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Short communication

Antagonism of 3,4-methylenedioxymethamphetamine-induced neurotoxicity in rat brain by 1-piperonylpiperazine

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The effects of 1-piperonylpiperazine and N,α-dimethylpiperonylamine, which are weak inhibitors for [^{3}H]5-hydroxytryptamine (5-HT) uptake, on 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity were examined. The reductions of serotonergic parameters in the rat cerebral cortex produced by multiple administration of MDMA (10 mg/kg) were attenuated significantly by coadministration of 6-nitroquipazine (10 mg/kg), paroxetine (10 mg/kg) or 1-piperonylpiperazine (20 mg/kg), but not by N,α-dimethylpiperonylamine (20 mg/kg). The present data suggest that 1-piperonylpiperazine might inhibit the MDMA-induced neurotoxicity by effect(s) other than 5-HT uptake inhibition.

MDMA (3,4-methylenedioxymethamphetamine); 5-HT uptake inhibitor; 1-Piperonylpiperazine; 5-HT (5-hydroxytryptamine, serotonin); Neurotoxicity; Brain (rat)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a psychedelic amphetamine analogue which has been used for recreational purposes, and MDMA is a selective neurotoxin of serotonergic neurons in rat brain (Battaglia et al., 1987, 1988; Schmidt, 1987; Stone et al., 1988; McKenna and Peroutka, 1990; Hashimoto and Goromaru, 1990, 1992a). Furthermore, it has been reported that 5-hydroxytryptamine (5-HT) uptake inhibitors blocked the neurotoxic effects of MDMA in the serotonergic neurons (Schmidt, 1987; Battaglia et al., 1988; Hashimoto and Goromaru, 1990), indicating that 5-HT uptake carrier plays an important role in the MDMA-induced neurotoxicity. It has been reported that 5-HT_2 receptor antagonists attenuate the neurotoxicity of MDMA in rat brain (Schmidt et al., 1990). Thus, although the neurotoxicity of MDMA in the serotonergic neurons is now well established, the underlying mechanisms of action are unknown (McKenna and Peroutka, 1990). Interestingly, MDMA had no neurotoxic effects on serotonergic neurons when administered i.c.v. (Molliver et al., 1989), suggesting that MDMA-induced neurotoxicity may be due to its metabolite(s) formed in the periphery. Recently, we found that the disposition and metabolism of [^{3}H]MDMA in brain and peripheral organs were altered by treatment with paroxetine (a potent 5-HT uptake inhibitor) or basic compounds (1-piperonylpiperazine and N,α-dimethylpiperonylamine) including a piperonyl (3,4-methylenedioxybenzyl) group (Hashimoto et al., submitted), suggesting that a specific mechanism for 3,4-methylenedioxyphenyl group might exist in brain and peripheral organs. MDMA, paroxetine, 1-piperonylpiperazine and N,α-dimethylpiperonylamine all possess the 3,4-methylenedioxyphenyl group. 1-Piperonylpiperazine and N,α-dimethylpiperonylamine are very weak inhibitors for [^{3}H]5-HT uptake (Hashimoto and Goromaru, 1992b). Therefore, the present study was undertaken to examine whether these drugs could affect the MDMA-induced neurotoxicity of 5-HT nerve terminals in rat brain. Furthermore, we also examined the effects of a potent 5-HT uptake inhibitor, 6-nitroquipazine, on the neurotoxicity of MDMA.

2. Materials and methods

2.1. Drug administration

Male Wistar rats (200–250 g) were maintained on a 12 h light-dark cycle with free access to food and water. Rats were divided into eight groups. Vehicle (1 ml/kg), MDMA (10 mg/kg), MDMA (10 mg/kg) plus

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drugs (6-nitroquipazine (10 mg/kg), paroxetine (10 mg/kg), 1-piperonylpiperazine (20 mg/kg), N,α-dimethylpiperonylamine (20 mg/kg)), 1-piperonylpiperazine (20 mg/kg) alone and N,α-dimethylpiperonylamine (20 mg/kg) alone were administered i.p. twice a day (9:00 and 19:00) for three consecutive days. Rats were killed by decapitation 1 week after last administration, and the brains were removed, rapidly dissected on ice and stored at −80°C.

