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N-*o*-Methoxyphenylpiperazine: a simple blocker of dopaminergic receptors in the brain

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N-*o*-Methoxyphenylpiperazine (MPP) is a moderately effective in vivo blocker of dopaminergic receptors. Its ability to increase the concentration of rat brain homovanillic acid (HVA) and the resulting time course for HVA were similar to the actions of clozapine. The increased concentration of HVA did not result from decreased outflow from brain because HVA also rapidly decreased after a subsequent injection of pargyline. MPP blocked the circling behaviour caused by apomorphine in mice with a unilateral striatal lesion, and MPP and apomorphine reciprocally blocked the occurrence of stereotypy and increased HVA in rats. Diazepam partially prevented the MPP-induced elevation of HVA. Thus, both biochemical and pharmacological evidence indicate the dopaminergic blocking action of MPP.

Although agonists of dopaminergic receptors are either simple (dopamine, *N*-methyldopamine) or complex (apomorphine) in chemical structure, only relatively complex structures have been reported to exhibit antagonist activity. The present report shows that *N*-*o*-methoxyphenylpiperazine (MPP) is an effective blocker of striatal dopaminergic receptors in rat brain and is apparently the simplest chemical structure known to exert dopaminergic blocking activity: the molar weights of MPP chlorpromazine and sulpiride are, respectively, 192, 319 and 341. This activity of MPP was revealed by several methods commonly used to demonstrate the dopaminergic blocking action of neuroleptics.

MPP is a drug that exhibited pronounced antihypertensive and weak sympatholytic activities in experimental animals (Morphis et al 1959). Blood pressures were also lowered in hypertensive patients by MPP (Page et al 1959), and this effect was sometimes accompanied by a strong sedation, and after large repeated doses, by disorientation and stupor. The structure of MPP has also been incorporated into neuroleptic drugs where, as the information presented here indicates, it is probably the functional moiety (Turek et al 1970; Castaner 1976).

MATERIALS AND METHODS

Drugs were administered intraperitoneally, except where noted, as solutions of their hydrochloride salts in 0·9% saline to male Long-Evans rats (Simonsen Labs.), 150–200 g. Following decapitation, paired striata or whole brains (minus cerebella) were homogenized in 0·4 M perchloric acid. Each neutralized extract was passed over a 0·65 × 3·0 cm column of Amberlite resin CG-50. Dopamine was eluted with 7·0 ml of 2% boric acid (Minard & Grant 1972) and analysed according to the I_2-trihydroxyindole method of Welch & Welch (1969). Homovanillic acid (HVA) was extracted from the column effluent and measured according to Murphy et al (1969). Many of the drugs were synthesized at Abbott Laboratories; others were purchased from commercial sources.

Unilateral intrastriatal injections of 6-hydroxydopamine (15 μg) were made freehand (Pycock et al 1975). Only those animals displaying proper turning behaviour to intraperitoneal injections of (+)-amphetamine (5 mg kg^{-1}) and apomorphine (2 mg kg^{-1}) were used.

RESULTS AND DISCUSSION

As may be seen in Table 1 for the simplest chemical structures, only MPP, its *N*-methyl derivative, and N^1-*o*-chlorophenyl-N_2-methylpiperazine increased the concentration of HVA in whole rat brain, an action that is known to mirror the occurrence of striatal dopaminergic blockade. Larger structures, *o*-ethoxyphenyl- and 1-naphthylpiperazines, exerted a similar activity. Increased HVA concentrations were also observed in the striata of five rats treated with MPP (150 μmol kg^{-1}, 34·2 mg kg^{-1} of MPP HCl) for 2 h (saline-treated/MPP-treated: HVA, 0·86 ± 0·05/2·76 ± 0·07; dopamine, 9·1 ± 0·4/8·1 ± 0·2 μg g^{-1}). *N*-*m*-Chlorophenylpiperazine was recently reported by Fuller & Snoddy (1977) to exert a related action, that of

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Table 1. Ability of derivatives of *N*-phenylpiperazine to elevate brain HVA.

