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STUDY OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED NEUROTOXICITY IN RAT BRAIN USING SPECIFIC *IN VIVO* BINDING OF [^{3}H]6-NITROQUIPAZINE

Kenji Hashimoto* and Tsuyoshi Goromaru

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University,
Fukuyama, Hiroshima, Japan

Abstract

The present study was undertaken to examine the 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity in the various regions of rat brain using specific *in vivo* binding of [^{3}H]6-nitroquipazine. The regional distribution of radioactivity in the rat brain 5 hr after i.v. administration of [^{3}H]6-nitroquipazine was highly correlated with that of [^{3}H]6-nitroquipazine binding *in vitro* ($r=0.977$, $P<0.001$). The multiple administration of MDMA (10 mg/kg, i.p., twice daily for three days) reduced significantly the specific *in vivo* binding of [^{3}H]6-nitroquipazine in the rat brain as well as the contents of serotonin and 5-HIAA in the rat brain. These results demonstrate that [^{3}H]6-nitroquipazine would

*Author correspondence, Present Address: Neuroscience Branch, National Institute on Drug Abuse, Addiction Research Center, P.O. Box 5180, Baltimore, Maryland 21224

be a suitable radioligand for studying *in vivo* neuropathological and neurochemical changes of serotonin nerve terminals in the brain regions of rats. It might be of great interest to study *in vivo* serotonin uptake sites in intact human brain using a 6-nitroquipazine derivative labeled by a positron-emitting radioisotope and positron emission tomography (PET).

Introduction

3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) is a ring-substituted amphetamine derivative which is chemically related to both hallucinogens and amphetamines (McKenna and Peroutka, 1990). MDMA has been used for recreational purposes (Peroutka, 1987). It is well known that MDMA produces the neurodegeneration of serotonergic neurons after administration of MDMA into rats. The MDMA-induced neurotoxicity is characterized by a decrease in the contents of serotonin and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA)(Battaglia et al., 1987; Commins et al., 1987; Battaglia et al., 1988; Schmidt, 1987; Schmidt and Taylor, 1987; Stone et al., 1987; Hashimoto and Goromaru, 1990a; De Souza et al., 1990), a decrease in the activity of tryptophan hydroxylase (Schmidt, 1987; Schmidt and Taylor, 1987; Stone et al., 1987) and a reduction of density of serotonin uptake sites labeled by [^{3}H]paroxetine (Battaglia et al., 1987; Battaglia et al., 1988; De Souza et al., 1990) or [^{3}H]6-nitroquipazine (Hashimoto and Goromaru, 1990a). Interestingly, it has been demonstrated that MDMA-induced neurotoxicity is antagonized by administration of serotonin uptake inhibitors (Schmidt, 1987; Battaglia et al., 1988; Hashimoto and Goromaru, 1990a), indicating that serotonin uptake carrier plays an important role in the neurodestruction of serotonergic neurons by MDMA. Furthermore, it has been that MDMA produces damage of serotonergic neuron in the brain of non-human primates

(Ricaurte et al., 1988). It is currently unknown whether humans who use MDMA sustain damage of serotonergic neurons in the brain. Unfortunately, there is no direct method for evaluating *in vivo* the damage of serotonergic neurons in the living human brain. It would be, therefore, of great interest to study *in vivo* serotonin uptake sites in the living human brain using a radioligand labeled by a positron-emitting radioisotope and positron emission tomography (PET).

Recently, it has been reported that [^{3}H]6-nitroquipazine is a suitable radioligand for studying serotonin uptake sites in the brain (Hashimoto and Goromaru, 1990b; Hashimoto and Goromaru, 1991), and that [^{3}H]6-nitroquipazine is a best radioligand for studying *in vivo* serotonin uptake sites in the mouse brain (Hashimoto and Goromaru, 1990c). Also, it is well known that mice are not sensitive to neurotoxicity of MDMA (Stone et al., 1987). Therefore, the present study was undertaken to determine whether *in vivo* binding of [^{3}H]6-nitroquipazine might be used to detect *in vivo* damage of serotonin nerve terminals produced by administration of MDMA into rats. Moreover, the potential for *in vivo* study of serotonin uptake sites in the intact human brain with PET was discussed.

