Neurochemical Study of Mafoprazine, a New Phenylpiperazine Derivative

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Abstract—Mafoprazine, a phenylpiperazine derivative, was neurochemically investigated in rats to determine its action mechanism. The rank order of affinity of mafoprazine for neuronal receptors was D2 > α1 > S2 > α2 >> D1 > β > mAC. The affinity of mafoprazine for D2 receptors (Ki=10.7 nM) was 2 times higher than that of azaperone, and 6 and 16 times lower than those of chlorpromazine and haloperidol, respectively, whereas the D2 receptor selectivity [D1/D2 (Ki value ratio)] of mafoprazine was 10, 9 and 2 times higher than those of chlorpromazine, azaperone and haloperidol, respectively. The affinity of mafoprazine for α2 receptors in terms of the ratio of the Ki values for D2 and α2 receptors (D2/α2) was 345, 26 and 3 times higher than those of haloperidol, chlorpromazine and azaperone, respectively. Mafoprazine slightly showed the inhibitory effect on dopamine-stimulated adenylate cyclase (IC50=52300 nM), and it had almost no affinity for β and mAC receptors. Mafoprazine significantly increased dopamine metabolites in the corpus striatum and nucleus accumbens, although to lesser extents as compared with azaperone and chlorpromazine. These results suggest that mafoprazine probably manifests its antipsychotic action mainly through D2 receptor blocking activity and α-adrenergic activity (α1 receptor blocking activity and α2 receptor stimulating activity).

Mafoprazine, 4'-[[3-[4-(o-fluorophenyl)-1-piperazinyl]propyl]oxy]-3'-methoxyacetanilide mesylate (Fig. 1), is a newly synthesized phenylpiperazine compound. It shows relatively potent anti-apomorphine and anti-methamphetamine actions in experimental animals, but does not induce typical catalepsy in monkeys. It is considered to be an antipsychotic drug with mild extrapyramidal side effects (M. Yamamura et al., unpublished data). In addition to these actions, mafoprazine shows taming effects such as inhibitory effects on long-term isolation-induced fighting behavior in mice and on hyperemotional behavior and muricide induced by olfactory bulbectomy in rats (M. Yamamura et al., unpublished data). Therefore, attempts have been made to use this drug as an anti-fighting drug for pigs. In the present study, mafoprazine was investigated neurochemically in terms of its effects on the rat brain receptors and monoamine-related substance levels to elucidate the mechanism of action in comparison with azaperone, a swine anti-fighting drug having a potent sedative effect (1), chlorpromazine and haloperidol.

Materials and Methods

1. Experimental animals: Nine- to 15-week-old male Sic:SD rats (body weights: 300–500 g) [Shizuoka Laboratory Animal Center] were used for the receptor binding experiments and for the determination of dopamine-stimulated adenylate cyclase activity. Nine- to 10-week-old male Sic:Wistar rats (body weights: 200–
250 g) [Shizuoka Laboratory Animal Center] were used for the determination of brain monoamines and their metabolites.

2. Drugs and reagents used: The drugs and reagents used in the experiments were: mafoprazine mesylate (Lot No. 303020, synthesized at Tanabe), azaperone (Stresnil®, Sankyo), chlorpromazine-HCl (Wintermin®, Shionogi), haloperidol (Janssen Pharmaceutica), (+)-butaclamol·HCl (Research Biochemicals), prazosin·HCl (synthesized at Tanabe), ketanserin tartrate (synthesized at Tanabe), phenotolamine mesylate (Regitine®, Ciba-Geigy), clonidine·HCl (Boehringer Ingelheim), yohimbine·HCl (Nakarai), (±)-propranolol (Nakarai), atropine sulfate (Schering Japan), 5-hydroxytryptamine [5-HT] creatinine sulfate complex (Sigma), dopamine [DA]·HCl (Sigma), 3,4-dihydroxyphenylacetic acid [DOPAC] (Sigma), homovanillic acid [HVA] (Sigma), 5-hydroxy-3-indoleacetic acid [5-HIAA] (Sigma), (-)-noradrenaline [NA] (Katayama), tetrahydrofuran [THF] (Katayama), 1-heptansulfonate Na (Chemuco), 3-isobutyl-1-methylxanthine [IBMX] (Sigma), ethyleneglycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid [EGTA] (Sigma), bovine serum albumin [BSA] (Sigma), ethylenediaminetetraacetic acid disodium salt [EDTA-Na₂] (Katayama), adenosine-5'-triphosphate [ATP] disodium salt (Yamas), guanosine-5'-triphosphate [GTP] trisodium salt (Yamas), 3-(N-morpholine) propanesulfonic acid [MOPS] (Wako Pure Chemical), and other reagents of analytical grade.

