Effects of Mafoprazine, a Phenylpiperazine Derivative, on the Central Dopaminergic System

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Abstract—The effects of mafoprazine, a new phenylpiperazine derivative, on the central dopaminergic system were studied. Mafoprazine, like chlorpromazine and haloperidol, reduced the apomorphine-induced cage-climbing behavior in mice, emesis in dogs and stereotyped behavior in monkeys; methamphetamine-induced hyperlocomotion and group toxicity in mice; and agitation in rats. Mafoprazine inhibited the unilateral circling behavior induced by methamphetamine and apomorphine in rats with 6-hydroxydopamine-induced lesions in the unilateral nigrostriatal neuronal tract. The potency of mafoprazine in these experiments was almost equal to that of chlorpromazine and about one-tenth that of haloperidol. The cataleptogenic activity of mafoprazine was lower than those of chlorpromazine and haloperidol. Mafoprazine potentiated clonidine-induced hypothermia. These results suggest that mafoprazine has a relatively selective postsynaptic dopamine D2-receptor blocking action in the nucleus accumbens compared with chlorpromazine and haloperidol and suggest that mafoprazine also has α2-adrenoceptor-stimulating actions.

From the results of studies on behavioral pharmacology such as aggressive behavior in long-term isolated mice, hyperemotionality and muricide in olfactory bulbectomized rats, gross behavior in dogs, and operant behavior (DRL response) in rats, we suggested that mafoprazine, 4’-[[3-[4-(o-fluorophenyl)-1-piperazinyl]propyl]oxy]-3’-methoxy-acetanilide mesylate, is a unique neuroleptic drug with an anti-aggressive action and a weak extrapyramidal effect (1). In addition, a neurochemical study on mafoprazine revealed that its D2 receptor selectivity (D1/D2, Ki value ratio) and affinity for α2-receptors in terms of D2/α2 (Ki value ratio) are much higher than those of chlorpromazine and haloperidol (2). However, the effects of mafoprazine on the central dopaminergic and adrenergic systems have not been studied in detail. In this study, therefore, we investigated the regional specificity of its dopaminergic action and its effect on clonidine-induced hypothermia as an action on the central α2-adrenergic system to elucidate further mechanisms of its potential neuroleptic and anti-aggressive actions.

Materials and Methods

1. Experimental animals: The experiments were performed on male Std:ddY mice (26–28 g), male Slc:Wistar (200–300 g) and Jcl:SD (190–200 g) rats, female and male cats (2.5–3.2 kg), male beagle dogs (8–10 kg) and male cynomogus monkeys (Macaca fuscicularis) (2.5–5 kg). Dogs and monkeys were used repeatedly after withdrawal for 1 week or more.

2. Drugs: Mafoprazine mesylate (Lot No. 303020, synthesized at the Organic Chemistry Research Lab. of Tanabe) is an odorless white crystalline powder with a bitter taste. Chlorpromazine hydrochloride (Shionogi) was the pure substance extracted from Wintemin® 10% powder and haloperidol was in the form of Serenace® injection. Other drugs used in the experiments were (+) apomorphine hydro-
chloride (Messrs. Meferlane Amith), methamphetamine hydrochloride (Dainippon), yohimbine hydrochloride (Nakarai Chemical), 6-hydroxydopamine hydrobromide (Sigma) and clonidine hydrochloride (Tokyo Kasei).

3. Preparation and administration of test drugs: All the test drugs were dissolved in distilled water and administered subcutaneously at a volume of 10, 5, 1 and 1 ml/kg body weight to mice, rats, dogs and monkeys, respectively.

4. Animal maintenance: Animals used were housed as follows: mice, 25 each in a plastic cage (42×26×15 cm); rats, one rat in one compartment (6 mm mesh, 14×24×16 cm) of a five-compartment stainless-steel wire cage (6 mm mesh, 75×25×14 cm); cats, 10 to 20 each in a stainless-steel cage (150×150×180 cm); beagle dogs, each in an individual stainless-steel cage (700×800×810 cm); monkeys, each in an individual stainless-steel cage (60×55×80 cm). All the animals were housed in animal rooms maintained at 23±1 °C with 55±5% humidity and illuminated for 12 hr (6:30 a.m.–6:30 p.m.).

Mice and rats were allowed free access to an ordinary pellet diet (CRF-1, Oriental Kobo) and tap water. Each cat was given 70–90 g, in the ratio of 3 to 2, of pellet diet (CS, Oriental Kobo) and canned cat food (T-2, Oriental Kobo) once daily (4:00 p.m.); each dog was given 300 g of pellet diet (DS, Oriental Kobo), once daily (4:00 p.m.); and each monkey was given 30 g of pellet diet (for monkeys, Oriental Kobo), 100 g of apple and 150 g of sweet potato, once daily (4:00 p.m.).

