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Drug effects on distribution of [^{3}H]3,4-methylenedioxymethamphetamine in mice

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The present study was undertaken to examine the drug interactions between 3,4-methylenedioxymethamphetamine (MDMA) and paroxetine or several compounds including the 3,4-methylenedioxybenzyl (piperonyl) group in mice The time course of radioactivity in the mouse brain after i v administration of the tracer amount (approximately 70 ng/kg) of [^{3}H]MDMA was altered significantly by coinjection of carrier MDMA (15 mg/kg) or by pretreatment with paroxetine (10 mg/kg, i p , 5 min) Furthermore, the radioactivity in the brain 60 min after injection of [^{3}H]MDMA was increased significantly by pretreatment with paroxetine, but not by pretreatment with 6-nitroquipazine, fluoxetine, clomipramine, GBR 12909 or desipramine, indicating that paroxetine-induced alteration of the brain radioactivity was not due to the inhibitory effect of 5-hydroxytryptamine (5-HT) uptake of paroxetine The radioactivity in the brain 60 min after injection of [^{3}H]MDMA was increased significantly by pretreatment with 3,4-methylenedioxyamphetamine (MDA), MDMA, 1-piperonylpiperazine and N,α-dimethylpiperonylamine, but not by pretreatment with piperonylacetone, piperonyl butoxide and piperonyl isobutyrate HPLC analyses indicated that the alteration of brain radioactivity 60 min after injection of [^{3}H]MDMA was, in part, due to inhibition in the metabolism of [^{3}H]MDMA to radioactive metabolite(s) The present results suggest that a specific mechanism for the 3,4-methylenedioxyphenyl group which rapidly alters the disposition and metabolism of [^{3}H]MDMA may exist in brain and peripheral organs of mice

MDMA (3,4-methylenedioxymethamphetamine), Paroxetine, 5-HT uptake inhibitors, 1-Piperonylpiperazine, Brain (mouse)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') is a novel psychedelic amphetamine analogue which has been used for recreational purposes (Shulgin, 1986; Peroutka, 1987; Barnes, 1988, McKenna and Peroutka, 1990). MDMA is a selective neurotoxin of 5-hydroxytryptamine (5-HT, serotonin) nerve terminals in the brain MDMA produces a decrease in the contents of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), a decrease in the activity of tryptophan hydroxylase and a reduction in the densities of 5-HT uptake sites labeled by [^{3}H]paroxetine or [^{3}H]6-nitroquipazine in the brain (Schmidt, 1987, Schmidt and Taylor, 1987, 1988, Commins et al., 1987, Stone et al , 1986, 1987a,b, Battaglia et al , 1987, 1988; Ricaurte et al , 1988; O'Hearn et al , 1988, Hashimoto and Goromaru, 1990a, 1992a) Interestingly, it has been reported that 5-HT uptake inhibitors block the neurotoxic effects of MDMA on 5-HT nerve terminals, indicating that the 5-HT transporter participates in the MDMA-induced neurotoxicity (Schmidt, 1987; Schmidt and Taylor, 1987, Battaglia et al., 1988, Hashimoto and Goromaru, 1990a). Although the neurotoxicity of MDMA in the 5-HT neuron is now well established, the underlying mechanisms of action remain unknown

Marked species differences in the MDMA-induced neurotoxicity were shown in mice and rats (Stone et al , 1987a, Logan et al , 1988, Peroutka, 1988, Battaglia et al., 1988), indicating that mice are relatively insensitive to the neurotoxic effects of MDMA These authors attribute the greater sensitivity of rats to metabolic differences between the two species (McKenna and Peroutka, 1990) Therefore, it is of interest to study the distribution and metabolism of MDMA in rats and mice. We have reported previously that in vivo binding of [^{3}H]paroxetine in the mouse brain is decreased significantly by pretreatment with 3,4-methylenedioxyamphetamine (MDA) or MDMA, whereas specific [^{3}H]paroxetine binding to mouse homogenates in

