

BRES 25360

Effects of benzylpiperazine derivatives on the neurotoxicity of 3,4-methylenedioxymethamphetamine in rat brain

Kenji Hashimoto, Harumi Maeda and Tsuyoshi Goromaru

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama (Japan)

(Accepted 23 June 1992)

Key words: 3,4-Methylenedioxymethamphetamine; Neurotoxicity; Benzylpiperazine; Serotonin; *p*-Nitrobenzylpiperazine; *p*-Chlorobenzylpiperazine; 1-Piperonylpiperazine

The neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA) in rat brain was attenuated significantly by coadministration of several benzylpiperazines (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine), which were weak inhibitors for [³H]6-nitroquipazine binding to the 5-hydroxytryptamine (5-HT) transporter in rat brain. These results suggest that these benzylpiperazines may inhibit the MDMA-induced neurotoxicity by a novel neuropharmacological effect other than 5-HT uptake inhibition.

3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) has been used for recreational purposes¹² and is a neurotoxin to serotonergic neurons in rat brain^{1–4,6,10,11,14–17}. It is well known that the neurotoxicity of MDMA is inhibited by treatment with 5-hydroxytryptamine (5-HT; serotonin) uptake inhibitors^{2,4,14–16}, indicating that the 5-HT transporter plays an important role in the neurodestruction of serotonergic neurons by MDMA. It has also been reported that selective 5-HT₂ receptor antagonists partially attenuate the MDMA-induced neurotoxicity in rat brain^{15,16}. Thus, although the neurotoxicity of MDMA in the serotonergic neurons is now well established, the underlying mechanisms of action are unknown. Recently, we found that the MDMA-induced neurotoxicity in rat brain was significantly attenuated by coadministration of 1-piperonylpiperazine (3,4-methylenedioxybenzylpiperazine), which was a weak inhibitor for [³H]5-HT uptake into rat brain synaptosomes and [³H]ketanserin binding to 5-HT₂ receptor^{7,8}. These results suggest that the antagonism of MDMA-induced neurotoxicity by 1-piperonylpiperazine might be due to effect(s) other than 5-HT uptake inhibition and 5-HT₂ receptor antagonism, although the pharmacological effects of 1-piperonylpiperazine and the mechanisms underlying

the antagonism by 1-piperonylpiperazine are currently unknown. Therefore, it may be of interest to examine the effects of several benzylpiperazine derivatives on the neurotoxicity of MDMA in rat brain. The present study was undertaken to study the effects of several benzylpiperazine derivatives on the neurotoxicity of MDMA in the serotonergic neurons of rat brain. Moreover, we compared these effects with the effects of desipramine (a very weak 5-HT uptake inhibitor) on the MDMA-induced neurotoxicity, and also examined the effects of these drugs on the [³H]6-nitroquipazine labeling of the 5-HT transporter in rat brain *in vitro*.

Male Wistar rats (200–250 g) were administered intraperitoneally with either vehicle (1 ml/kg), MDMA (10 mg/kg) or MDMA (10 mg/kg) plus drugs (10 mg/kg) twice a day (09.00 h and 19.00 h) for 3 consecutive days. Rats were killed by decapitation 1 week after the last administration. Brains were rapidly removed and dissected on ice. The contents of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral cortex were measured by high performance liquid chromatography (HPLC) with electrochemical detection as described previously^{4,6–8}. The IC₅₀ values of drugs on [³H]6-nitroquipazine binding to cortical membranes of rat at 37°C were determined by the previous report⁵

with a slight modification. MDMA HCl, *p*-chlorobenzylpiperazine HCl, *p*-methoxybenzylpiperazine HCl and *p*-nitrobenzylpiperazine HCl were synthesized in our laboratory. 1-Benzylpiperazine (Wako Pure Chemical Co., Tokyo, Japan) and 1-piperonylpiperazine (Aldrich Chemical Co., Milwaukee, WI) were used as HCl salt. Desipramine HCl was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were purchased commercially. The statistical evaluation of multiple data was performed by a one-way analysis of variance, followed by the Fisher's PLSD test.