Materials and Methods

[^{3}H]6-Nitroquipazine (2.1 TBq/mmol(58.0 Ci/mmol)) was synthesized by the method of previous report (Hashimoto and Goromaru, 1990d) with a slight modification. MDMA hydrochloride was synthesized in our laboratory. Male Wistar rats (200-250 g) were housed in a 12-h light-dark cycle with free access to food and water. Rats were administered i.p. with either vehicle (1 ml/kg) or MDMA (10 mg/kg) twice a day (9:00 and 19:00) for three consecutive days. Rats were used 1 week after last administration. *In vivo* binding of [^{3}H]6-nitroquipazine in the rat brain was measured by the method of previous report (Hashimoto and Goromaru, 1990c). Rats

were administered i.v. with 0.5 ml of [^{3}H]6-nitroquipazine (185 kBq(5 μCi)). The animals were killed by decapitation 5 hr after administration of [^{3}H]6-nitroquipazine. Blood, hypothalamus, midbrain, striatum, hippocampus, medulla oblongata, cerebral cortex and cerebellum were removed and dissected on ice by the method of Glowinski and Iversen (1966). Tissue samples were weighed, each sample being incinerated by an automatic combustion system (Aloka, ASC-113, Tokyo, Japan). The percentage of the injected dose per gram tissue (% dose/g tissue) in each sample was determined by a liquid scintillation counter (Aloka, LSC-1000, Tokyo, Japan). The contents of serotonin and 5-HIAA in the regions of rat brain were measured by means of a high-performance liquid chromatography with electrochemical detection as reported previously (Hashimoto and Goromaru, 1990a; Hashimoto and Goromaru, 1990b). The statistical significance of results was evaluated by the Student's t-test. The criterion for significance was $P<0.05$.

Results

Table 1 shows the distribution of radioactivity in the rat blood and brain regions 5 hr after i.v. administration of [^{3}H]6-nitroquipazine. The radioactivity in the rat brain after administration of [^{3}H]6-nitroquipazine was in the order (highest to lowest) hypothalamus > midbrain > striatum > hippocampus > cerebral cortex > medulla oblongata > cerebellum. There was a good correlation ($r=0.997$, $P<0.001$) between the *in vivo* distribution of [^{3}H]6-nitroquipazine in the rat brain and regional distribution of [^{3}H]6-nitroquipazine binding *in vitro* in the rat brain (Hashimoto and Goromaru, 1990b). The distributions of radioactivity in the hypothalamus, midbrain, striatum, hippocampus and cerebral cortex 5 hr after administration of [^{3}H]6-nitroquipazine were significantly decreased by the multiple administration of MDMA (10 mg/kg), whereas those of blood and cerebellum were not altered (table 1). Although a small reduction was found in the

medulla oblongata, this change was not statistically significant.

The radioactivity in the cerebellum could reflect non-specific binding and free radioligand, as the cerebellum has very low levels of [^{3}H]6-nitroquipazine binding (Hashimoto and Goromaru, 1990b). Therefore, we have subtracted the radioactivity in the cerebellum from that in the other regions to determine the specific *in vivo* binding of [^{3}H]6-nitroquipazine in the brain regions. As shown in table 2, the treatment with MDMA caused significant reductions in the specific *in vivo* binding of [^{3}H]6-nitroquipazine in the hypothalamus, medulla oblongata and cerebral cortex when compared with the respective vehicle-treated controls. The reductions of specific *in vivo* binding of [^{3}H]6-nitroquipazine in the hypothalamus, midbrain, hippocampus and cerebral cortex (more than 60 %) were greater than those found in the striatum (50 %) and medulla oblongata (36 %).