Methods

1. Effect on apomorphine-induced cage-climbing behavior in mice: Groups of 10 mice were used. Immediately after s.c. administration of the test drugs, mice were given 2.5 mg/kg of apomorphine, s.c. Five mice were placed together in a wire cage (6 mm mesh, 14×24×16 cm). Cage-climbing behavior was observed for 3 min from 30 min after apomorphine administration. The ED50 value was calculated from the number of mice that failed to climb up the cage walls.

2. Effect on apomorphine-induced vomiting in dogs: A total of 6 dogs that vomited 6 times or more within 30 min after s.c. administration of 0.1 mg/kg of apomorphine were selected beforehand and used randomly as a group of 3 or 6. After being fasted for 18 to 20 hr, dogs were given 300 g of pellet diet. The test drugs and apomorphine (0.1 mg/kg) were simultaneously administered subcutaneously 15 min after complete ingestion of all pellets, and the number of dogs vomiting at least once during the observation period of 60 min after apomorphine administration was counted. Dogs were used repeatedly after withdrawal for 1 week or more. The ED50 value was calculated from the number of dogs showing no vomiting during the observation period.

3. Effect on apomorphine-induced stereotypy in monkeys: A total of 6 monkeys were used randomly as a group of 2. The test drugs were s.c. administered 30 min before s.c. administration of 1 mg/kg of apomorphine. Behavior was observed according to the method of Peng and Wang (3) for 60 min after apomorphine administration. Monkeys were used repeatedly after withdrawal for 1 week or more.

4. Effect on methamphetamine-induced hyperlocomotion in mice: Groups of 10 mice were used. Immediately after s.c. administration of the test drugs, mice were injected with 1 mg/kg of methamphetamine, i.p. Each mouse was placed in an open polyethylene bucket-type vessel (inside base diameter: 20 cm, height: 18 cm, capacity: 730 cm³), and locomotor activity was measured for 60 min thereafter with an Ambulometer® (Ohara Ika, AMB-10). The ED50 value was calculated from the number of mice with less than 100 counts/hr.

5. Effect on methamphetamine-induced agitation in rats: Agitation was observed for 5 min from 40 min after s.c. injection of 10 mg/kg of methamphetamine. The intensity of agitation was scored according to the method of Janssen et al. (4) as graded responses using “zero to three” scoring scales: agitation score 0: absent, i.e., virtually no body movements; agitation score 1: slight, i.e., weak sporadic movements; agitation score 2: moderate, i.e., vigorous ambulation, rearing and jumping with significant interruptions; agitation score 3: high, i.e., continuous and general motor hyperactivity. The rats with agitation score 3 were selected beforehand and used as a group of 5. The test drugs were
s.c. injected 15 min before administration of methamphetamine. The ED50 value was calculated from the number of rats scoring 0 during the observation period.

6. Effect on methamphetamine-induced group toxicity in mice: Groups of 10 mice were used. Immediately after s.c. administration of the test drugs, mice were i.p. injected with 50 mg/kg of methamphetamine, and all the mice of each group were placed together in a frame of light gray opaque vinyl chloride (14 x 12 x 13 cm). The ED50 value was calculated from the number of mice surviving for 24 hr after administration of the test drugs.

7. Effect on circling behavior in rats: Male rats (Jcl:SD) weighing 190–200 g were used. Rats anesthetized with sodium pentobarbital (50 mg/kg, i.p.) were secured in a stereotaxic instrument (Narishige, ST-7). The right nigrostriatal dopaminergic tract (atlas by König and Klippel (5): A: 4.6, L: 1.8, H: -2.2 mm) was lesioned by infusion of 8 μg/4 μl/rat of 6-hydroxydopamine (6-OHDA) dissolved in cold saline with 0.2% L-ascorbic acid at a rate of 1 μl/min through a catheter needle (outside diameter: 0.45 mm) connected to a microsyringe (TERUMO®). About 6 months after infusion of 6-OHDA, a total of 24 rats that showed pronounced circling only towards the lesioned side (ipsilateral circling) in response to i.p. administration of methamphetamine and circlings only towards the intact side (contralateral circling) in response to i.p. administration of apomorphine were chosen beforehand and used randomly as a group of 6.

Ten min after i.p. injection of 3 mg/kg of methamphetamine, mafoprazine or chlorpromazine was s.c. injected; and 30 min later, the circling behavior was observed for 5 min. Haloperidol was s.c. injected 20 min before methamphetamine injection; and 60 min later, the circling behavior was observed for 5 min. On the other hand, apomorphine was injected at 1 mg/kg, i.p., at 30, 30 and 60 min after administration of mafoprazine, chlorpromazine and haloperidol, respectively. The number of circlings was counted for 5 min from 20 min after apomorphine injection. Rats were used repeatedly after withdrawal for 2 weeks.