Table I shows the effects of drugs on the reductions of 5-HT and 5-HIAA produced by multiple administration of MDMA. The contents of 5-HT and 5-HIAA in the cerebral cortex of rats after multiple administration of MDMA (10 mg/kg, i.p.) were decreased significantly to 17% and 20% of control, respectively. The coadministration of *p*-nitrobenzylpiperazine (10 mg/kg), *p*-chlorobenzylpiperazine (10 mg/kg) and 1-piperonylpiperazine (10 mg/kg) significantly attenuated the reductions of 5-HT and 5-HIAA produced by treatment with MDMA (10 mg/kg). *p*-Nitrobenzylpiperazine was the most potent blocker for the MDMA-

TABLE II

Drug effects on [3 H]6-nitroquipazine binding to cortical membranes of rats

The IC₅₀ values of drugs on [3 H]6-nitroquipazine binding to cortical membranes at 37°C were determined as described in the text. The values are the mean of 3 determinations done in duplicate, the S.D. of which are less than 10%.

	IC ₅₀ (μM)
1-Benzylpiperazine	122.6
1-Piperonylpiperazine	78.7
<i>p</i> -Chlorobenzylpiperazine	25.0
<i>p</i> -Methoxybenzylpiperazine	49.3
<i>p</i> -Nitrobenzylpiperazine	10.67
Desipramine	1.63

induced neurotoxicity, followed by *p*-chlorobenzylpiperazine and 1-piperonylpiperazine. However, the reductions of 5-HT and 5-HIAA in the cerebral cortex by MDMA were not altered significantly by coadministration of 1-benzylpiperazine (10 mg/kg), *p*-methoxybenzylpiperazine (10 mg/kg) and desipramine (10 mg/kg).

Table II shows the IC₅₀ values of drugs on [3 H]6-nitroquipazine binding to cortical membranes of rat at 37°C. The benzylpiperazine derivatives examined were very weak inhibitors for [3 H]6-nitroquipazine binding to cortical membranes, and were less potent as compared with desipramine. Thus, it seems that these benzylpiperazine derivatives are weak inhibitors of [3 H]6-nitroquipazine-labeling of the 5-HT transporter in vitro.

The major finding of the present study is that the neurotoxicity of MDMA in the rat brain was attenuated significantly by coadministration of benzylpiperazine derivatives (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine). The present data show that 1-piperonylpiperazine (10 mg/kg) is less active at inhibiting the neurotoxicity of MDMA as compared with a previous report⁸, since the half-dose (10 mg/kg) of the previous report⁸ was used in the present experiment. The data of IC₅₀ values of drugs on [3 H]6-nitroquipazine binding suggest that benzylpiperazine derivatives used in the present study were weak inhibitors of [3 H]6-nitroquipazine binding to the 5-HT transporter in vitro. We have also demonstrated recently that these benzylpiperazine derivatives were weak inhibitors of [3 H]5-HT uptake into rat brain synaptosomes (Hashimoto et al., submitted). The coadministration of desipramine could not attenuate the MDMA-induced neurotoxicity in rat brain, although desipramine is a more potent 5-HT uptake inhibitor than the benzylpiperazine derivatives examined in this study. Moreover, the MDMA-induced neurotoxicity could not be attenuated significantly by coadministra-

TABLE I

Drug effects on the MDMA-induced neurotoxicity in rat brain

Vehicle (1 ml/kg), MDMA (10 mg/kg) or MDMA (10 mg/kg) plus drugs (10 mg/kg) were administered intraperitoneally into rats twice a day for 3 consecutive days. Rats were killed by decapitation 1 week after last administration, and the contents of 5-HT and 5-HIAA in the cerebral cortex of rats were measured by HPLC. Values are the mean ± S.D. of 4 rats. Values in parentheses represent percent of vehicle group.