Haloperidol and (+)-butaclamol were dissolved in 0.005 M tartaric acid and ethanol, respectively, each to a concentration of 2 mM; each solution was diluted with distilled water prior to use. The injections were directly diluted with distilled water. The other drugs were dissolved in distilled water and diluted appropriately. The [³H]-labeled compounds used were [³H]-SCH23390 (specific activity (sp. act.): 80.4 Ci/mmol), [³H]-spiroperidol (sp. act.: 29.5 Ci/mmol), [³H]-prazosin (sp. act.: 20.2 Ci/mmol), [³H]-clonidine·HCl (sp. act.: 48.8 Ci/mmol), [³H]-dihydroalprenolol·HCl (sp. act.: 99.9 Ci/mmol), [³H]-ketanserin·HCl (sp. act.: 72.3 Ci/mmol) and [³H]-(−)-quinuclidinyl benzilate (sp. act.: 36 Ci/mmol) [New England Nuclear (NEN)].

3. Receptor binding experiments: After decapitation, the whole brain was removed and specific regions of the brain were dissected on ice. The corpus striatum was used in the dopamine receptor (D₁, D₂) binding experiment, the cerebral cortex for the muscarinic cholinergic receptor (mACH) binding experiment, and the frontal cortex for the serotonin receptor (S₂) binding experiment. The tissue was weighed. Then 0.25 M sucrose was added to the tissue for the S₂ receptor binding experiment, and 25 mM Tris HCl buffer containing 5 mM EDTA-Na₂ (pH 7.4, 0°C) was added to the tissues for the other experiments. The tissues were homogenized using a Potter-Elvehjem glass homogenizer (Teflon pestle) (for the D₁, D₂, α₁ and mACH binding experiments) or a Polytron (PT-10, dial setting 7, 10 sec×2). Thereafter, membrane preparations were prepared in accordance with the reference mentioned in Table 1. The [³H]-ligands used in the receptor binding experiments were, as shown in Table 1, [³H]-SCH23390 for D₁ receptors, [³H]-spiroperidol ([³H]-SPL) for D₂ receptors, [³H]-prazosin ([³H]-PRZ) for α₁ receptors, [³H]-clonidine ([³H]-CLN) for α₂ receptors, [³H]-dihydroalprenolol ([³H]-DHA) for β receptors, [³H]-ketanserin ([³H]-KET) for S₂ receptors, and [³H]-(−)-quinuclidinyl benzylate ([³H]-QNB) for mACH receptors. To each membrane preparation was added the corresponding [³H]-ligand and the test drug, and the mixture was incubated for a predetermined time under the conditions given in Table 1. The assay was terminated by filtration through a Whatman GF/F glass fiber filter under vacuum and washed four times with 3 ml portions of ice-cold buffer. The radioactivity on the filter was measured with a liquid scintillation spectrometer (TRI-CARB 4640, Packard) using 5 ml of Aquasol 2 (NEN).

Specific binding was calculated as the amount of the difference between total binding and nonspecific binding (the binding amount in the presence of the cold ligand described in Table 1). The 50% inhibitory concentration (IC₅₀) of the test drug relative to the specific binding of each labeled ligand
| Receptor   | Ligand                  | Conc. (nM) | Area            | Conc. (mg/ml) | Time (min) | Temperature (°C) | Volume (ml) | Buffer                        | Drug used to define non-specific binding |
|------------|-------------------------|------------|-----------------|---------------|------------|------------------|-------------|-------------------------------|------------------------------------------|
| Dopamine-1 | [³H]-SCH23390 [Ref. 2] | 0.3        | Corpus striatum | 2.5           | 30         | 37               | 1.0         | 50 mM Tris-HCl** (pH 7.4)     | (+)-Butaclamol 10 µM                      |
| Dopamine-2 | [³H]-Spiroperidol [Ref. 3] | 0.15       | Corpus striatum | 1.6           | 30         | 37               | 2.0         | 20 mM Tris-25 mM MOPS*** (pH 7.4) | (+)-Butaclamol 1 µM                       |
| Adrenergic | α₁                      | 0.5        | Cerebral cortex | 2.5           | 30         | 25               | 2.0         | 50 mM Tris-HCl* (pH 7.7)       | Phentolamine 10 µM                         |
|            | α₂                      | 2.0        | Cerebral cortex | 5.0           | 30         | 25               | 2.0         | 50 mM Tris-HCl* (pH 7.5)       | Clonidine 1 µM                             |
|            | β                       | 1.0        | Cerebral cortex | 5.0           | 20         | 23               | 2.0         | 50 mM Tris-HCl* (pH 8.0 at 25°C) | (+)-Propranolol 20 µM                      |
| Serotonin-2 | [³H]-Ketanserin [Ref. 7] | 0.4        | Frontal cortex  | 1.6           | 15         | 37               | 2.5         | 50 mM Tris-HCl* (pH 7.4)       | 5-HT 100 µM                                |
| Muscarinic cholinergic | [³H]-Quinuclidinyl benzilate [Ref. 8] | 0.1        | Cerebral cortex | 0.375         | 60         | 37               | 2.0         | 25 mM Tris-HCl* (pH 7.4)       | Atropine 10 µM                             |