8. Cataleptogenic effect: 1) Mice: Groups of 10 mice were used. Mice were forced to hold both their forelimbs on a horizontal wire (1 mm in diameter) stretched 5 cm above the floor at 15, 30, 180 and 300 min after s.c. administration of the test drugs. Mice sustaining the forced posture for more than 30 sec were judged to be cataleptic. The ED50 value was calculated from the number of mice showing catalepsy at the time of maximal activity.

2) Rats: Groups of 5 rats were used. Rats were forced to hold their right forelimbs on a gum stopper (9 cm high) at 30, 30 and 60 min after s.c. administration of mafoprazine chlorpromazine and haloperidol, respectively. Rats sustaining the forced posture for more than 30 sec were judged to be cataleptic. The ED50 value was calculated from the number of rats showing catalepsy.

3) Monkeys: A total of 8 male monkeys were used randomly as a group of 2. Their general behaviors were observed at the fixed times over 5 hr after s.c. administration of the test drugs, and the forced posture of their limbs was rated according to the method of Ito et al. (6). Monkeys were used repeatedly after withdrawal for 1 week or more.

9. Effect on clonidine-induced hypothermia in mice: Groups of 10 mice were used. Mice were given 0.25 mg/kg of clonidine, i.p., immediately after s.c. administration of the test drugs. Rectal temperature was measured with a thermister (Nihon Kohden, MGA-III) 30 min before and 60 min after administration of the test drugs. The experiment was performed in a room controlled to 23 ± 1°C in temperature and 55 ± 5% in humidity.

Statistical analysis
The ED50 and 95% confidence limits were calculated according to the probit method. Significant differences were evaluated by Student's t-test.

Results
1. Effect on apomorphine-induced cage-climbing behavior in mice: All the mice treated with apomorphine alone showed cage-climbing behavior during the 3-min observation period at 30 min after the treatment. Mafoprazine dose-dependently inhibited the behavior at 0.05 mg/kg or more, s.c., with an ED50 value of 0.38 mg/kg, s.c. The inhibitory effect of chlorpromazine was similar to that of
Table 1. Effect of mafoprazine and reference drugs on apomorphine-induced cage-climbing behavior in mice

| Drugs      | Doses mg/kg, s.c. | No. of mice | No. of mice without climbing | ED50 (95% C.L.) mg/kg, s.c. |
|------------|-------------------|-------------|-----------------------------|-----------------------------|
| Control    | —                 | 10          | 0                           | —                           |
| Mafoprazine| 0.02              | 10          | 0                           | 0.38                        |
|            | 0.05              | 10          | 1                           |                             |
|            | 0.1               | 10          | 2                           | (0.22–0.88)                 |
|            | 0.2               | 10          | 3                           |                             |
|            | 0.5               | 10          | 5                           |                             |
|            | 1                 | 10          | 8                           |                             |
| Chlorpromazine| 0.02            | 10          | 0                           |                             |
|            | 0.05              | 10          | 1                           |                             |
|            | 0.1               | 10          | 3                           | 0.41                        |
|            | 0.2               | 10          | 3                           | (0.23–1.12)                 |
|            | 0.5               | 10          | 4                           |                             |
|            | 1                 | 10          | 8                           |                             |
| Haloperidol| 0.005             | 10          | 0                           | 0.016                       |
|            | 0.01              | 10          | 2                           |                             |
|            | 0.02              | 10          | 7                           | (0.012–0.022)               |
|            | 0.05              | 10          | 10                          |                             |

Apomorphine (2.5 mg/kg) was injected s.c. at 30 min after administration of mafoprazine and chlorpromazine and 60 min after administration of haloperidol. ED50 was calculated from the number of mice that failed to climb up the cage walls for 3 min from 30 min after apomorphine.

mafoprazine at the same dose range, whereas haloperidol dose-dependently inhibited the behavior at 0.01 mg/kg or more s.c. (Table 1). Compared in terms of the ED50 values, the inhibitory activity of mafoprazine was equal to that of chlorpromazine and 1/24 that of haloperidol.

2. Effect on apomorphine-induced vomiting in dogs: Apomorphine induced vomiting in all 6 dogs at 0.1 mg/kg, s.c., within 30 min after administration. Subcutaneous injection of mafoprazine inhibited the vomiting in 2 of 3 dogs, 4 of 6 dogs and all of 3 dogs at 0.1, 0.3 and 1 mg/kg, but not at 0.03 mg/kg: the ED50 value was 0.13 mg/kg. Chlorpromazine and haloperidol also inhibited the vomiting at 1 mg/kg or more and 0.01 mg/kg or more, s.c., respectively (Table 2). When the ED50 values are compared, the inhibitory activity of mafoprazine was 13 and 1/10 times stronger than those of chlorpromazine and haloperidol, respectively.