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vitro is not altered by treatment with MDA or MDMA (Hashimoto and Goromaru, 1990b,c) It may therefore be, of interest to study the interaction between paroxetine and MDMA in mouse brain

As shown in fig 1, MDA, MDMA and paroxetine possess a 3,4-methylenedioxyphenyl group. This suggests that a mechanism for the 3,4-methylenedioxyphenyl group may exist in the brain The present study was undertaken to examine the drug interactions between MDMA and paroxetine or several compounds including the 3,4-methylenedioxybenzyl (piperonyl) group in mice using a radiotracer technique The major finding of the present study is that the distribution and metabolism of MDMA in mouse brain and peripheral organs were altered significantly by pretreatment with paroxetine or the basic compounds including the piperonyl group, suggesting that a specific mechanism for the 3,4-methylenedioxyphenyl group which rapidly alters the disposition and metabolism of MDMA might exist in mouse brain and peripheral organs Portions of these data were presented at the Fifth Annual Meeting of the Japanese Society for the Study of Xenobiotics (Tokushima, October, 1990) (Hashimoto et al., 1990b) and the 111th Annual Meeting of the Pharmaceutical Society of Japan (Tokyo, March, 1991)

2. Materials and methods

2 1 Animals

Male ddy mice weighing 30–35 g were used The animals were housed in groups of ten animals per cage They were maintained under standard conditions (light on from 6:00 to 18:00 h, room temperature 23 ± 1°C, humidity 55 ± 5%) with free access to food and water

2 2 Materials

[^{3}H]MDMA (3.15 TBq/mmol) was prepared as previously described (Hashimoto et al , 1990a) MDMA hydrochloride and MDA hydrochloride were synthesized from 3,4-methylenedioxy-2-propanone (piperonylacetone) (Fluka, Switzerland) and methylamine or ammonium acetate as described by Braun et al (1980) N,α-Dimethylpiperonylamine hydrochloride was synthesized from 3,4-methylenedioxyacetophenone (Tokyo Kasei, Tokyo, Japan) and methylamine as described above 6-Nitroquipazine maleate was synthesized as described previously (Hashimoto and Goromaru, 1990d) The drugs were obtained from the following sources paroxetine hydrochloride (Beecham Pharma-