*: Containing 0.05% BSA. **: Containing 5 mM MgSO₄, 0.5 mM EDTA-Na₂. ***: Containing 5 mM MgSO₄, 0.02% ascorbic acid.
was determined by probit analysis from the corresponding inhibition rates at 4 to 6 concentrations. In addition, the specific binding at 7 to 10 concentrations of the \([^{3}\text{H}]\)-ligand were previously determined, and the resulting saturation curve was subjected to Scatchard analysis (9) to find the \(K_d\) value of the ligand. Using this \(K_d\) value, the \(K_i\) value was calculated from the equation of Cheng-Prusoff (10): 
\[
K_i = \frac{IC_{50}}{1 + L/K_d}
\]
where \(K_d\) is the dissociation constant, \(K_i\) the inhibition constant and \(L\), the concentration of \([^{3}\text{H}]\)-ligand (nM). Three independent series of receptor binding experiments were performed in triplicate.

4. Effect on dopamine-stimulated adenylylate cyclase activity: The method of Markstein (11) was partially modified and used. After decapitation, the whole rat brain was removed and the corpus striatum was dissected on ice. The tissue was homogenized in 100 volumes of ice-cold 10 mM Tris-acetate buffer containing 2 mM EGTA (pH 7.4, 0°C) with a Potter-Elvehjem glass homogenizer (Teflon pestle) and centrifuged at 20,000×g for 10 min. The pellet was washed twice with the same volume of buffer and then resuspended in 199 volumes of buffer to obtain a membrane suspension. This membrane suspension was rapidly frozen with liquid nitrogen and stored at −80°C until use.

A mixture containing 120 mM Tris-acetate buffer (pH 7.4, 30°C), 5 mM MgCl₂, 1.125 mM EGTA, 0.25 mM IBMX, 0.15% BSA, 156.25 μM DA, the test drug and 100 μl of membrane suspension (0.5 mg wet tissue) in a volume of 400 μl was preincubated at 4°C for 13.5 min and transferred to a constant temperature bath at 30°C, immediately followed by addition of 100 μl of a 2.5 mM ATP-0.5 mM GTP mixture (pH 7.4) to initiate the reaction. After 3 min of incubation, the reaction mixture was immersed in a 95°C water bath for 3.5 min to terminate the reaction and centrifuged at 10,000×g for 5 min. The cyclic AMP in the supernatant was assayed with a cyclic AMP assay kit (Yamas). The mean value of 2 to 5 independent experiments performed in duplicate was taken, and the 50% inhibitory concentration (IC₅₀) of the test drug for dopamine-stimulated adenylylate cyclase was determined by probit analysis of the inhibition rates.

5. Effect on rat brain monoamines and their metabolite contents: One hr after the subcutaneous administration of the test drug, the rats were killed by microwave irradiation [3 kW, 2.1 sec (Microwave applicator TMW-6402A, Toshiba)] focused on the head of the animals and then chilled in liquid nitrogen to the extent of not freezing the brain tissue. The whole brain was then removed, and on a filter paper soaked with saline, the frontal cortex, corpus striatum and nucleus accumbens were dissected on ice. By reference to the brain atlas of König and Klippel (12), the nucleus accumbens was dissected from a slice (thickness about 1.5 mm) by making two coronal cuts, using a stainless steel pipe having an internal diameter of 1.6 mm. All brain tissues were stored at −80°C until use.