3. Effect on apomorphine-induced stereotypy in monkeys: All 6 monkeys injected s.c. with 1 mg/kg of apomorphine exhibited the typical stereotyped behaviors listed for the control animals in Table 3. Although mafoprazine had no effect on the stereotypy at 0.2 mg/kg, s.c., it inhibited the behaviors except for chattering at 0.5 mg/kg, s.c., and completely at 1 mg/kg, s.c. Chlorpromazine and haloperidol inhibited the stereotypy completely at 5 and 0.1 mg/kg, s.c., respectively (Table 3).

4. Effect on methamphetamine-induced hyperlocomotion in mice: Locomotor activity for 60 min after i.p. injection of 1 mg/kg of methamphetamine (control II) was about 3 times higher than that of mice given saline instead of methamphetamine (control I). Mafoprazine dose-dependently inhibited methamphetamine-induced hyperlocomotion at 0.1 mg/kg or more, s.c., with an ED50 value of 0.45 mg/kg. Chlorpromazine and haloperidol also inhibited the hyperlocomotion dose-dependently at 1 mg/kg or more and 0.02 mg/kg or more, s.c., respectively (Table 4). As compared in terms of the ED50 values, the inhibitory activity of mafoprazine was about 6 and 1/8 times stronger than those of chlor-
Table 2. Effect of mafoprazine and reference drugs on apomorphine-induced vomiting in dogs

| Drugs          | Doses mg/kg, s.c. | No. of dogs | No. of dogs without vomiting | ED50 (95% C.L.) mg/kg, s.c. |
|---------------|------------------|-------------|-------------------------------|-----------------------------|
| Control       | ---              | 6           | 0                             | ---                         |
| Mafoprazine   | 0.03             | 3           | 0                             | 0.13                        |
|               | 0.1              | 3           | 2                             |                             |
|               | 0.3              | 6           | 4                             | (0.012–0.41)                |
|               | 1                | 3           | 3                             |                             |
| Chlorpromazine| 0.3              | 3           | 0                             | 1.73                        |
|               | 1                | 3           | 1                             |                             |
|               | 3                | 3           | 2                             | (0.24–12.32)                |
|               | 10               | 3           | 3                             |                             |
| Haloperidol   | 0.003            | 3           | 0                             |                             |
|               | 0.01             | 3           | 2                             | 0.012                       |
|               | 0.03             | 3           | 2                             | (0.001–0.055)               |
|               | 0.1              | 3           | 3                             |                             |

Aromorphine (0.1 mg/kg) was injected s.c. at 30 min after administration of mafoprazine and chlorpromazine and 60 min after administration of haloperidol. ED50 was calculated from the number of dogs that showed no vomiting within 60 min after apomorphine administration.

Table 3. Effect of mafoprazine and reference drugs on apomorphine-induced stereotypy in monkeys

| Drugs          | Doses mg/kg, s.c. | Findings (No. of monkeys: observed/tested) |
|---------------|------------------|------------------------------------------|
| Control       | ---              | Stereotypy (continuous biting, licking, chattering and/or repetitive movements of the bilateral body swinging) with twisted tongue, piloerection, tail-reaction and vocalization (6/6) |
| Mafoprazine   | 0.2              | Almost the same as the control (2/2)      |
|               | 0.5              | Only slight chattering (2/2)              |
|               | 1                | Complete antagonism to apomorphine-induced stereotypy (2/2) |
| Chlorpromazine| 0.5              | Almost the same as the control (2/2)      |
|               | 1                | Moderate intensity of the stereotypy (biting and licking) (1/2), slight chattering and vocalization (1/2) |
|               | 2                | Only slight chattering (2/2)              |
|               | 5                | Complete antagonism to apomorphine-induced stereotypy (2/2) |
| Haloperidol   | 0.05             | Chattering and vocalization (1/2), only slight chattering (1/2) |
|               | 0.1              | Complete antagonism to apomorphine-induced stereotypy (2/2) |

A total of 6 male monkeys (Macaca irus) were repeatedly used as a group of 2 for each dose. Drugs were administered 30 min before s.c. administration of apomorphine (1 mg/kg). Behavior was observed for 1 hr after apomorphine administration.

