Reduction of [^{3}H]6-nitroquipazine-labelled 5-hydroxytryptamine uptake sites in rat brain by 3,4-methylenedioxymethamphetamine

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Summary – 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) is a known neurotoxin to 5-hydroxytryptamine (5-HT; serotonin) nerve terminals. It has recently been demonstrated that [^{3}H]6-nitroquipazine is a new radioligand for studying the 5-HT transport system in brain. Therefore, we examined the effects of repeated systemic administration (10 mg/kg ip, twice daily for 3 d) of MDMA on [^{3}H]6-nitroquipazine-labelled 5-HT uptake sites in rat brain. Marked reductions in the concentrations of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) were observed in the cerebral cortex 1 week after the last injection of MDMA. In addition, the density of [^{3}H]6-nitroquipazine-labelled 5-HT uptake sites was significantly decreased by MDMA. Furthermore, the reduction of 5-HT and 5-HIAA content and the density of [^{3}H]6-nitroquipazine-labelled 5-HT uptake sites by MDMA were significantly prevented by co-administration of 6-nitroquipazine (5 mg/kg), a very potent and selective 5-HT uptake inhibitor. The present results indicate that the 5-HT uptake carrier plays an important role in the neurotoxic action of MDMA.

3,4-Methylenedioxymethamphetamine / 5-Hydroxytryptamine uptake sites / [^{3}H]6-Nitroquipazine binding / rat brain

Introduction

3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) is a unique analog of amphetamine with widespread recreational use (Shulgin, 1986; Peroutka 1987; McKenna and Peroutka, 1990). MDMA is a selective neurotoxin to 5-hydroxytryptamine (5-HT; serotonin) nerve terminals in rat brain. MDMA induces a decrease of the concentrations of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) in rat brain (Battaglia *et al,* 1987; Stone *et al,* 1987; Battaglia *et al,* 1988; Commins *et al,* 1987; Schmidt, 1987; Schmidt and Taylor, 1987; Schmidt and Taylor, 1988; the reduction of tryptophan hydroxylase activity, rate-limiting enzyme of 5-HT synthesis (Schmidt and Taylor, 1987, 1988; Stone *et al,* 1987), and the reduction in the density of 5-HT uptake sites labelled by [^{3}H] paroxetine (Battaglia *et al,* 1987; Battaglia *et al,* 1988).

6-Nitroquipazine is a very potent and selective inhibitor of 5-HT (Vaatstra *et al,* 1981). Furthermore, it has been reported that 6-nitroquipazine is a more potent 5-HT uptake inhibitor than paroxetine (Classen *et al,* 1984). Recently, we reported the preparation of [^{3}H]6-nitroquipazine with high specific activity (Hashimoto and Goromaru, 1990a). Furthermore, it has been demonstrated that [^{3}H]6-nitroquipazine binding to rat cortical membranes is selectively inhibited by 5-HT uptake inhibitors and the potency of various drugs to inhibit [^{3}H]6-nitroquipazine binding closely correlated with their inhibitory effect upon [^{3}H]5-HT uptake into synaptosomes (Hashimoto and Goromaru, 1990b, 1990c; Hashimoto and Goromaru, submitted). Thus, [^{3}H]6-nitroquipazine is a new excellent radioligand for studying the 5-HT uptake sites in rat brain (Hashimoto and Goromaru, 1990b, 1990c, Hashimoto and Goromaru, submitted). Therefore, we explored the use of [^{3}H]6-nitroquipazine for studying neurodegeneration of 5-HT nerve terminals in rat brain after administration of MDMA. Moreover, it has been reported that 5-HT uptake inhibitors prevented MDMA-induced neurotoxicity (Schmidt, 1987; Schmidt and Taylor, 1987; Battaglia *et al,* 1988). We also studied whether 6-nitroquipazine could prevent the MDMA-induced neurotoxicity in rat brain or not.