6-Nitroquipazine maleate, MDMA HCl and N,α-dimethylpiperonylamine HCl were synthesized in our laboratory. Paroxetine HCl was donated from Beecham Pharmaceutical, (Surrey, UK). 1-Piperonylpiperazine (Aldrich Chemical Co., Milwaukee, WI) was used as HCl. Other chemicals were purchased commercially.

2.2. Determination of 5-HT and 5-HIAA

The concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral cortex of rats were determined by high performance liquid chromatography with electrochemical detection as described previously (Hashimoto and Goromaru, 1990).

2.3. Determination of 5-HT uptake sites in the brain

Specific [^{3}H]6-nitroquipazine (67.8 Ci/mmol, synthesized in our laboratory) (30–1500 pM) binding to cortical membranes of rats treated with vehicle or drugs was measured by a previously reported method (Hashimoto and Goromaru, 1990) with a slight modification. A maximal number of binding sites (B_{max}) and an apparent equilibrium dissociation constant (K_d) were determined by Scatchard plot analysis.

2.4. Statistics

The statistical evaluation of the multigroup was performed by a one-way analysis of variance, followed by the Scheffe's test for multiple comparisons. The criterion for significance was $P < 0.05$.

3. Results

The contents of 5-HT and 5-HIAA and the density (B_{max}) of [^{3}H]6-nitroquipazine-labeled 5-HT uptake sites in the cerebral cortex of rats were decreased significantly by the multiple administration of MDMA (10 mg/kg), as shown in table 1. The coadministration of two potent and selective 5-HT uptake inhibitors (6-nitroquipazine (10 mg/kg) and paroxetine (10 mg/kg)) significantly antagonized the reductions of serotonergic parameters in the cerebral cortex by MDMA. Furthermore, the coadministration of 1-piperonylpiperazine (20 mg/kg) significantly attenuated the reductions of serotonergic parameters produced by MDMA. However, the reductions of 5-HT and 5-HIAA and the density of [^{3}H]6-nitroquipazine-labeled 5-HT uptake sites in the rat brain by MDMA were not affected by coadministration of N,α-dimethylpiperonylamine (20 mg/kg). The K_d values of [^{3}H]6-nitroquipazine binding to rat cortical membranes were not significantly altered by treatment with drugs used

TABLE 1

Effects of drugs on the reductions of serotonergic parameters in the rat cerebral cortex produced by multiple administration of MDMA.

Vehicle or drugs were administered i.p. into rats twice a day for three consecutive days. Rats were killed by decapitation 1 week after last administration, and the contents of 5-HT and 5-HIAA and the density (B_{max}; fmol/mg protein) of [^{3}H]6-nitroquipazine-labeled 5-HT uptake sites in the cerebral cortex of rats were determined as described in Materials and methods. Values are the mean ± S.D. of five rats.

	ng/mg tissue		fmol/mg protein
	5-HT	5-HIAA	5-HT Uptake sites
Vehicle	0.137 ± 0.01 (100%)	0.125 ± 0.01 (100%)	314.9 ± 15.1 (100%)
MDMA	0.057 ± 0.02 [a] (42%)	0.050 ± 0.03 [a] (40%)	82.0 ± 34.1 [a] (26%)
MDMA + 6-nitroquipazine	0.136 ± 0.02 [c] (99%)	0.117 ± 0.01 [c] (94%)	320.5 ± 64.0 [c] (102%)
MDMA + paroxetine	0.116 ± 0.02 [c] (85%)	0.151 ± 0.02 [c] (121%)	301.4 ± 23.9 [c] (96%)
MDMA + 1-piperonylpiperazine	0.106 ± 0.03 [b] (77%)	0.103 ± 0.02 [b] (82%)	193.8 ± 34.1 [b] (62%)
MDMA + N,α-dimethylpiperonylamine	0.058 ± 0.03 [a] (42%)	0.039 ± 0.01 [a] (31%)	68.1 ± 34.1 [a] (22%)
1-Piperonylpiperazine	0.145 ± 0.04 (106%)	0.153 ± 0.04 (122%)	309.2 ± 25.9 (98%)
N,α-Dimethylpiperonylamine	0.139 ± 0.03 (102%)	0.121 ± 0.04 (97%)	324.5 ± 30.1 (103%)