5. Effect on methamphetamine-induced agitation in rats: Methamphetamine induced remarkable agitation lasting for about 60 min at 10 mg/kg, s.c. Mafoprazine dose-dependently inhibited the agitation at 1 mg/kg or more, s.c., with an ED50 value of 1.64 mg/kg. Chlorpromazine and haloperidol also inhibited the agitation dose-dependently at 1 mg/kg or more and 0.01 mg/kg or more, s.c., respectively. A comparison of the ED50 values indicates that the inhibitory activity of mafopra-
### Table 4. Effect of mafoprazine and reference drugs on methamphetamine-induced hyperlocomotion in mice

| Drugs        | Doses mg/kg, s.c. | Methamphetamine 1 mg/kg, i.p. | Locomotor activity (counts/60 min) | N* | ED50 (95% C.L.) mg/kg, s.c. |
|--------------|-------------------|--------------------------------|-----------------------------------|----|---------------------------|
| Control I    | —                 | —                              | 101.1±11.2                        | —  | —                         |
| Control II   | —                 | +                              | 337.0±53.4                        | 0  | —                         |
| Mafoprazine  | 0.05 +            | 261.8±29.1                     | 0                                 | 1  | 0.45                      |
|              | 0.1 +             | 173.8±17.0*                    | 1                                 | (0.36–0.61) |
|              | 0.2 +             | 138.4±18.0**                   | 1                                 |  (1.73–5.07) |
|              | 0.5 +             | 90.4±19.9***                   | 5                                 |  (0.036–0.079) |
|              | 1 +               | 25.5±4.3***                    | 10                                |    |                           |
| Chlorpromazine | 0.2 +            | 250.2±30.8                     | 0                                 | 2.61 |                           |
|              | 0.5 +             | 240.0±30.5                     | 0                                 | 0.054 |                          |
|              | 1 +               | 201.0±24.0*                    | 1                                 | (0.036–0.079) |
|              | 2 +               | 132.3±18.7**                   | 4                                 |  (1.73–5.07) |
|              | 5 +               | 65.0±10.6***                   | 8                                 |  (1.11–3.66) |
| Haloperidol  | 0.01 +            | 276.9±16.8                     | 0                                 |  0.05 |                          |
|              | 0.02 +            | 173.6±16.2**                   | 1                                 | (0.97–3.25) |
|              | 0.05 +            | 114.8±19.5**                   | 5                                 |  (1.11–3.66) |
|              | 0.1 +             | 71.3±16.8***                   | 7                                 |  (1.73–5.07) |
|              | 0.2 +             | 50.4±5.6***                    | 10                                |    |                           |

Groups of 10 mice were used. *The number of mice showing less than 100 counts/60 min. Control I: saline. Control II: methamphetamine alone. Hyperlocomotor activity was individually measured by the Ambulometer for 60 min after methamphetamine injection. Mafoprazine, chlorpromazine and haloperidol were administered 30, 30 and 60 min before methamphetamine injection. ED50 was calculated from the number of mice showing less than 100 counts/60 min. *P<0.05, **P<0.01, ***P<0.001: Significantly different from the Control II.

### Table 5. Effect of mafoprazine and reference drugs on methamphetamine-induced agitation in rats

| Drugs        | Doses mg/kg, s.c. | No. of rats | No. of rats scoring 0 | ED50 (95% C.L.) mg/kg, s.c. |
|--------------|-------------------|-------------|----------------------|---------------------------|
| Mafoprazine  | 0.5               | 5           | 0                    |                           |
|              | 1                 | 5           | 1                    |                           |
|              | 2                 | 5           | 3                    | 1.64                      |
|              | 5                 | 5           | 5                    | (0.97–3.25) |
|              | 10                | 6           | 5                    |                           |
| Chlorpromazine | 0.5              | 5           | 0                    |                           |
|              | 1                 | 5           | 1                    |                           |
|              | 2                 | 5           | 2                    | 1.90                      |
|              | 5                 | 5           | 5                    | (1.11–3.66) |
|              | 10                | 5           | 5                    |                           |
| Haloperidol  | 0.003             | 5           | 0                    |                           |
|              | 0.01              | 5           | 1                    |                           |
|              | 0.03              | 5           | 1                    | 0.04                      |
|              | 0.1               | 5           | 4                    | (0.02–0.10) |
|              | 0.3               | 5           | 5                    |                           |

Drugs were administered 15 min before methamphetamine injection (10 mg/kg, s.c.). *Agitation was assessed according to the rating scale for behavioral measurement by Janssen et al. (see text). ED50 was calculated from the number of rats scoring 0 for 5 min from 40 min after methamphetamine injection.
prazine was almost equal to that of chlorpromazine and about 1/40 that of haloperidol (Table 5).

6. Effect on methamphetamine-induced group toxicity in mice: Within 24 hr after i.p. injection of 50 mg/kg of methamphetamine, 9 out of 10 mice died when all the mice were placed together in a small cage (14 x 12 x 13 cm), whereas none of the 10 mice died when placed individually in a cage. Mafoprazine dose-dependently reduced the group toxicity at 1 mg/kg or more, s.c. Chlorpromazine and haloperidol also reduced the group toxicity dose-dependently at 0.3 mg/kg or more and 0.03 mg/kg or more, respectively. The dose required to reduce the number of deaths by 50% (ED50 value) was 1.73 mg/kg for mafoprazine, 1.44 mg/kg for chlorpromazine and 0.24 mg/kg for haloperidol (Table 6).