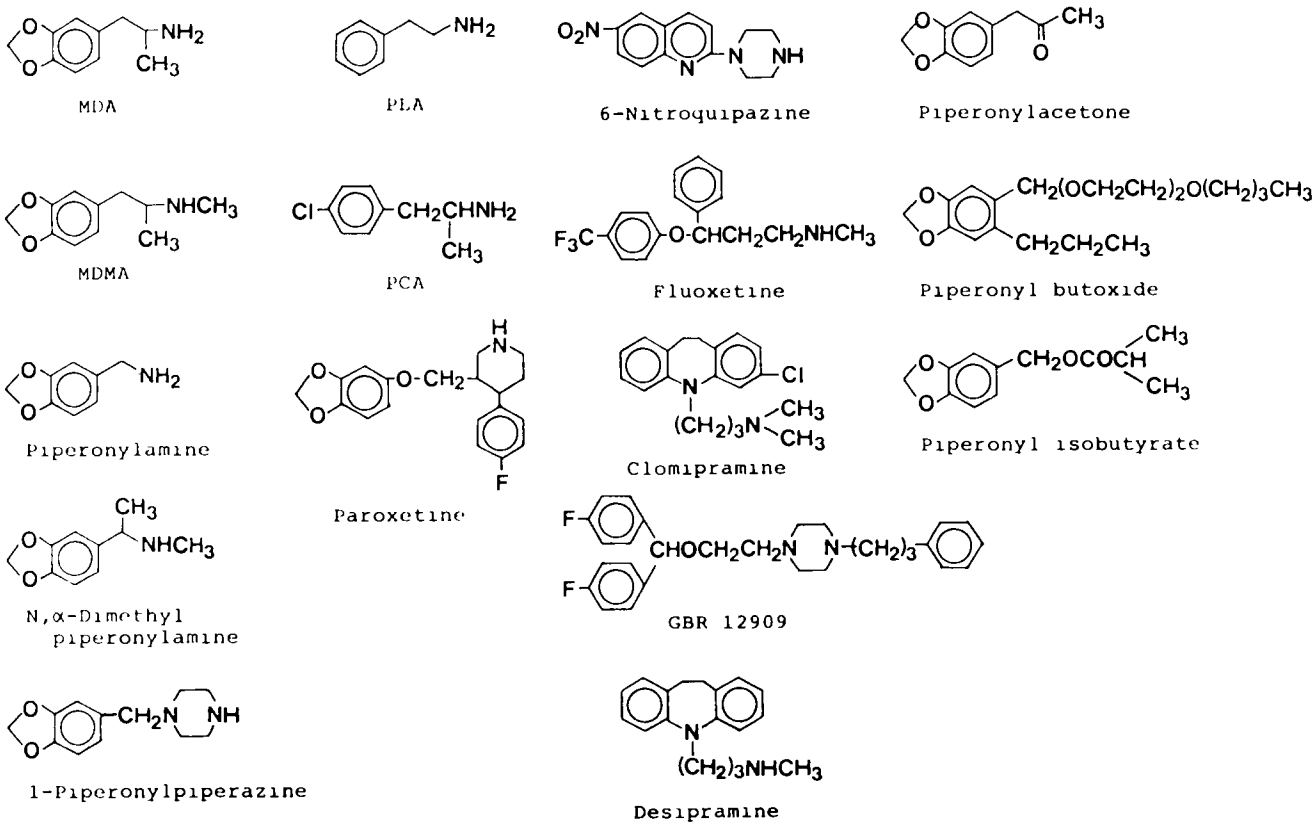

Fig 1 Chemical structure of compounds used in the present study

TABLE 1

Time course of radioactivity in mice after i v administration of [^{3}H]MDMA

A tracer amount (about 70 ng/kg) of [^{3}H]MDMA was administered i v to mice The time course of radioactivity in mice was measured as described in Materials and methods The values are the mean ± S D of three mice

	% Dose/g tissue				
	1 min	5 min	15 min	30 min	60 min
Blood	1 49 ± 0 08	0 79 ± 0 03	0 75 ± 0 17	0 77 ± 0 22	0 55 ± 0 06
Heart	5 22 ± 0 29	2 30 ± 0 11	1 50 ± 0 14	0 87 ± 0 05	0 49 ± 0 03
Lung	11 8 ± 1 23	5 11 ± 1 02	2 90 ± 0 62	1 82 ± 0 07	1 02 ± 0 07
Liver	3 00 ± 0 19	8 31 ± 0 03	12 3 ± 0 51	13 4 ± 1 46	12 1 ± 0 76
Spleen	3 04 ± 0 75	4 00 ± 0 53	3 57 ± 0 25	1 45 ± 0 38	0 62 ± 0 03
Kidney	21 5 ± 3 41	15 1 ± 1 55	8 27 ± 0 57	4 38 ± 0 61	2 21 ± 0 49
Brain	3 47 ± 0 45	4 12 ± 0 04	3 02 ± 0 21	1 44 ± 0 12	0 49 ± 0 02

ceutical, Surrey, UK), fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN), clomipramine hydrochloride (Ciba-Geigy, Takarazuka, Japan), GBR 12909 (Research Biochemical, Natick, MA), desipramine hydrochloride, p-chloroamphetamine (PCA) hydrochloride, and pargyline hydrochloride (Sigma Chemical Co , St. Louis, MO), 1-piperonylpiperazine, piperonyl isobutyrate, piperonylamine (Aldrich Chemical Co , Milwaukee, WI), piperonyl butoxide (Tokyo Kasei, Tokyo, Japan) and phenethylamine (PEA) hydrochloride (Wako Pure Chemical, Tokyo, Japan). Other chemicals were purchased commercially.