Each tissue was homogenized in 500 μl of 0.2 M HClO₄ and 100 μl of 0.05 M EDTA-Na₂ (1/4 volume each for the nucleus accumbens) by sonication and centrifuged at 27,000×g for 20 min. The supernatant as such was used as a sample for determination of monoamine-related substances, and the pellet was dissolved in a 0.5 M NaOH solution for determination of protein concentration. Distilled water was administered to control animals.

For the determination of monoamines and their metabolites, a reverse phase column (ODS-T, Yanagimoto) was used for separation and 50 μl of the sample was injected into a high performance liquid chromatograph (L-2000 or L-4000W, Yanagimoto) equipped with an electrochemical detector (VMD-101 or VMD-501, Yanagimoto) under the following conditions. For the determination of DA, DOPAC, HVA, 5-HT and 5-HIAA, a 0.1 M sodium acetate buffer (pH 4.6) containing 10% methanol, 0.5% THF, 0.25 mM 1-heptanesulfonate•Na and 40 mg/l EDTA-Na₂ was used as the mobile phase, and the determinations were carried out at the flow rate of 0.6 ml/min and the applied potential of +500 mV and +700 mV (vs. Ag/AgCl) (VMD-501). For the determination of NA, a 0.075 M sodium citrate buffer (pH 3.6) containing 5% methanol, 2 mM 1-heptanesulfonate-Na and 40 mg/l EDTA-Na₂ was used as the mobile phase, and the determination was carried out at the flow rate of 0.67 ml/min and the ap-
plied potential of +800 mV (vs. Ag/AgCl). The protein content of each tissue was determined by the method of Lowry et al. (13). The values were expressed in ng/mg protein.

The results were expressed as the mean±standard error. For statistical analysis, one-way analysis of variance followed by the Sheffe-test for multiple comparisons were used.

Results

1. Saturation assays of $[^{3}H]$-ligand binding

Saturation assays were performed in the concentration range of 0.125-3 nM for $[^{3}H]$-SCH23390, 0.05-1 nM for $[^{3}H]$-SPL, 0.05-2 nM for $[^{3}H]$-PRZ, 0.1-3 nM for $[^{3}H]$-CLN, 0.1-2 nM for $[^{3}H]$-DHA, 0.1-3 nM for $[^{3}H]$-KET and 0.03-1 nM for $[^{3}H]$-ONB. The $K_d$ and $B_{max}$ values of $[^{3}H]$-SCH23390 in the corpus striatum were 0.460±0.026 nM and 84.3±1.3 pmol/g tissue, respectively; those of $[^{3}H]$-SPL in the corpus striatum, 47.2±2.6 pM and 28.1±1.8 pmol/g tissue, respectively; those of $[^{3}H]$-PRZ in the cerebral cortex, 82.3±12.1 pM and 15.0±0.6 pmol/g tissue, respectively; those of $[^{3}H]$-CLN in the cerebral cortex, 1.68±0.09 nM and 7.50±0.46 pmol/g tissue, respectively; those of $[^{3}H]$-DHA in the cerebral cortex, 0.482±0.010 nM and 9.97±0.28 pmol/g tissue, respectively; those of $[^{3}H]$-KET in the frontal cortex, 0.489±0.038 nM and 11.1±0.1 pmol/g tissue, respectively; and those of $[^{3}H]$-ONB in the cerebral cortex, 19.7±0.6 pM and 113±1 pmol/g tissue, respectively.

2. Inhibition studies for $[^{3}H]$-ligand binding

1) Effect on $D_1$ receptors: The inhibitory effect of mafoprazine on $[^{3}H]$-SCH23390 binding ($K_i$=2330 nM) was 61, 36 and 4 times less potent than those of chlorpromazine, haloperidol and azaperone, respectively (Table 2).

2) Effect on $D_2$ receptors: The inhibitory effect of mafoprazine on $[^{3}H]$-SPL binding ($K_i$=10.7 nM) was twice as potent as that of azaperone, but 16 and 6 times less potent than those of haloperidol and chlorpromazine, respectively (Table 2).

3) Effect on $\alpha_1$ receptors: The inhibitory effect of mafoprazine on $[^{3}H]$-PRZ binding ($K_i$=12.6 nM) was 10, 3 and 2 times less potent than those of chlorpromazine, azaperone and haloperidol, respectively (Table 2).