7. Effect on circling behavior in rats: Rats with a 6-OHDA-induced lesion in the unilateral nigrostriatal dopaminergic tract exhibited about 50 ipsilateral circlings for 5 min from 40 min after i.p. injection of 3 mg/kg of methamphetamine and about 33 contralateral circlings for 5 min from 30 min after i.p. injection of 1 mg/kg of apomorphine. Mafoprazine significantly decreased the number of methamphetamine- and apomorphine-induced circlings at 1 and 0.675 mg/kg, s.c., respectively. Chlorpromazine and haloperidol also significantly decreased both the numbers (Table 7). The inhibitory effect of mafoprazine was almost equal to or slightly inferior to that of chlorpromazine and about 1/10 that of haloperidol.

8. Cataleptogenic activity: 1) Mice: Mafoprazine dose-dependently induced a cataleptogenic effect at 5 mg/kg or more, s.c. Even at 20 mg/kg, s.c., however, 3 of 10 mice showed no catalepsy. The effect reached the maximum between 30 and 60 min and disappeared between 180 and 360 min after administration. On the other hand, chlorpromazine and haloperidol dose-dependently induced more marked catalepsy than mafoprazine at 2 mg/kg or more and 0.1 mg/kg or more, s.c., respectively. Almost all the mice given 10 mg/kg or more of chlorpromazine and 2 mg/kg or more of haloperidol showed catalepsy lasting for over 3 hr, respectively (Table 8). When compared in terms of the ED50 values, the cataleptogenic activity of mafoprazine in mice was about 1/5 that of chlorpromazine and haloperidol.

Table 6. Effect of mafoprazine and reference drugs on methamphetamine-induced group toxicity in mice

| Drugs   | Doses mg/kg, s.c. | No. of mice | No. of survivals | ED50 (95% C.L.) mg/kg, s.c. |
|---------|-------------------|-------------|-----------------|-----------------------------|
| Control I | —                 | 10          | 10              | —                           |
| Control II | —                 | 10          | 1               | —                           |
| Mafoprazine | 0.3               | 10          | 0               | 1.73                        |
|          | 1                 | 10          | 2               | (0.94–3.20)                 |
|          | 3                 | 10          | 8               |                             |
|          | 10                | 10          | 10              |                             |
| Chlorpromazine | 0.3               | 10          | 3               | 1.44                        |
|          | 1                 | 10          | 4               | (0.16–6.78)                 |
|          | 3                 | 10          | 6               |                             |
|          | 10                | 10          | 8               |                             |
| Haloperidol | 0.03              | 10          | 3               | 0.24                        |
|          | 0.1               | 10          | 4               | (0.12–0.40)                 |
|          | 0.3               | 10          | 7               |                             |
|          | 1                 | 10          | 9               |                             |

Control I: under isolated condition, Control II: under grouped condition. Methamphetamine (50 mg/kg) was injected i.p. at 30 min after administration of mafoprazine and chlorpromazine and 60 min after administration of haloperidol. ED50 was calculated from the number of surviving mice for 24 hr after methamphetamine administration.
Table 7. Effect of mafoprazine and reference drugs on methamphetamine- and apomorphine-induced circlings in rats with unilateral striatal 6-OHDA lesions

| Drugs     | Methamphetamine-induced ipsilateral circlings | Apomorphine-induced contralateral circlings |
|-----------|----------------------------------------------|--------------------------------------------|
|           | Doses mg/kg, s.c. | Total counts for 5 min (mean±S.E.) | Doses mg/kg, s.c. | Total counts for 5 min (mean±S.E.) |
|           | Pre          | Post          | Pre          | Post          |
| Mafoprazine | 0.1          | 51.8±11.3     | 74.7±9.2     | 0.3           | 33.7±6.2      | 25.0±4.6     |
|           | 0.3          | 48.7±5.3      | 27.0±8.9     | 0.45          | 33.7±6.2      | 17.8±3.6     |
|           | 1            | 51.5±11.6     | 3.0±4.0**    | 0.675         | 33.7±4.4      | 6.8±2.6***   |
| Chlorpromazine | 0.3         | 50.0±7.8      | 45.8±9.3     | 0.1           | 33.2±5.1      | 31.5±4.5     |
|           | 1            | 51.8±11.3     | 20.5±6.0*    | 0.3           | 32.0±2.9      | 14.0±3.8**   |
|           | 3            | 48.7±5.3      | 9.7±3.5***   | 1             | 34.2±6.0      | 8.2±0.9***   |
| Haloperidol | 0.01         | 51.5±11.6     | 67.3±6.8     | 0.01          | 33.7±4.9      | 29.2±4.8     |
|           | 0.03         | 48.5±5.3      | 31.5±6.2     | 0.03          | 33.7±4.9      | 20.0±2.9*    |
|           | 0.1          | 50.0±7.8      | 0.3±0.3***   | 0.1           | 32.7±2.4      | 11.0±5.9**   |

Groups of 6 rats were used. Circlings were counted for 5 min from 40 min after i.p. administration of methamphetamine (3 mg/kg) or from 20 min after i.p. administration of apomorphine (1 mg/kg). *P<0.05, **P<0.01, ***P<0.001: Significantly different from each corresponding pre-administration value.