2 3 Distribution of radioactivity in mice after i.v administration of [^{3}H]MDMA

Mice were administered i.v with 0 2 ml of [^{3}H]-MDMA solution (approximately 37 kBq) The mice were killed by decapitation at various times after injection of [^{3}H]MDMA Organs were removed and weighed, each sample being incinerated by an automated sample combustion system (Aloka, ASC-113, Tokyo, Japan), and the percentage of injected dose per gram tissue (% dose/g) in each sample was determined by a liquid scintillation counter (Aloka, LSC-1000, Tokyo, Japan)

In order to examine the effects of several drugs on the distribution of [^{3}H]MDMA in mice, drugs were administered i.p into mice 5 min before i.v. administration of [^{3}H]MDMA The radioactivity in the mice after injection of [^{3}H]MDMA was determined as described above. The dose of drugs was expressed as the free base. Piperonyl acetone, piperonyl butoxide and piperonyl isobutyrate were dissolved in 10% Tween 80 solution. Other drugs were dissolved in distilled water. 1-Piperonylpiperazine and piperonylamine were used as the hydrochloride salt.

2 4 HPLC analysis of radioactivity in mice after i v administration of [^{3}H]MDMA

0 2 ml aliquot of the tracer amount (approximately 700 ng/kg) of [^{3}H]MDMA (approximately 370 kBq) was administered i.v to the mice, which were then killed by decapitation 1 min or 60 min after injection of [^{3}H]MDMA. The tissues were removed and homogenized with 1 ml of 0 1 N $HClO_4$ containing 0.1% cysteine. After centrifugation at $4000 \times g$ for 10 min (4°C), the fractions of supernatant were filtered through a microfilter system (Air Press-30, Tosoh, Tokyo, Japan) and the filtrate analyzed by high performance liquid chromatography (HPLC) (column, TSKgel ODS-80 Tm

TABLE 2

Time course of radioactivity in mice after i v administration of [^{3}H]MDMA (15 mg/kg)

Carrier-added [^{3}H]MDMA (15 mg/kg) was administered i v The time course of radioactivity in mice was measured as described in Materials and methods The values are the means ± S D of three mice

	% Dose/g tissue				
	1 min	5 min	15 min	30 min	60 min
Blood	1 51 ± 0 17	1 09 ± 0 15	0 80 ± 0 10	0 84 ± 0 08	0 84 ± 0 17
Heart	4 60 ± 0 63	3 08 ± 0 53	2 50 ± 0 10	2 38 ± 0 05	2 02 ± 0 30
Lung	6 94 ± 0 93	6 84 ± 0 58	6 04 ± 0 28	4 91 ± 0 78	3 57 ± 1 32
Liver	4 11 ± 1 22	7 52 ± 1 08	7 58 ± 1 06	7 51 ± 0 43	6 67 ± 0 88
Spleen	4 65 ± 1 26	5 80 ± 0 27	4 67 ± 0 14	4 54 ± 1 02	3 57 ± 0 59
Kidney	16 7 ± 1 96	9 99 ± 0 79	10 4 ± 2 62	7 85 ± 0 46	6 10 ± 0 89
Brain	3 77 ± 0 20	4 91 ± 0 80	4 82 ± 0 11	4 27 ± 0 21	3 59 ± 0 60

TABLE 3

Effect of paroxetine on time course of radioactivity in mice after i v administration of [^{3}H]MDMA

Paroxetine (10 mg/kg) was administered i p to mice 5 min before i v administration of a tracer amount (about 70 ng/kg) of [^{3}H]MDMA The time course of radioactivity in mice was measured as described in Materials and methods The values are the mean ± S D of three mice