TABLE 1

Distribution of radioactivity in the blood and brain of rats 5 hr after i.v. administration of [^{3}H]6-nitroquipazine

	% Dose/g tissue	
	Control	MDMA
Blood	0.05±0.01	0.04±0.01(-20%)
Hypothalamus	0.66±0.02	0.30±0.09(-55%)**
Midbrain	0.57±0.01	0.24±0.06(-58%)**
Striatum	0.51±0.03	0.29±0.07(-43%)*
Hippocampus	0.44±0.03	0.22±0.05(-50%)**
Medulla oblongata	0.41±0.03	0.29±0.08(-29%)
Cerebral cortex	0.43±0.02	0.22±0.05(-49%)**
Cerebellum	0.13±0.03	0.10±0.03(-23%)

Rats were administered i.p. with either vehicle (1 ml/kg) or MDMA (10 mg/kg) twice a day for three consecutive days. The distributions of radioactivity in the rats 5 hr after i.v. administration of [^{3}H]6-nitroquipazine were determined at 1 week after the last administration of MDMA.
Values are mean ± S.D. of four rats.
*P<0.01, **P<0.001 when compared with control group (Student's t-test).

TABLE 2

Effects of MDMA on the specific *in vivo* binding of [^{3}H]6-nitroquipazine and serotonin and 5-HIAA contents in the rat brain

	% Dose/g tissue	ng/mg tissue	
	Specific *in vivo* binding	Serotonin	5-HIAA
Hypothalamus			
Control	0.52±0.05	0.34±0.09	0.46±0.11
MDMA	0.20±0.06(-62%)***	0.15±0.02(-56%)*	0.31±0.01(-33%)
Midbrain			
Control	0.44±0.02	0.18±0.03	0.17±0.03
MDMA	0.14±0.03(-68%)***	0.09±0.01(-50%)**	0.11±0.01(-35%)**
Striatum			
Control	0.37±0.06	0.23±0.02	0.33±0.07
MDMA	0.18±0.05(-51%)***	0.17±0.02(-26%)*	0.26±0.03(-21%)
Hippocampus			
Control	0.31±0.04	0.10±0.02	0.15±0.03
MDMA	0.12±0.03(-61%)***	0.04±0.01(-60%)**	0.07±0.01(-53%)**
Medulla oblongata			
Control	0.27±0.01	0.17±0.03	0.16±0.02
MDMA	0.17±0.05(-37%)*	0.14±0.03(-18%)	0.14±0.03(-13%)
Cerebral cortex			
Control	0.29±0.01	0.15±0.03	0.06±0.01
MDMA	0.11±0.02(-62%)***	0.07±0.01(-53%)**	0.03±0.01(-50%)**

The specific *in vivo* binding of [^{3}H]6-nitroquipazine in the brain regions was determined by subtracting the radioactivity in the cerebellum from that in the other regions.
Values are mean ± S.D. of four rats.
*P<0.05, **P<0.01, ***P<0.001 when compared with control group (Student's t-test).

Furthermore, the reduction of serotonin and 5-HIAA contents in the hypothalamus, midbrain, hippocampus and cerebral cortex were greater than those found in the striatum and medulla oblongata (table 2). Although a small decrease of serotonin was found in the medulla oblongata, the change was not statistically significant.