	5-HT (ng / mg tissue)	5-HIAA (ng / mg tissue)
Vehicle	0.18 ± 0.03	0.15 ± 0.01
MDMA	0.03 ± 0.01 (17%) **	0.03 ± 0.02 (20%) **
MDMA + 1-benzyl- piperazine	0.03 ± 0.01 (17%) **	0.03 ± 0.01 (20%) **
MDMA + 1-piperonyl- piperazine	0.07 ± 0.01 (39%) ***+	0.06 ± 0.02 (40.0%) ***+
MDMA + <i>p</i> -chloro- benzyl- piperazine	0.09 ± 0.05 (50%) ****	0.09 ± 0.03 (60%) ****
MDMA + <i>p</i> -methoxy- benzyl- piperazine	0.03 ± 0.01 (17%) **	0.04 ± 0.01 (27%) **
MDMA + <i>p</i> -nitro- benzyl- piperazine	0.12 ± 0.04 (67%) ***+	0.12 ± 0.03 (80%) **
MDMA + desipramine	0.03 ± 0.01 (17%) **	0.03 ± 0.01 (20%) **

* $P < 0.05$, ** $P < 0.01$ when compared with vehicle group. + $P < 0.05$, ++ $P < 0.01$ when compared with MDMA alone group.

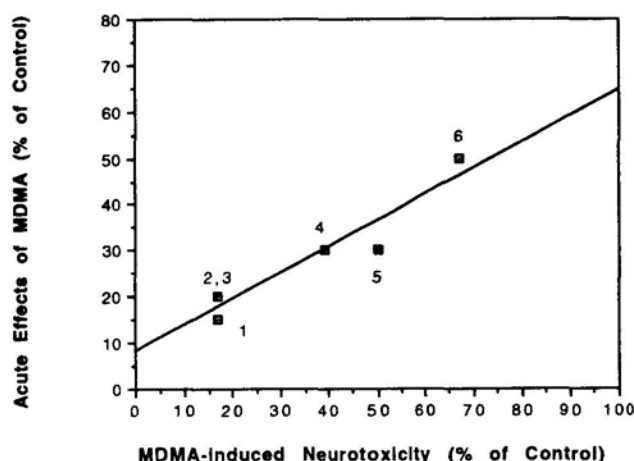


Fig. 1. Correlation between potency for inhibition of the MDMA-induced neurotoxicity and potency for inhibition of acute effects of MDMA. Data of the MDMA-induced neurotoxicity were taken from Table I (5-HT contents). Data of the acute effects of MDMA on the concentration of 5-HT were taken from our data (Hashimoto et al., submitted). The data of MDMA alone and MDMA plus 5 benzylpiperazine derivatives were used. 1, MDMA; 2, MDMA + 1-benzylpiperazine; 3, MDMA + *p*-methoxybenzylpiperazine; 4, MDMA + 1-piperonylpiperazine; 5, MDMA + *p*-chlorobenzylpiperazine; 6, MDMA + *p*-nitrobenzylpiperazine. The correlation coefficient (r) = 0.95 and slope = 0.56 for data presented.

tion of imipramine, which was a more potent 5-HT uptake inhibitor than desipramine and benzylpiperazine derivatives (Hashimoto, unpublished data). Taken together, these benzylpiperazine derivatives might inhibit the MDMA-induced neurotoxicity by effect(s) other than 5-HT uptake inhibition.

It is well known that the acute neurochemical effect of MDMA is its carrier-mediated release of 5-HT^{9,16}, and it is suggested that the protective effects of 5-HT uptake inhibitors might be attributable to their ability to inhibit 5-HT release induced by MDMA⁹. Recently, we have found that these benzylpiperazines (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine) significantly attenuated the acute effects of MDMA on concentration of 5-HT in rat brain (Hashimoto et al., submitted). Interestingly, the potencies of these benzylpiperazines for blocking the acute effects of MDMA were highly correlated with the potencies for blocking the MDMA-induced neurotoxicity ($y = 0.56x - 8.0$, $r = 0.95$, $P < 0.001$) (Fig. 1). Taken together, the present results suggest that the 5-HT transporter might play a role in the inhibition of the acute neurochemical effects of MDMA by these benzylpiperazines, and that the inhibition of acute effects by these drugs might lead to the inhibition of MDMA-induced neurotoxicity. Moreover, we have found recently that specific *in vivo* binding of [³H]6-nitroquipazine to the 5-HT transporter in brain was decreased significantly by pretreatment with *p*-nitro-

benzylpiperazine, but not by 1-benzylpiperazine (Hashimoto, unpublished data). Unlike 5-HT uptake inhibitors, it does not seem that these benzylpiperazines interact directly with the 5-HT transporter in the brain. Although the exact sites at which these benzylpiperazines act remain to be elucidated, the results from the present study suggest that the 5-HT transporter might be involved in the inhibition of MDMA-induced neurotoxicity by these benzylpiperazines, and that these benzylpiperazines might modulate the 5-HT transporter *in vivo* after administration of drugs. Thus, it appears that these benzylpiperazines may possess a novel neuropharmacological effect in the central nervous system. Further studies on this hypothesis are necessary. Moreover, previous reports^{13,18} suggest that 5-HT uptake inhibition may be important for modulation and attenuation of the rewarding effects of amphetamine and related compounds. It would therefore be of great interest to study the effects of these benzylpiperazines on the rewarding effects of amphetamine and related compounds.