Z	N_1-Z-piperazine R*	N_1-Z-N_4-methylpiperazine R*
o-Methoxyphenyl	3·6**	3·2**
Phenyl	0·9	1·0
o-Hydroxyphenyl	—	1·0
m-Methoxyphenyl	0·9	1·0
p-Methoxyphenyl	0·8	1·0
o-Methylphenyl	0·9	—
o-Chlorophenyl	—	2·6**
o-Ethoxyphenyl	3·4**	—
1-Naphthyl	—	2·8**

Groups of rats received intraperitoneal injections of saline (5 animals) or test drug (3 animals: 150 u mol kg^{-1}). They were decapitated 2 hours later and the brains (minus cerebella) were analysed.
* Ratio of concentration of HVA in brains of drug-treated group to that of saline-treated group.
** Difference in HVA concentrations between drug-treated and saline-control group was at $P < 0{\cdot}01$.

increasing the brain concentration of dihydroxyphenylacetic acid.

The time course for the effect of MPP upon the brain HVA resembled that seen with clozapine (Fig. 1), a known antischizophrenic drug.

Both MPP and clozapine also exhibited similar potencies when expressed as ED100 values, the dose required to increase the HVA concentration by 100% after 2 h, although both drugs were less potent in this test than chlorpromazine (ED100's of 35, 52 and 2·8 μmol kg^{-1} body weight, respectively). These values were obtained by measurement of brain HVA at 2 h after the administration of graded doses of drugs to groups of animals.

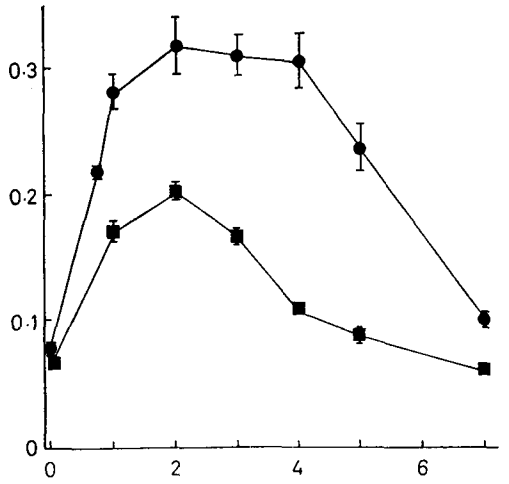

FIG. 1. Drug-induced elevation of HVA in rat brain. ■ MPP (75 μmol kg^{-1}). ● Clozapine (150 μmol kg^{-1}). There were five animals in each saline-control group (zero-time values) and three animals in each drug-treated group. Ordinate: HVA (μg g^{-1} brain). Abscissa: time (h).

MPP at 150 μmol kg^{-1} completely blocked the turning behaviour expected from apomorphine in mice having unilateral striatal lesions. Apomorphine (2 mg kg^{-1}) was administered 25 min after MPP and rotational behaviour was noted 15 min later. The brain HVA concentration was also elevated in intact mice by intraperitoneal administration of MPP at a dose of 150 μmol kg^{-1}.

To further demonstrate receptor blockade by MPP, the reciprocal antagonism with apomorphine was examined using stereotypy and elevation of HVA in rats as parameters. The test was conducted according to that described by Lahti et al (1972), and as shown in Table 2, the simultaneous injection of apomorphine completely blocked the increased value of HVA expected from MPP. This antagonism was also evident in the behaviour of the animals: those receiving the simultaneous injection of MPP and apomorphine quickly exhibited (within 15 min) a pronounced stereotyped behaviour which disappeared within 55 min. Those receiving only apomorphine exhibited the expected stereotypic response (sniffing, chewing, gnawing) for approximately 95 min. Thus, MPP blocked the stereotypy produced by apomorphine and, conversely, apomorphine blocked the HVA increase produced by MPP.