4) Effect on $\alpha_2$ receptors: The inhibitory effect of mafoprazine on $[^{3}H]$-CLN binding ($K_i$=101 nM) was 21, 6 and 4 times more

| $[^{3}H]$-ligand (receptor type) | Inhibition of $[^{3}H]$-ligand binding ($K_i$, nM) | Reference compound |
|---------------------------------|-----------------------------------------------|-------------------|
| $[^{3}H]$-SCH23390 ($D_1$)     | 2330                                          | —                 |
| $[^{3}H]$-SPL ($D_2$)          | 10.7                                          | —                 |
| $[^{3}H]$-PRZ ($\alpha_1$)     | 12.6                                          | Prazosin 0.0618   |
| $[^{3}H]$-CLN ($\alpha_2$)     | 101                                           | Yohimbine 37.1    |
| $[^{3}H]$-Dihydroalprenolol ($\beta$) | 15100                                        | (±)-Propranolol 2.04 |
| $[^{3}H]$-Ketanserin ($S_2$)   | 36.9                                          | Ketanserin 0.477  |
| $[^{3}H]$-ONB (mACH)           | 18800                                         | Atropine 0.354    |

$K_i$ values were determined using the relationship $K_i=IC_{50}/(1+(L/K_d))$, where $IC_{50}$ is the drug concentration necessary to displace 50% of specifically bound $[^{3}H]$-ligand. $K_i$: inhibition constant, $L$: concentration of $[^{3}H]$-ligand used in the assay, $K_d$: dissociation constant.
potent than those of haloperidol, azaperone and chlorpromazine, respectively (Table 2).

5) Effect on β receptors: The inhibitory effects of all test drugs were very weak, and their Kᵢ values were 10,000 nM or more (Table 2).

6) Effect on S₂ receptors: The inhibitory effect of mafoprazine on [³H]-KET (Kᵢ=36.9 nM) was 17.8 and 2 times less potent than those of chlorpromazine, azaperone and haloperidol, respectively (Table 2).

7) Effect on mACh receptors: The inhibitory effect of mafoprazine on [³H]-QNB binding was very weak (Kᵢ=18800 nM). Haloperidol and azaperone had relatively weak inhibitory effects, showing Kᵢ values of 2170 and 556 nM, respectively. On the other hand, the inhibitory effect of chlorpromazine on [³H]-QNB binding (Kᵢ=19.4 nM) was relatively strong (Table 2).

3. Effect on dopamine-stimulated adenylate cyclase activity

Mafoprazine had a very weak inhibitory effect on dopamine-stimulated adenylate cyclase activity in the corpus striatum (IC₅₀=52300 nM). Azaperone was similarly weak (IC₅₀=29800 nM), whereas chlorpromazine and haloperidol had relatively strong inhibitory effects, with the IC₅₀ values of 740 nM and 990 nM, respectively (Table 3).

4. Effects on brain monoamines and their metabolite contents

1) Frontal cortex: Mafoprazine at 10 mg/kg decreased NA and 5-HIAA significantly, but slightly (20% and 16%, respectively). Mafoprazine at 1–10 mg/kg hardly influenced the DA, DOPAC, HVA and 5-HT contents. Azaperone at 1–10 mg/kg caused a dose-dependent decrease of NA (17–63%). An increase in the DA metabolites (DOPAC 53%, HVA 53%) was found at 10 mg/kg. No dose-dependent decrease in 5-HIAA (22–30%) was found at 1 and 3 mg/kg. Chlorpromazine at 10 mg/kg tended to increase DA, DOPAC and HVA, but caused no remarkable change in NA, 5-HT or 5-HIAA (Table 4).

2) Corpus striatum: Mafoprazine at 1–10 mg/kg tended to decrease NA and increased the DA metabolites (DOPAC, 46–86%; HVA, 35–56%). The DOPAC- and HVA-increasing effects of mafoprazine became nearly maximal at 3 mg/kg, showing no further change even at 10 mg/kg. DA, 5-HT and 5-HIAA showed no notable change. Azaperone at 1–10 mg/kg caused a decreasing tendency of NA and a dose-dependent decrease of DA (10–42%) and increase of the DA metabolites (DOPAC, 53–156%; HVA, 43–130%). No remarkable change was found in 5-HT or 5-HIAA. With chlorpromazine at doses of 3 and 10 mg/kg, a tendency of decreased NA and remarkable increase in the DA metabolites (DOPAC, 198–247%; HVA, 121–156%) were found, whereas no notable change was found in 5-HT or 5-HIAA content (Table 5).