- 1 Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J. and De Souza, E.B., 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [³H]paroxetine-labeled serotonin uptake sites, *J. Pharmacol. Exp. Ther.*, 242 (1987) 911-916.
- 2 Battaglia, G., Yeh, S.Y. and De Souza, E.B., MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons, *Pharmacol. Biochem. Behav.*, 29 (1988) 269-274.
- 3 Commins, D.L., Vosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R. and Seiden, L.S., Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain, *J. Pharmacol. Exp. Ther.*, 241 (1987) 338-345.
- 4 Hashimoto, K. and Goromaru, T., Reduction of [³H]6-nitroquipazine-labelled 5-hydroxytryptamine uptake sites in rat brain by 3,4-methylenedioxymethamphetamine, *Fundam. Clin. Pharmacol.*, 4 (1990) 635-641.
- 5 Hashimoto, K. and Goromaru, T., High affinity binding of [³H]6-nitroquipazine to cortical membranes in the rat: inhibition by 5-hydroxytryptamine and 5-hydroxytryptamine uptake inhibitors, *Neuropharmacology*, 30 (1991) 113-117.
- 6 Hashimoto, K. and Goromaru, T., Study of 3,4-methylenedioxymethamphetamine-induced neurotoxicity in rat brain using specific *in vivo* binding of [³H]6-nitroquipazine, *Res. Commun. Subst. Abuse*, 13 (1992) in press.
- 7 Hashimoto, K. and Goromaru, T., Reversal of acute effects of 3,4-methylenedioxymethamphetamine in rat brain by 1-piperonylpiperazine, *Res. Commun. Subst. Abuse*, 13 (1992) in press.
- 8 Hashimoto, K., Maeda, H. and Goromaru, T., Antagonism of 3,4-methylenedioxymethamphetamine-induced neurotoxicity in rat brain by 1-piperonylpiperazine, *Eur. J. Pharmacol.*, in press.
- 9 Hekmatpanah, C.R. and Peroutka, S.J., 5-Hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents, *Eur. J. Pharmacol.*, 177 (1990) 95-98.
- 10 McKenna, D.J. and Peroutka, S.J., Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), *J. Neurochem.*, 54 (1990) 14-22.
- 11 O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J. and Molliver, M.E., Methylenedioxyamphetamine (MDA) and meth-

- ylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity, *J. Neurosci.*, 8 (1988) 2788-2803.
- 12 Peroutka, S.J., Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') on an undergraduate campus, *N. Eng. J. Med.*, 317 (1987) 1542-1543.
 - 13 Ritz, M.C. and Kuhar, M.J., Relationship between self-administration of amphetamine and monoamine receptors in brain: comparison with cocaine, *J. Pharmacol. Exp. Ther.*, 248 (1989) 1010-1017.
 - 14 Schmidt, C.J., Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 240 (1987) 1-7.
 - 15 Schmidt, C.J., Abbate, G.M., Black, C.K. and Taylor, V.L., Selective 5-hydroxytryptamine₂ receptor antagonists protect against the neurotoxicity of methylenedioxymethamphetamine in rats, *J. Pharmacol. Exp. Ther.*, 255 (1990) 478-483.
 - 16 Schmidt, C.J., Levin, J.A. and Lovenberg, W., In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, *Biochem. Pharmacol.*, 36 (1987) 747-755.
 - 17 Stone, D.M., Johnson, M., Hanson, G.R. and Gibb, J.W., Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 247 (1988) 79-87.
 - 18 Yu, D.S.L., Smith, F.L., Smith, D.C. and Lyness, W.H., Fluoxetine-induced attenuation of amphetamine self-administration in rats, *Life Sci.*, 39 (1986) 1383-1388.