	% Dose/g tissue				
	1 min	5 min	15 min	30 min	60 min
Blood	1 48 ± 0 12	1 04 ± 0 10	1 00 ± 0 16	1 02 ± 0 15	0 69 ± 0 04
Heart	5 18 ± 0 17	2 92 ± 0 28	2 34 ± 0 37	1 98 ± 0 25	1 31 ± 0 16
Lung	12 3 ± 0 09	4 71 ± 0 99	3 68 ± 1 25	2 89 ± 0 80	3 30 ± 0 41
Liver	4 07 ± 1 11	6 14 ± 0 57	7 50 ± 1 72	7 35 ± 1 84	4 14 ± 0 71
Spleen	4 58 ± 1 44	4 40 ± 0 22	5 20 ± 1 51	4 23 ± 0 91	2 59 ± 0 44
Kidney	20 8 ± 5 28	17 3 ± 1 50	11 9 ± 2 93	9 45 ± 1 39	7 08 ± 1 16
Brain	4 45 ± 0 10	4 72 ± 0 61	4 88 ± 0 87	3 95 ± 0 70	2 40 ± 0 27

(5 μm, 4 6 mm i d × 15 cm), mobile phase; acetonitrile. 1% aqueous triethylamine acetate (pH 4 0) = 1 9, flow rate, 1 ml/min, detector, UV (254 nm)) The radioactivity in each fraction was determined by a liquid scintillation counter.

2 5 *Statistics*

The statistical evaluation of the multigroup data 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

Table 1 shows the time course of radioactivity in the blood, heart, lung, liver, spleen, kidney and brain after i v administration of a tracer amount (approximately 70 ng/kg) of [^{3}H]MDMA High accumulations of radioactivity in the lung and kidney were found 1 min after injection of [^{3}H]MDMA Intermediate uptake of radioactivity in the brain was observed, and the maximum peak of radioactivity in the brain occurred 5 min after injection of [^{3}H]MDMA

Table 2 shows the time course of radioactivity in the blood, heart, lung, liver, spleen, kidney and brain after i.v administration of carrier-added [^{3}H]MDMA (15 mg/kg) The observed behavior following i v administration of a large amount of MDMA (15 mg/kg) into mice has been described as excitatory in nature (locomotor activity, tremor, jerking, etc) The distributions of radioactivity in the mice after injection of [^{3}H]MDMA were altered by coinjection of a large amount (15 mg/kg) of MDMA The radioactivity in the lung 1 min after injection of [^{3}H]MDMA was decreased, suggesting that lung tissue has a saturable uptake system for basic amines Particularly, the distribution of radioactivity in the brain was changed by coinjection of the carrier (15 mg/kg) The time course of radioactivity in the brain was altered significantly by coinjection of MDMA (15 mg/kg), whereas the radioactivity in the blood was altered only slightly

The effect of a potent 5-HT uptake inhibitor paroxetine on the distribution of radioactivity in the blood, heart, lung, liver, spleen, kidney and brain after i v

TABLE 4

Effects of monoamine uptake inhibitors on distribution of radioactivity in mice 60 min after i v administration of [^{3}H]MDMA

Vehicle (10 ml/kg) or drugs (10 mg/kg) were administered i p to mice 5 min before i v administration of [^{3}H]MDMA The distributions of radioactivity in mice 60 min after injection of [^{3}H]MDMA were measured as described in Materials and methods The values are the mean ± S D of three mice

	% Dose/g tissue				
	Vehicle	Paroxetine	6-Nitro-quipazine	GBR 12909	Desipramine
Blood	0 63 ± 0 09	0 64 ± 0 15	0 55 ± 0 11	0 57 ± 0 08	0 56 ± 0 05
Heart	0 57 ± 0 15	1 12 ± 0 08 [a]	0 46 ± 0 07	0 43 ± 0 03	0 48 ± 0 04
Lung	0 79 ± 0 79	1 54 ± 0 10 [a]	0 79 ± 0 10	0 79 ± 0 06	0 88 ± 0 13
Liver	9 67 ± 7 15	6 45 ± 2 86	12 5 ± 6 40	11 5 ± 4 06	7 88 ± 7 16
Spleen	0 48 ± 0 04	0 32 ± 0 10	0 55 ± 0 12	0 12 ± 0 05 [a]	0 12 ± 0 03 [a]
Kidney	1 77 ± 0 06	4 09 ± 0 59 [a]	1 76 ± 0 25	2 05 ± 0 74	2 81 ± 0 69
Brain	0 36 ± 0 06	1 88 ± 0 13 [a]	0 40 ± 0 06	0 36 ± 0 05	0 50 ± 0 11