[a] $P < 0.01$ as compared with vehicle-treated group. [b] $P < 0.05$, [c] $P < 0.01$ as compared with MDMA-treated group.

in this study (data not shown). Moreover, the multiple administration of 1-piperonylpiperazine (20 mg/kg) or N,α-dimethylpiperonylamine (20 mg/kg) alone did not alter the serotonergic parameters in the rat brain.

4. Discussion

As reported previously, it is well known that 5-HT uptake inhibitors could antagonize the neurotoxicity of serotonergic neurons produced by MDMA (Schmidt, 1987; Battaglia et al., 1988; Hashimoto and Goromaru, 1990; Schmidt et al., 1990). The antagonism of MDMA-induced neurotoxicity by 6-nitroquipazine or paroxetine is consistent with other reports demonstrating that 5-HT uptake carrier plays an important role in the neurodestruction of serotonergic neurons by MDMA (Schmidt, 1987; Battaglia et al., 1988; Hashimoto and Goromaru, 1990; Schmidt et al., 1990). The major finding of present study is that 1-piperonylpiperazine could attenuate the neurotoxicity of serotonergic neurons by MDMA. The IC_{50} values of 1-piperonylpiperazine and N,α-dimethylpiperonylamine for [^{3}H]5-HT uptake into rat brain synaptosomes were 35,700 nM and 118,100 nM, respectively (Hashimoto and Goromaru, 1992b). Thus, it seems that 1-piperonylpiperazine has no inhibitory effect of 5-HT uptake. It has been reported that selective 5-HT_2 receptor antagonists attenuated significantly the neurotoxicity of MDMA, suggesting that 5-HT_2 receptor antagonists interfere with the maintenance of MDMA-induced, carrier-mediated dopamine release by blocking the stimulation of dopamine synthesis produced by MDMA (Schmidt et al., 1990). Stone et al. (1988) also reported that GBR 12909, a selective dopamine uptake inhibitor, attenuated significantly the MDMA-induced neurotoxicity, suggesting a possible role for MDMA-induced dopamine release in the neurodestruction of serotonergic neurons produced by MDMA (Stone et al., 1988; Schmidt et al., 1990). However, 1-piperonylpiperazine has no inhibitory effects for [^{3}H]ketanserin binding to 5-HT_2 receptor or [^{3}H]dopamine uptake into synaptosomes of striatum (Hashimoto, unpublished data). Recently, Hekmatpanah and Peroutka (1990) suggested that the protective effect of 5-HT uptake inhibitors might be attributable to their ability to inhibit 5-HT release induced by MDMA. However, 1-piperonylpiperazine and N,α-dimethylpiperonylamine had no effect on the [^{3}H]5-HT release from rat brain synaptosomes or on the MDMA-induced [^{3}H]5-HT release from rat brain synaptosomes (Hashimoto, unpublished data). Taken together, the present data suggest that 1-piperonylpiperazine might inhibit the MDMA-induced neurotoxicity by effect(s) other than 5-HT uptake inhibition and 5-HT_2 receptor antagonism. Interestingly, 1-piperonylpiperazine significantly attenuated the neurodestruction of serotonergic neurons in rat brain produced by other amphetamine derivatives (p-chloroamphetamine and fenfluramine) (Hashimoto, unpublished data). Thus, 1-piperonylpiperazine may be a suitable drug for studying the neurotoxicity of serotonergic neurons produced by amphetamine derivatives, although the mechanism underlying the inhibition of MDMA-induced neurotoxicity by 1-piperonylpiperazine is currently unknown. Further studies on pharmacological effects of 1-piperonylpiperazine are necessary.