3) Nucleus accumbens: Mafoprazine at 1–10 mg/kg increased the DA metabolites (DOPAC, 18–52%; HVA, 18–40%), but had no notable influence on NA, DA, 5-HT or 5-HIAA content. The DOPAC- and HVA-increasing effect of mafoprazine, as in the corpus striatum, reached the maximum at 3 mg/kg, and no further increase was found at 10 mg/kg. Azaperone caused a tendency of decreased DA at 3 and 10 mg/kg and increase in the DA metabolites (DOPAC, 31–62%) at 1–10 mg/kg. No notable change was found in NA, 5-HT or 5-HIAA content. The DA metabolite-increasing effect of azaperone became nearly maximal at 3 mg/kg. Chlor-
Table 4. Effects of mafoprazine, azaperone and chlorpromazine on monoamines and their metabolite contents in rat frontal cortex

| Drugs       | Dose mg/kg, s.c. | N | NA       | DA       | DOPAC    | HVA*    | 5-HT    | 5-HIAA  |
|-------------|------------------|---|----------|----------|----------|---------|---------|---------|
| Control     |                  | 8 | 3.57±0.10| 1.00±0.04| 0.36±0.03| 0.57±0.06| 14.73±0.45| 3.42±0.08|
| Mafoprazine | 1                | 8 | 3.56±0.08| 0.96±0.09| 0.27±0.03| 0.46±0.03| 15.07±0.39| 2.99±0.08|
|             | 3                | 8 | 3.30±0.05| 1.13±0.04| 0.34±0.02| 0.52±0.04| 14.95±0.50| 3.06±0.09|
|             | 10               | 8 | 2.84±0.07***| 1.03±0.08| 0.37±0.05| 0.55±0.03| 14.85±0.53| 2.87±0.07*|
| Azaperone   | 1                | 8 | 2.96±0.10**| 1.05±0.05| 0.36±0.03| 0.61±0.06| 16.29±0.57| 2.41±0.05***|
|             | 3                | 8 | 2.38±0.07***| 1.08±0.04| 0.43±0.02| 0.70±0.02| 14.97±0.44| 2.66±0.06***|
|             | 10               | 8 | 1.31±0.06***| 0.86±0.08| 0.55±0.03*| 0.93±0.07*| 13.47±0.42| 3.89±0.08|
| Chlorpromazine | 3         | 7 | 3.44±0.16| 1.16±0.11| 0.39±0.02| 0.53±0.04| 14.94±0.42| 3.19±0.17|
|             | 10               | 6 | 3.74±0.11| 1.41±0.13| 0.47±0.03| 0.70±0.04| 14.43±0.41| 3.10±0.14|

One-way ANOVA followed by the Sheffé-test. *: P<0.05, **: P<0.01, ***: P<0.001. significant difference from the control. *All values were log-transformed prior to ANOVA.

Table 5. Effects of mafoprazine, azaperone and chlorpromazine on monoamines and their metabolite contents in rat corpus striatum

| Drugs       | Dose mg/kg, s.c. | N | NA       | DA       | DOPAC    | HVA     | 5-HT    | 5-HIAA  |
|-------------|------------------|---|----------|----------|----------|---------|---------|---------|
| Control     |                  | 8 | 0.93±0.05| 99.28±3.03| 8.74±0.32| 8.28±0.36| 6.39±0.18| 5.37±0.19|
| Mafoprazine | 1                | 8 | 0.77±0.04| 98.01±2.74| 12.78±0.66**| 11.17±0.52*| 5.68±0.23| 5.26±0.21|
|             | 3                | 8 | 0.88±0.08| 92.87±2.89| 15.40±0.58***| 12.31±0.39***| 6.23±0.29| 5.47±0.21|
|             | 10               | 8 | 0.71±0.04| 92.21±2.88| 16.25±0.51***| 12.91±0.47***| 6.07±0.15| 5.21±0.21|
| Azaperone   | 1                | 8 | 0.71±0.05| 90.87±1.86| 13.33±0.45***| 11.83±0.48**| 5.57±0.16| 4.87±0.14|
|             | 3                | 8 | 0.64±0.05| 80.36±1.76***| 17.46±0.43***| 15.05±0.41***| 5.18±0.18| 4.93±0.14|
|             | 10               | 8 | 0.68±0.07| 57.19±2.63***| 22.40±0.70***| 19.02±0.54***| 5.37±0.31| 6.01±0.29|
| Chlorpromazine | 3         | 7 | 0.80±0.06| 105.67±2.43| 26.07±0.79***| 18.29±0.47***| 6.16±0.42| 6.25±0.27|
|             | 10               | 6 | 0.77±0.12| 101.33±1.90| 30.32±0.82***| 21.22±0.56***| 6.02±0.44| 6.13±0.43|