Diazepam is known to partially block the increase in brain HVA expected from neuroleptics, presumably by a GABA-mimetic action upon the striatal-nigral control pathway of dopamine synthesis (Keller et al 1976). The effect of MPP upon brain HVA was also prevented to a significant extent when diazepam was administered 30 min beforehand (Table 3). This observation was consistent with the previous evidence that the action of MPP is via dopaminergic blockade.

Table 2. Antagonism by apomorphine of the MPP-induced elevation of brain HVA.

Treatment	HVA concentration μg g^{-1} brain
Saline	0·071 ± 0·003
MPP	0·222 ± 0·007*
Apomorphine	0·059 ± 0·002*
MPP + apomorphine	0·077 ± 0·003

Groups of 5 rats received intraperitoneal injections of saline, MPP (75 μmol kg^{-1}), apomorphine (33 μmol kg^{-1}) or a single solution containing both drugs. Animals were decapitated 2 hours later and brains (minus cerebella) were analysed. Values are expressed as a mean ± s.e.m.
* $P < 0{\cdot}01$ with respect to saline-control value.

Table 3. Antagonism by diazepam of the MPP-induced elevation of brain HVA.

Treatment	HVA concn $\mu g\ g^{-1}$ brain
Saline/saline	0·079 ± 0·004
Diazepam/saline	0·068 ± 0·002
Saline/MPP	0·197 ± 0·015*
Diazepam/MPP	0·153 ± 0·005**

Groups of 5 rats received intraperitoneal injections of either diazepam (35 μmol kg^{-1}) or saline, and this was followed 30 min later by injections of either MPP (75 μmol kg^{-1}) or saline. Animals were decapitated 2 hours later, and brains (minus cerebella) were analysed. Values are expressed as a mean ± s.e.m.
* $P < 0.01$ for 0·197 vs 0·079.
** $P < 0.025$ for 0·153 vs 0·197.

Finally, it was shown that the increased concentration of HVA following the administration of MPP was not due to interference with its outward movement from the brain. This was demonstrated by the observation that the MPP-induced elevation in HVA rapidly declined after inhibition of further HVA synthesis by an injection of pargyline (Table 4). The experimental procedure was based upon that of Tagliamonte et al (1975).

Table 4. Null effect of MPP upon the outflow of HVA from brain.

Treatment	HVA concn $\mu g\ g^{-1}$ brain
Saline/saline at 100 min	0·064 ± 0·002
Saline/pargyline at 100 min	0·036 ± 0·003
MPP at 60 min	0·340 ± 0·009*
MPP/saline at 100 min	0·373 ± 0·008
MPP/pargyline at 100 min	0·166 ± 0·002**

Groups of 5 rats received intraperitoneal injections of either MPP (150 μmol kg^{-1}) or saline and this was followed 60 min later by injections of either saline or pargyline hydrochloride (36 μmol kg^{-1}). One group of MPP-treated animals was decapitated at 60 min, and the other groups were decapitated 40 min later; brains (minus cerebella) were analysed. There were 5 animals in each group. Values are expressed as a mean ± s.e.m.
* $P < 0.01$ for 0·340 vs 0·064.
** $P < 0.01$ for 0·166 vs 0·373.

The experiments described here provide evidence that MPP blocks unidentified dopaminergic receptors in the brain; its simple structure and its lack of stereoisomerism may offer further insights into the nature of such receptors. With the use of Dreiding Stereomodels, the two nitrogen atoms and the benzene ring of MPP can be superimposed upon those of chlorpromazine, and MPP itself has been incorporated into the structures of three neuroleptics; fluanisone, SU-17595A* (Turek et al 1970), and LR-511† (Castaner 1976). Moreover, *N*-methylpiperazine is a part of the clozapine molecule. Thus, MPP also offers an opportunity to design antischizophrenic drugs with desired pharmacological properties by structural additions to a simple active moiety.

*3-(4-*o*-Methoxyphenyl-1-piperazinyl)-4′-morpholinopropiophenone di HCl
†4-*p*-Fluorophenyl-5H-(*N*′-*o*-methoxyphenyl-piperazinoethyl)-4-oxazolin-2-one

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