Material and Methods

Drugs

[^{3}H]6-Nitroquipazine (2.26 TBq/mmol) and 6-nitroquipazine maleate were synthesized as described previously (Hashimoto and Goromaru, 1990a). MDMA hydrochloride was synthesized from 3,4-methylenedioxyphenyl-2-propanone (Fluka AG, Switzerland) and methylamine, as described previously (Braun *et al,* 1980). Paroxetine hydrochloride was donated by Beecham Pharmaceuticals (Surrey, UK). Other chemicals were purchased commercially.

Animals

Male Wistar strain rats (200-230 g) were housed in a 12-h light-dark cycle with free access to food and water. Rats were administered intraperitoneally with either vehicle, MDMA (10 mg/kg) or MDMA (10 mg/kg) plus 6-nitroquipazine (5 mg/kg) twice a d (12-h interval) for 3 consecutive d. Rats were sacrificed 1 week after the last injection. The cerebral cortex of the rat brains was dissected on ice and stored at $-80°C$ until use.

Determination of 5-HT and 5-HIAA

5-HT and 5-HIAA contents in the cerebral cortex of rat brains were measured using a high-performance liquid chromatography (HPLC) with electrochemical detection. The tissues were weighed and homogenized with 0.1 N $HClO_4$ containing 0.1% cysteine. *N*-Acetyl 5-HT (100 ng/ml) was added as an internal standard to allow recoveries to be determined. After centrifugation at 4000 *g* for 15 min (4°C), the fractions of supernatant were filtered through a

0.2 μm microfilter system (Millipore Ltd, Tokyo, Japan) and 20 μl was injected into a reverse-phase column [TSKgel ODS-80T$_M$ (5 μm, 4.6 mm id × 25 cm)] and eluted with a mobile phase [monochloroacetic acid (0.15 M), disodium EDTA (0.2 mM), 1-octanesulfonic acid (0.1 mM) in 15% methanol at pH 2.9], at a flow rate of 0.8 ml/min. The detector potential was set at + 0.80 V. The 5-HT and 5-HIAA were quantified by comparison with standards of known concentration.

Membrane preparation

Briefly, cerebral cortex was homogenized in 50 vol of ice-cold buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4) using a Kinematica Polytron homogenizer (Lüzern, Switzerland) at setting 5 for 30 s. The homogenate was centrifuged at 48 000 *g* for 10 min (4°C). The resulting pellet was resuspended in 50 vol of ice-cold buffer and recentrifuged. The final pellet was resuspended in the same buffer to a protein concentration of 1-1.5 mg/ml as measured using the method of Lowry *et al* (1951).

[³H]6-Nitroquipazine binding

Aliquots of membrane suspension were incubated with [³H]6-nitroquipazine (30-1,500 pM) at 22°C in a final vol of 1 ml for 2 h. After the addition of 4 ml of ice-cold buffer, the homogenates were rapidly filtered through Whatman GF/C filters using a 24 channel cell harvester (Brandel, Gaithersburg, MD, USA). Filters were routinely pre-treated with 0.05% polyethyleneimine before use. Finally, the filters were washed with three 5 ml rinses of ice-cold buffer. The radioactivity trapped by the filters was determined by a liquid scintillation counter (Aloka, LSC-1000). Non-specific binding was estimated in the presence of 1 μM paroxetine.

Data analysis and statistics

The data from equilibrium saturation were analyzed by means of the least squares linear regression method.

The statistical significance of the results was evaluated by 1 way analysis of variance (ANOVA) with the Scheffe's multiple range test. The criterion for significance was $P < 0.05$.

Results

The repeated systemic administration of MDMA (10 mg/kg, ip, twice daily for 3 d) reduced rat cerebral cortical levels of 5-HT and 5-HIAA to 55.2% and 50.0% of control values, respectively (table I). These measurements were made 1 week after the last injection of MDMA. The MDMA-induced reductions of 5-HT contents in the cerebral cortex were significantly ($P < 0.01$) prevented by co-administration of 6-nitroquipazine (5 mg/kg). The difference in 5-HT contents between the vehicle-treated rats and MDMA plus 6-nitroquipazine-treated rats were not significant. In addition, the MDMA-induced reduction in 5-HIAA levels were significantly ($P <$

0.05) prevented by 6-nitroquipazine, and were significantly different when compared to the vehicle-treated rats, as shown in table I.