One-way ANOVA followed by the Sheffé-test. *: P<0.05, **: P<0.01, ***: P<0.001, significant difference from the control.
Table 6: Effects of mafoprazine, azaperone and chlorpromazine on monoamines and thier metabolite contents in rat nucleus accumbens

| Drugs       | Dose mg/kg. s.c. | N | NA      | DA   | DOPAC* | HVA   | 5-HT  | 5-HIAA  |
|-------------|------------------|---|---------|------|--------|-------|-------|---------|
| Control     | —                | 8 | 2.58±0.47 | 62.38±3.09 | 8.77±0.43 | 5.58±0.41 | 8.33±0.42 | 4.66±0.12 |
| Mafoprazine | 1                | 8 | 2.17±0.45 | 66.37±2.25 | 10.34±0.70 | 6.57±0.21 | 8.41±0.61 | 4.34±0.20 |
|             | 3                | 8 | 2.46±0.37 | 68.75±0.57 | 13.11±0.62*** | 8.27±0.28* | 8.26±0.42 | 4.43±0.08 |
|             | 10               | 8 | 2.05±0.19 | 67.11±1.64 | 13.31±0.44*** | 8.05±0.21* | 9.05±0.55 | 4.23±0.09 |
| Azaperone   | 1                | 8 | 2.62±0.28 | 64.13±1.76 | 11.52±0.60 | 7.58±0.40 | 9.49±0.47 | 4.31±0.15 |
|             | 3                | 8 | 2.24±0.35 | 54.19±2.09 | 12.62±0.50** | 10.07±0.37*** | 7.87±0.42 | 4.12±0.22 |
|             | 10               | 8 | 2.71±0.29 | 39.70±0.94 | 14.17±0.49*** | 10.88±0.50*** | 8.02±0.69 | 4.86±0.16 |
| Chlorpromazine | 3           | 7 | 2.34±0.40 | 74.09±1.58 | 20.39±0.86*** | 12.84±0.73*** | 7.41±1.04 | 5.16±0.19 |
|             | 10               | 6 | 2.99±0.65 | 66.72±2.59 | 20.69±1.83*** | 12.85±0.59*** | 7.75±0.86 | 5.21±0.27 |

One-way ANOVA followed by the Sheffé-test. *: P<0.05, **: P<0.01, ***: P<0.001. significant difference from the control. aAll values were log-transformed prior to ANOVA.
promazine slightly increased DA at 3 mg/kg and increased DOPAC and HVA by about 130% both at 3 and 10 mg/kg, but had no notable influence on NA, 5-HT or 5-HIAA content (Table 6).

Discussion

The affinity of mafoprazine for neuronal receptors in rat brain was in the order: $D_2 > \alpha_1 > S_2 > 5-HT > D_1, > \beta > mACH$. Mafoprazine showed the highest affinity for $D_2$ receptors. However, even this affinity for $D_2$ receptors was 16 and 6 times lower than that of haloperidol and chlorpromazine, respectively. On the other hand, the selectivity of mafoprazine for $D_2$ receptors in terms of $D_1/D_2$ ($K_i$ value ratio) was about 9 times as high as that of chlorpromazine and even about twice that of haloperidol which has a relatively high $D_2$ selectivity. The inhibitory effect of mafoprazine on dopamine-stimulated adenylyl cyclase ($IC_{50}=52300$ nM) was less potent than that of chlorpromazine or haloperidol. This also suggests that mafoprazine has higher $D_2$ receptor selectivity than the reference drugs, since $D_1$ receptors associate with adenylyl cyclase (14).