Table 8. Cataleptogenic activity of mafoprazine and reference drugs in mice

| Drugs     | Doses mg/kg, s.c. | No. of mice | No. of cataleptic mice | ED50 (95% C.L.) mg/kg, s.c. |
|-----------|-------------------|-------------|------------------------|-----------------------------|
|           |                   | 15a         | 30                     | 60 | 180 | 300 | min |
| Mafoprazine | 2                | 10          | 0                      | 0  | 0   | 0   | 0   |
|           | 5                | 10          | 2                      | 3  | 2   | 0   | 0   | 13.26 |
|           | 10               | 10          | 3                      | 4  | 3   | 3   | 1   | (8.08–36.93) |
|           | 20               | 10          | 4                      | 6  | 7   | 4   | 0   |             |
| Chlorpromazine | 1               | 10          | 0                      | 0  | 0   | 0   | 0   |             |
|           | 2                | 10          | 0                      | 1  | 3   | 0   | 0   | 2.70 |
|           | 5                | 10          | 3                      | 6  | 9   | 3   | 1   | (2.02–3.90) |
|           | 10               | 10          | 5                      | 6  | 10  | 10  | 3   |             |
|           | 20               | 10          | 7                      | 8  | 10  | 10  | 6   |             |
| Haloperidol | 0.1              | 10          | 0                      | 1  | 2   | 0   | 0   |             |
|           | 0.2              | 10          | 1                      | 2  | 3   | 1   | 1   |             |
|           | 0.5              | 10          | 3                      | 3  | 6   | 4   | 2   | 0.44 |
|           | 1                | 10          | 6                      | 4  | 7   | 7   | 5   | (0.28–0.64) |
|           | 2                | 10          | 7                      | 8  | 10  | 9  | 8   |             |
|           | 5                | 10          | 10                     | 10 | 10  | 10  | 10  |             |

*aTime after drug administration. ED50 was calculated from the number of cataleptic mice at the time of the maximal activity.

about 1/30 that of haloperidol.

2) Rats: Mafoprazine, chlorpromazine and haloperidol induced catalepsy dose-dependently at 5 mg/kg or more, 1 mg/kg or more, and 0.03 mg/kg or more, respectively. As compared with each ED50, the cataleptogenic effect of mafoprazine in rats was about 1/4 that of chlorpromazine and about 1/120 that of haloperidol (Table 9).

3) Monkeys: Subcutaneous injection of
mafoprazine to 2 monkeys produced slight sedation without catalepsy at 1 mg/kg and sedation with transient immobilization at 2 mg/kg or more in both animals. However, neither of the monkeys could maintain a forced posture even at 20 mg/kg, s.c. In contrast, both of 2 monkeys subcutaneously given chlorpromazine or haloperidol retained the forced posture at 3 mg/kg or more and 0.2 mg/kg or more, respectively, and they demonstrated typical extrapyramidal signs such as mask-like faces, hypokinesia of the limbs, spontaneous abnormal posture characterized by involuntarily rotatory movements of the head and/or the body, and maintenance of imposed abnormal posture with rigidity at 10 and 0.5 mg/kg, respectively.

9. Effect on clonidine-induced hypothermia in mice: Intraperitoneal injection of 0.25 mg/kg of clonidine lowered body temperature by about 2°C within 60 min after administration. Mafoprazine dose-dependently and significantly potentiated clonidine-induced hypothermia at 0.1 mg/kg, s.c., but it had no significant influence on normal body temperature. On the other hand, chlorpromazine and haloperidol had no potentiating effect on clonidine-induced hypothermia at 0.1 mg/kg, s.c., producing no significant influence on normal body temperature (Table 10).

