** $P < 0.01$ compared with control

In vivo Studies

mCPP produced a dose dependent decrease of food intake (Table 1) without gross behavioral changes, except at the highest dose at which animals showed some motor signs consisting mainly of hindlimb adduction, piloerection, back rigidity and backward locomotion.

The effect of mCPP on food intake was significantly counteracted by pretreatment with methergoline (Table 1). The motor syndrome induced by the highest dose of mCPP was also prevented by such pretreatment. Neither penfluridol, phentolamine nor propranolol significantly modified the effect of mCPP on food intake (Table 2). The effect of mCPP was unchanged in animals lesioned in the MR or treated with 6-OHDA; the anorectic activity of mCPP was significantly enhanced in animals lesioned in the VNB (Table 3). Histology showed that the lesions were generally confined to the desired area; a few animals in which the lesion was not properly located were not included in the final evaluation of the results. VNB lesions produced a significant decrease of NA in the forebrain without affecting either DA or 5 HT (NA levels in control and

Table 2. Effect of mCPP on food intake of rats pretreated with penfluridol, phentolamine or propranolol

Pretreatment	Food intake (g/rat/h)	
	Saline	mCPP
Vehicle	9.2 ± 0.8	3.9 ± 1.1*
Penfluridol	9.5 ± 0.4	1.4 ± 0.8*
Control	10.4 ± 0.9	4.6 ± 0.3*
Phentolamine	8.2 ± 1.4	3.1 ± 1.0*
Propranolol	9.3 ± 0.5	3.1 ± 0.8*

Each value represents the mean ± S.E. of 1 h food intake for 6 rats
Penfluridol (2.5 mg/kg p.o.), phentolamine (5 mg/kg i.p.) or propranolol (5 mg/kg i.p.) were adminstered respectively 18, 1 and 0.5 h before mCPP (2.5 mg/kg/i.p.)
* $P < 0.01$ compared with saline

Table 3. Effect of mCPP on food intake of rats with electrolytic lesions of the ventral noradrenergic bundle (VNB) or nucleus medianus raphe (MR) or treated with 6-hydroxy-dopamine (6-OHDA)

Treatment (mg/kg i.p.)	Food intake (g/rat/h)			
	Control	VNB lesioned	MR lesioned	6-OHDA
Saline	8.4 ± 0.4	7.6 ± 0.6	8.1 ± 0.7	7.2 ± 0.6
mCPP 2.5	3.0 ± 0.6	0.8 ± 0.2*	1.8 ± 0.8	2.6 ± 0.5

Each value represents the mean ± S.E. of 6 animals
* $P < 0.01$ compared with control
Control: sham operated or vehicle treated

lesioned rats were respectively 0.40 ± 0.02 and 0.25 ± 0.02 μg/g ± S.E.); MR lesions produced a selective decrease of forebrain 5 HT (5 HT concentrations were 0.36 ± 0.01 in control and 0.09 ± 0.02 μg/g in MR lesioned rats). The intraventricular injection of 6-OHDA caused a marked decrease of catecholamine levels without significant effects on 5 HT (control NA 0.58 ± 0.04 and DA 7.4 ± 0.2 μg/g; 6-OHDA NA 0.15 ± 0.04 and DA 1.50 ± 0.37 μg/g).

Discussion

mCPP inhibits synaptosomal uptake of 5 HT and NA to the same extent while the effect of DA is less marked. The effects on 5 HT uptake is not likely to contribute significantly to its anorectic activity. In fact, at a dose of 2.5 mg/kg, which markedly inhibits food intake (Garattini, 1979), mCPP only poorly inhibits 5 HT uptake in the rat brain as indicated by its poor effectiveness in counteracting the reduction of brain 5 HT induced by fenfluramine (F) (Samanin et al., 1979a); 5 HT uptake inhibitors have been shown to specifically block the effect of F on brain 5 HT (Ghezzi et al., 1973; Samanin et al., 1977a).

In a previous study (Garattini et al., 1976a), mCPP completely prevented the effect of fenfluramine on brain 5 HT at a dose of 25 mg/kg, which is ten times that producing maximal anorectic effect. That the action of mCPP on 5 HT uptake does not contribute significantly to its anorectic activity is also suggested by the fact that the effect of 2.5 mg/kg was not significantly modified by raphe lesions. Raphe lesions markedly deplete brain 5 HT and reduce the effect of fenfluramine (Samanin et al., 1972), a 5 HT releaser and uptake inhibitor (Fuxe et al., 1975; Garattini et al., 1975). It has recently been suggested that 5 HT uptake inhibition is not a relevant mechanism for the anorexia induced by drugs acting on 5 HT (Samanin et al., 1979a).

In the present experiments, methergoline caused marked reduction of the mCPP effect, supporting the hypothesis that 5 HT receptor stimulation is important for its effects on food intake. Considering the ability of mCPP to displace ^{3}H-5 HT specifically bound to brain membranes, it is likely that it acts as a 5 HT agonist in the brain, this being important for its anorectic activity.

mCPP can also inhibit NA uptake but this is not likely to contribute to mCPP anorexia since neither VNB lesions, which completely prevent amphetamine anorexia (Ahlskog and Hoebel, 1973; Samanin et al., 1977b), nor phentolamine, propranolol or 6-OHDA significantly counteracted the effect of mCPP on food intake. On the contrary, VNB lesions tended to increase the anorectic activity of mCPP. Whether this is due to a VNB induced supersensitivity to the sites specifically involved in the effect of mCPP or to other factors remains to be clarified.

The specificity of the interaction of mCPP with 5 HT, at anorectic doses, is also illustrated by the fact that at 2.5 mg/kg the drug significantly reduced brain 5 HIAA concentrations without affecting striatal HVA (Samanin et al., 1979b) and by the finding that penfluridol does not modify its anorectic activity.

In conclusion, mCPP is a powerful anorectic agent in rats; this effect very probably depends on its ability to act as a 5 HT agonist in the central nervous system. The specificity of the effects on 5 HT suggests that this compound could prove an important tool for studies aimed at elucidating the functional role of 5 HT in the CNS.

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