Figure 1 shows typical data of a Scatchard plot analysis of [^{3}H]6-nitroquipazine binding to rat cortical membranes. Non-specific binding was estimated in the presence of 1 μM paroxetine. The concentration of paroxetine is appropriate as previously reported (Hashimoto and Goromaru, 1990b, 1990c; Hashimoto and Goromaru, submitted). As shown in figure 1 and table II, MDMA significantly reduced the B_{max} of [^{3}H]6-nitroquipazine binding (45.2% of control) to rat cortical membranes. Futhermore, the MDMA-induced reductions in the density of [^{3}H]6-nitroquipazine binding to cortical membranes were significantly prevented by co-administration of

Table I. Effects of MDMA and MDMA plus 6-nitroquipazine on 5-HT and 5-HIAA contents in cerebral cortex of rat brain. 5-HT and 5-HIAA levels in the cerebral cortex were measured using HPLC. The dosage for MDMA was 10 mg/kg and for 6-nitroquipazine 5 mg/kg. Measurements were made 1 wk following the last injection.

	ng/mg tissue	
	5-HT	*5-HIAA*
Vehicle	0.146 ± 0.01	0.194 ± 0.02
MDMA (10 mg/kg)	0.081 ± 0.03**	0.097 ± 0.02**
MDMA (10 mg/kg) + 6-nitroquipazine (5 mg/kg)	0.144 ± 0.01$^+$	0.151 ± 0.02*$^+$

Values are mean ± SD of 4 rats. * $P < 0.05$, ** $P < 0.01$ when compared with vehicle-treated group (Scheffe's test). $^+$ $P < 0.05$, $^{++}$ $P < 0.01$ when compared with MDMA-treated group (Scheffe's test).

Table II. Effects of MDMA and MDMA plus 6-nitroquipazine on [^{3}H]6-nitroquipazine binding to rat cortical membranes. The density (B_{max}) and affinity (K_d) of [^{3}H]6-nitroquipazine-labelled 5-HT uptake sites in cortical membranes was determined by Scatchard analysis. The dosage for MDMA was 10 mg/kg and for 6-nitroquipazine was 5 mg/kg. Measurements were made 1 wk following the last injection.

	B_{max} *(fmol/mg protein)*	K_d *(pM)*
Vehicle	626.8 ± 78.4	90.4 ± 8.8
MDMA (10 mg/kg)	283.6 ± 54.5*	78.0 ± 4.5
MDMA (10 mg/kg) + 6-nitroquipazine (5 mg/kg)	671.9 ± 113.5**	85.7 ± 6.6

The data presents the mean ± SD of 4 rats, each analyzed in duplicate. * $P < 0.05$ when compared with vehicle-treated group (Scheffe's test). ** $P < 0.01$ when compared with MDMA-treated group (Scheffe's test).

6-nitroquipazine (5 mg/kg). In addition, the B_{max} values between the vehicle-treated group and MDMA plus 6-nitroquipazine-treated group were not significant. Furthermore, the K_d values were not significantly altered by treatment with MDMA or MDMA plus 6-nitroquipazine.

Discussion

The present results show that the density of 5-HT uptake sites labelled by [3H]6-nitroquipazine, a new radioligand for 5-HT uptake sites, was significantly reduced by repeated systemic administration of MDMA, concomitant with the reduction in 5-HT levels. These data are consistent with previous reports for other radioligand [3H]paroxetine, demonstrating that the systemic administration of MDMA significantly reduced the density of [3H]paroxetine-labelled 5-HT uptake sites in rat brain (Battaglia *et al,* 1987; Battaglia *et al,* 1988).

The finding of the present results is that neurodegeneration of 5-HT nerve terminals by MDMA involves the 5-HT uptake carrier, as blockage of the carrier by co-administration of 6-nitroquipazine which can prevent the reduction in 5-HT levels and 5-HT uptake sites labelled by [3H]6-nitroquipazine following MDMA treatment.