Many antipsychotic drugs have a relatively high affinity for $\alpha$ receptors, and their clinical sedative and antihypertensive effects are considered to be due to $\alpha_1$ blocking activity (15, 16). In the present study, too, all test drugs including mafoprazine showed relatively high affinity for $\alpha_1$ receptors. Though the affinity of mafoprazine for $\alpha_1$ receptors was 2–10 times lower than that of the reference drugs, it was almost equal to that for $D_2$ receptors. Mafoprazine is known to have an inhibitory effect on the pressor response to electric stimulation of the posterior hypothalamus and an antagonistic effect on the pressor response to adrenaline or noradrenaline (M. Yamamura et al., unpublished observation). Therefore, mafoprazine is considered to have both central and peripheral $\alpha_1$ blocking activities. The affinity of mafoprazine for $\alpha_2$ receptors, when expressed in terms of $D_2/\alpha_2$ ($K_i$ value ratio), was 345, 26 and 3 times higher than those of haloperidol, chlorpromazine and azaperone, respectively. It augments the hypothermia induced by the $\alpha_2$ agonist clonidine at the doses where it fails to affect the normothermia in mice (M. Yamamura et al., unpublished observation), suggesting that mafoprazine probably has an agonistic effect on $\alpha_2$ receptors. These results further suggest that the action of mafoprazine as an antipsychotic drug is based mainly on $D_2$ receptor blocking activity and $\alpha$-adrenergic activity ($\alpha_1$ receptor blocking activity and $\alpha_2$ receptor stimulating activity). On the other hand, the affinity of azaperone for $\alpha_1$ receptors was the highest among all the receptors examined and 6 times as high as that for $D_2$ receptors. Azaperone strongly inhibits the lethal effect of adrenaline and noradrenaline in rats (1). Therefore, the sedative effect of azaperone in the pig is suggested to be partly due to $\alpha_1$ receptor blocking activity. Actually, azaperone strongly antagonizes the blood pressure elevation and decrease in heart rate caused by phenylephrine. Based on this fact, it is inferred that the preventive effect of azaperone on stress-induced lethality in the pig is mainly due to its peripheral $\alpha$-receptor blocking activity (17).

Generally, antipsychotic drugs are considered to enhance the turnover of dopamine at presynaptic nerve endings (18) and increase DA metabolites (19, 20) because of their dopamine receptor blocking activity. In the present study, too, mafoprazine and all reference drugs significantly increased the DA metabolites (DOPAC and HVA) in the corpus striatum and nucleus accumbens. When the relative potency of each test drug was investigated in terms of % increase of DA metabolites found after administration of 10 mg/kg of each drug, mafoprazine was less potent than azaperone and chlorpromazine both in the corpus striatum and in the nucleus accumbens. Moreover, the % increase of DA metabolites by mafoprazine was maximal at 3 mg/kg both in the corpus striatum and in the nucleus accumbens. These results suggest that the DA metabolite-increasing effect of mafoprazine is less potent than that of azaperone or chlorpromazine, not only in potency but also in efficacy. Among the dopaminergic neuronal systems, the nigrostriatal system is considered to be involved in the development of stereotyped behavior, rotational behavior and self-stimulation in animals and suppression of this dopaminergic neuronal system is considered to cause the
manifestation of extrapyramidal symptoms (21). The DA-metabolites-increasing effect of mafoprazine in the corpus striatum is less potent than those of chlorpromazine and haloperidol, which is probably related to its mild extrapyramidal side effects in experimental animals (M. Yamamura et al., unpublished observation). On the other hand, the mesolimbic system is an important site of action of antipsychotic drugs and is said to be related with the behavior and mental function of animals (21). Therefore, the finding that mafoprazine increased DA metabolites in the nucleus accumbens to a smaller extent than did the reference drugs indicates its less potent antipsychotic activity. However, mafoprazine showed an antagonistic effect almost comparable to those of chlorpromazine on apomorphine-induced cage-climbing behavior in mice and stereotyped behavior in monkeys, and methamphetamine-induced increased spontaneous motor activity and grouping toxicity in mice and stereotyped behavior in rats (M. Yamamura et al., unpublished observation). Therefore, the aforementioned \( \alpha \)-adrenergic activity as well as \( D_2 \) receptor blocking activity is considered to contribute to the development of the antipsychotic activity of mafoprazine. Incidentally, the azaperone-induced decrease in NA in the frontal cortex and decrease in DA in the corpus striatum and nucleus accumbens may be related to its potent sedative action.

In conclusion, unlike haloperidol, which has a high affinity for dopamine receptors, particularly \( D_2 \) receptors, azaperone, which is highly selective for \( \alpha_1 \) receptors, or chlorpromazine, which has a small \( D_1/D_2 \) ratio (Ki value ratio) and a relatively high affinity for mACH receptors, mafoprazine is a new antipsychotic drug having moderate \( D_2 \) receptor blocking activity and relatively potent \( \alpha_1 \) receptor blocking and \( \alpha_2 \) receptor agonistic activities.

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