Discussion

The present results suggest that [^{3}H]6-nitroquipazine is a suitable radioligand for studying *in vivo* serotonin uptake sites in the rat brain. The major findings of present results are as follows. First, the regional distribution of radioactivity in the rat brain 5 hr after i.v. administration of [^{3}H]6-nitroquipazine was highly correlated with the regional distribution of [^{3}H]6-nitroquipazine binding *in vitro* in the rat brain (Hashimoto and Goromaru, 1990b). Second, MDMA, which is a neurotoxin of serotonin nerve terminals, produced the marked reduction of specific *in vivo* binding of [^{3}H]6-nitroquipazine in the rat brain, concomitant with the reduction of serotonin and 5-HIAA levels. Scheffel and Ricaurte (1990) reported recently that [^{3}H]paroxetine could be used to label *in vivo* serotonin uptake sites in the rat brain, and that the damage of serotonin nerve terminals produced by MDMA could be detected using *in vivo* [^{3}H]paroxetine binding. The radioactivity detected in the hypothalamus of rat brain after i.v. administration of [^{3}H]6-nitroquipazine or [^{3}H]paroxetine was 0.66 % dose/g tissue (5 hr after injection)(table 1) or 0.17 % dose/g tissue (4 hr after injection)(Scheffel and Ricaurte, 1990), respectively. Moreover, the peak radioactivity detected in the mouse brain after i.v. administration of [^{3}H]6-nitroquipazine or [^{3}H]paroxetine was 12 % dose/g tissue (30 min after injection)(Hashimoto and Goromaru, 1990c) or 4.2 % dose/g tissue (60 min after injection)(Hashimoto and Goromaru, 1990e; Hashimoto and Goromaru, 1990f), respectively. Thus, [^{3}H]6-nitroquipazine has better penetration across the blood-brain barrier than [^{3}H]paroxetine, as reported previously (Hashimoto and Goromaru, 1990e). Therefore, [^{3}H]6-nitroquipazine seems to be superior to [^{3}H]paroxetine as a potential radioligand for labeling *in vivo* serotonin uptake sites in the brain. Furthermore, this study is encouraging for the use of a 6-nitroquipazine derivative as a radioligand for *in vivo* labeling of serotonin uptake sites in the

intact human brain with PET. Studies are currently underway to evaluate the possibility of several quipazine derivatives for *in vivo* study of serotonin uptake sites using PET (Hashimoto and Goromaru, 1992).

In the present study, some differences in the effects of MDMA on serotonergic parameters in the discrete regions of rat brain were observed. For example, the reduction of specific *in vivo* binding of [^{3}H]6-nitroquipazine in the medulla oblongata was smaller than those observed in the other regions. Furthermore, the reductions of serotonin and 5-HIAA in the striatum and medulla oblongata were also smaller than those in the other regions. These data suggest that serotonergic neurons in the medulla oblongata appear to be less sensitive to the neurotoxic effects of MDMA. The regional differences in the neurotoxic effects of MDMA have also been reported previously (Battaglia et al., 1987; De Souza et al., 1990; O'Hearn et al., 1988; Scheffel and Ricaurte, 1990). De Souza et al. (1990) have suggested that there is some neuroanatomical and morphological specificity to the neurotoxic effects of MDMA, as evidenced by predominant reductions in the serotonin uptake sites labeled by [^{3}H]paroxetine in the brain regions containing primarily serotonin terminals, while regions containing serotonin axons of passage and cell bodies are relatively unaffected. The discrepancy of reductions in the between specific *in vivo* binding of [^{3}H]6-nitroquipazine and serotonin levels was observed in the striatum and medulla oblongata. These data suggest that measurement of serotonin levels as an index of the damage of serotonergic neurons might underestimate the actual magnitude of the neurotoxic effects of MDMA. These findings are consistent with the results from Battaglia et al. (1987). The reasons underlying the discrepancy found in the striatum and medulla oblongata are at present unclear.

In conclusion, the present study suggests that [^{3}H]6-nitroquipazine is a suitable radioligand for labeling *in vivo* serotonin uptake sites in the rat brain, and that specific *in vivo* binding of

[^{3}H]6-nitroquipazine would be able to use to measure *in vivo* the extent of neurodegeneration of serotonin nerve terminals. Therefore, it might be of great interest to study *in vivo* serotonin uptake sites in the intact human brain using a 6-nitroquipazine derivative labeled by a positron-emitting radioisotope and PET.

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