### Table 9. Cataleptogenic activity of mafoprazine and reference drugs in rats

| Drugs      | Doses mg/kg, s.c. | No. of rats | No. of cataleptic rats | ED50 (95% C.L.) mg/kg |
|------------|-------------------|-------------|------------------------|----------------------|
| Mafoprazine| 2                 | 5           | 0                      |                      |
|            | 5                 | 5           | 1                      | 8.03                 |
|            | 10                | 5           | 3                      | (4.58–13.10)         |
|            | 20                | 5           | 5                      |                      |
| Chlorpromazine | 0.5           | 5           | 0                      |                      |
|            | 1                 | 5           | 1                      |                      |
|            | 2                 | 5           | 2                      | 1.90                 |
|            | 5                 | 5           | 5                      | (1.13–3.48)          |
|            | 10                | 5           | 5                      |                      |
| Haloperidol | 0.01             | 5           | 0                      |                      |
|            | 0.03              | 5           | 1                      | 0.068                |
|            | 0.1               | 5           | 3                      | (0.031–0.149)        |
|            | 0.3               | 5           | 5                      |                      |

ED50 was calculated from the number of cataleptic rats at 30 min after administration of mafoprazine and chlorpromazine and 60 min after administration of haloperidol.
Table 10. Effect of mafoprazine and reference drugs on clonidine-induced hypothermia in mice

| Drugs        | Doses (mg/kg, s.c.) | Clonidine (0.25 mg/kg, i.p.) | Rectal temperature (°C, mean±S.E.) 60 min^b |
|--------------|---------------------|-----------------------------|------------------------------------------|
| Control I    | —                   | —                           | 0.05±0.19                                |
| Control II   | —                   | +                           | -1.90±0.17                               |
| Mafoprazine  | 0.1                 | —                           | -0.26±0.08                               |
|              | 0.1                 | +                           | -2.63±0.20*                              |
|              | 1                   | —                           | -0.34±0.14                               |
|              | 1                   | +                           | -3.02±0.25**                             |
| Chlorpromazine| 0.1               | —                           | -0.36±0.10                               |
|              | 0.1                 | +                           | -1.99±0.16                               |
|              | 1                   | —                           | -1.03±0.25***                            |
|              | 1                   | +                           | -3.06±0.22***                            |
| Haloperidol  | 0.1                 | —                           | -0.24±0.08                               |
|              | 0.1                 | +                           | -1.87±0.23                               |
|              | 1                   | —                           | -0.91±0.17***                            |
|              | 1                   | +                           | -2.53±0.29***                            |

Groups of 10 mice were used. aDifference from basal value. bTime after administration of clonidine.
Control I: saline, Control II: clonidine alone. Clonidine was injected 15 min after administration of mafoprazine and chlorpromazine and 60 min after administration of haloperidol. ***P<0.001: Significantly different from control I. +P<0.05, ++P<0.01, +++P<0.001: Significantly different from control II.

accumbens. Considering these observations, the inhibitory effect of mafoprazine on methamphetamine-induced agitation is thought to result from the blockade of post-synaptic dopamine receptors in the nucleus accumbens. On the other hand, apomorphine-induced stereotypy is reported to be inhibited by pretreatment with 6-OHDA into the corpus striatum, but not into the nucleus accumbens or olfactory tubercle (11-14). Thus, the stereotypy induced by apomorphine is suggested to be due to stimulation of post-synaptic dopamine receptors in the corpus striatum. The ratio of the ED50 of antagonism against apomorphine-induced cage climbing behavior in mice to the ED50 of antagonism against methamphetamine-induced hyperlocomotion in mice was 3 to 5 times higher for mafoprazine than for chlorpromazine and haloperidol. Therefore, mafoprazine is considered to have higher selectivity for post-synaptic dopamine receptors in the nucleus accumbens than for that in the corpus striatum as compared with chlorpromazine and haloperidol.

The cataleptogenic activity of mafoprazine was 5 and 13 times lower in mice and 4 and 120 times lower in rats than those of chlorpromazine and haloperidol, respectively, when compared in terms of their ED50 values as a measure of potency. Furthermore, in monkeys, mafoprazine unlike these reference drugs, produced no typical catalepsy even at a high dose of 20 mg/kg, s.c. Mafoprazine showed higher ratios of the ED50 for cataleptogenic activity in mice and rats to the ED50 for inhibitory activity on methamphetamine-induced hyperlocomotion in mice and to the ED50 for inhibitory activity on methamphetamine-induced agitation in rats than chlorpromazine and haloperidol, suggesting that the extrapyramidal effect of mafoprazine is lower than those of the reference drugs.

At the dose producing no significant action on normothermia in mice, mafoprazine potentiated clonidine-induced hypothermia, whereas chlorpromazine and haloperidol did not. The neurochemical study (2) revealed that the affinity of mafoprazine for α2-receptors was 26 and 345 times higher than those of chlorpromazine and haloperidol, respectively, when expressed in terms of D2/α2 (K1 value
ratio). This suggests that mafoprazine probably has an agonistic effect on $\alpha_2$-receptors. These results further suggest that the action of mafoprazine as a neuroleptic drug is based mainly on $D_2$-receptor blocking activity as well as $\alpha_2$-receptor stimulating activity.

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