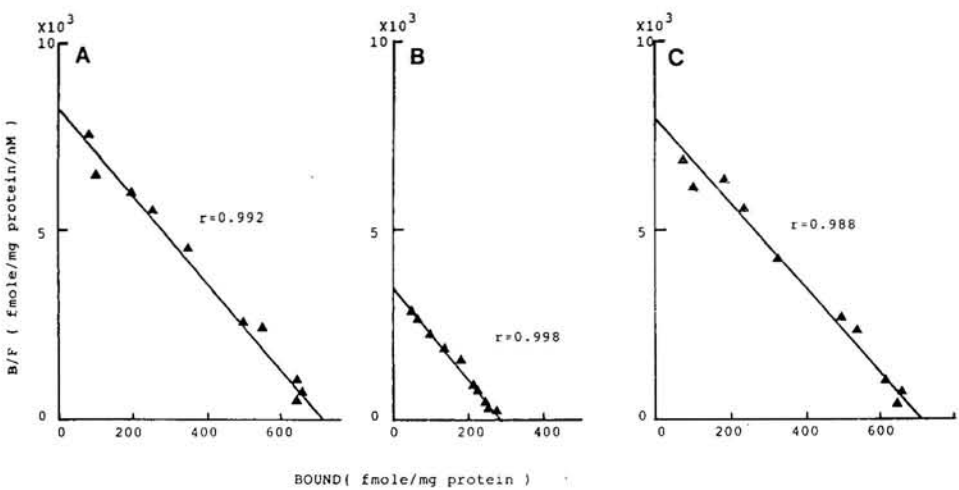

Fig 1. The [3H]6-nitroquipazine binding to rat cortical membranes. Membranes were incubated with various concentrations of [3H]6-nitroquipazine (30-1500 pM) for 2 h at 22°C. Non-specific binding was estimated in the presence of 1 μM paroxetine. Present results of a Scatchard plot analysis are from a typical experiment and values are the mean of duplicate determinations. (A): vehicle-treated group; K_d = 81.6 pM, B_{max} = 714.8 fmol/mg protein, (B): MDMA (10 mg/kg)-treated group; K_d = 84.6 pM, B_{max} = 288.5 fmol/mg protein, (C): MDMA (10 mg/kg) plus 6-nitroquipazine (5 mg/kg)-treated group; K_d = 84.7 pM, B_{max} = 715.1 fmol/mg protein.

These data are consistent with previous reports for other potent 5-HT uptake inhibitors (fluoxetine, citalopram) (Schmidt, 1987; Schmidt and Taylor, 1987; Battaglia *et al,* 1988). Moreover, Hekmatpanah and Peroutka (1990) reported that a significant correlation exists between drug affinities for the [^{3}H]paroxetine-labelled 5-HT uptake sites and their ability to inhibit MDMA-induced release of 5-HT, suggesting that the 5-HT uptake carrier plays a significant role in the MDMA-induced 5-HT release. Furthermore, the data demonstrating reduction in 5-HIAA level following MDMA plus 6-nitroquipazine administration but no significant decreases in 5-HT level and [^{3}H]6-nitroquipazine-labelled 5-HT uptake sites suggest that the changes in 5-HT metabolic activity by MDMA may occur independently of the processes destructing 5-HT nerve terminals.

In summary, the present results indicate that the density of 5-HT uptake sites labelled by [^{3}H]6-nitroquipazine was significantly decreased by repeated systemic administration of MDMA. Furthermore, the ability of 6-nitroquipazine, a potent 5-HT uptake inhibitor, to prevent the MDMA-induced neurotoxicity suggests that the 5-HT uptake carrier is involved in the mechanism of neurodestruction of 5-HT nerve terminals by MDMA. Thus, [^{3}H]6-nitroquipazine would be a suitable radioligand for the study of neuropathological and neurochemical changes of 5-HT nerve terminals in the brain.

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