It has also been demonstrated that metabolite(s) of either MDMA or an endogenous substance may play a possible role in the neurotoxicity of MDMA (Molliver et al., 1989; McKenna and Peroutka, 1990). Recently, we found that the disposition and metabolism of [^{3}H]MDMA in brain and peripheral organs were altered by treatment with 1-piperonylpiperazine or N,α-dimethylpiperonylamine (Hashimoto et al., submitted). However, the present study shows that the MDMA-induced neurotoxicity was attenuated significantly by coadministration of 1-piperonylpiperazine, but not by coadministration of N,α-dimethylpiperonylamine. Thus, it is currently unknown whether 1-piperonylpiperazine inhibit the metabolism of MDMA to neurotoxic agent(s).

In conclusion, the present results suggest that 1-piperonylpiperazine could attenuate significantly the neurotoxicity of serotonergic neurons produced by MDMA, and that the antagonism of MDMA-induced neurotoxicity by 1-piperonylpiperazine might be due to pharmacological effect(s) other than 5-HT uptake inhibition and 5-HT_2 receptor antagonism. Further detailed studies on this hypothesis are necessary.

References

Battaglia, G., S.Y. Yeh, E. O'Hearn, M.E. Molliver, M.J. Kuhar and E.B. De Souza, 1987, 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites, J. Pharmacol. Exp. Ther. 242, 911.

Battaglia, G., S.Y. Yeh and E.B. De Souza, 1988, MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons, Pharmacol. Biochem. Behav. 29, 269.

Commins, D.L., G. Vosmer, R.M. Virus, W.L. Woolverton, C.R. Schuster and L.S. Seiden, 1987, Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain, J. Pharmacol. Exp. Ther. 241, 338.

Hashimoto, K. and T. Goromaru, 1990, Reduction of [^{3}H]6-nitroquipazine-labelled 5-hydroxytryptamine uptake sites in rat brain by 3,4-methylenedioxymethamphetamine, Fund. Clin. Pharmacol. 4, 635.

Hashimoto, K. and T. Goromaru, 1992a, Study of 3,4-methylenedioxymethamphetamine-induced neurotoxicity in rat brain using specific in vivo binding of [^{3}H]6-nitroquipazine, Res. Commun. Subst. Abuse, in press.

Hashimoto, K. and T. Goromaru, 1992b, Reversal of acute effects of 3,4-methylenedioxymethamphetamine in rat brain by 1-piperonylpiperazine, Res. Commun. Subst. Abuse, in press.

Hekmatpanah, C.R. and S.J. Peroutka, 1990, 5-Hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents, Eur. J. Pharmacol. 177, 95.

McKenna, D.J. and S.J. Peroutka, 1990, Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), J. Neurochem. 54, 14.

Molliver, M.E., L.A. Mamounas and M.A. Wilson, 1989, Effects of neurotoxic amphetamines on serotonergic neurons: immunocytochemical studies, in: NIDA Research Monograph Series 94 – Pharmacology and Toxicology of Amphetamines and Designer Drugs, eds. K. Asghar and E.B. De Souza (National Institute on Drug Abuse, Rockville, MD) p. 270.

Schmidt, C.J., 1987, Neurotoxicity of the psychedelic amphetamine methylenedioxymethamphetamine, J. Pharmacol. Exp. Ther. 240, 1.

Schmidt, C.J., G.M. Abbate, C.K. Black and V.L. Taylor, 1990, Selective 5-hydroxytryptamine$_2$ receptor antagonists protect against the neurotoxicity of methylenedioxymethamphetamine in rats, J. Pharmacol. Exp. Ther. 255, 478.

Stone, D.M., M. Johnson, G.R. Hanson and J.W. Gibb, 1988, Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine, J. Pharmacol. Exp. Ther. 247, 79.