[a] $P < 0.01$ when compared with vehicle group

TABLE 5

Drug effects on distribution of radioactivity in blood and brain 60 min after i v administration of [^{3}H]MDMA

Vehicle (10 ml/kg) or drugs (exp 1, 10 mg/kg, exp 2, 15 mg/kg, exp 3, piperonyl compounds (20 mg/kg), pargyline (100 mg/kg), exp 4, 20 mg/kg) were administered i p to mice 5 min before i v administration of [^{3}H]MDMA The distributions of radioactivity in blood and brain 60 min after injection of [^{3}H]MDMA were measured as described in Materials and methods The values are the mean ± S D of three mice

	% Dose/g tissue	
	Blood	Brain
Exp 1		
Vehicle	0 77 ± 0 21	0 70 ± 0 14
Paroxetine	0 54 ± 0 09	1 83 ± 0 34 [b]
Fluoxetine	0 54 ± 0 08	0 97 ± 0 09
Clomipramine	0 57 ± 0 18	0 84 ± 0 08
Exp 2		
Vehicle	0 58 ± 0 08	0 58 ± 0 01
MDA	0 76 ± 0 24	2 35 ± 0 87 [b]
MDMA	0 45 ± 0 07	1 98 ± 0 18 [a]
PCA	0 61 ± 0 27	0 66 ± 0 11
PEA	0 52 ± 0 12	0 57 ± 0 09
Exp 3		
Vehicle	0 55 ± 0 01	0 53 ± 0 07
1-Piperonyl piperazine	0 40 ± 0 04	1 71 ± 0 44 [b]
N,α-Dimethyl-piperonylamine	0 56 ± 0 20	1 77 ± 0 07 [b]
Piperonylamine	0 49 ± 0 06	0 46 ± 0 04
Piperonylamine + Pargyline	0 44 ± 0 13	1 02 ± 0 44 [a c]
Pargyline	0 47 ± 0 01	0 57 ± 0 08
Exp 4		
Vehicle	0 67 ± 0 12	0 44 ± 0 10
Piperonyl acetone	0 76 ± 0 17	0 57 ± 0 11
Piperonyl butoxide	0 66 ± 0 08	0 63 ± 0 04
Piperonyl isobutyrate	0 75 ± 0 13	0 57 ± 0 16

[a] $P < 0.05$ [b] $P < 0.01$ when compared with vehicle group [c] $P < 0.05$ when compared with piperonylamine group

administration of [^{3}H]MDMA is shown in table 3 The radioactivity in each organ 1 min after i v. administration of [^{3}H]MDMA was not altered by pretreatment with paroxetine (10 mg/kg, i p , 5 min). However, the time course of radioactivity in each organ was altered by pretreatment with paroxetine, particularly the time course of brain radioactivity, as shown in table 3. The alteration of brain radioactivity induced by pretreatment with paroxetine was almost identical to the alteration induced by coinjection of MDMA (15 mg/kg) Thus, the clearance rate of radioactivity from mouse brain after injection of [^{3}H]MDMA was decreased by coadministration of carrier MDMA (15 mg/kg) or pretreatment with paroxetine

The effects of monoamine uptake inhibitors on distribution of [^{3}H]MDMA in the mice are shown in table 4 and table 5 (exp. 1). As shown in table 4, the radioactivity in the heart, lung, kidney and brain 60 min after i v administration of [^{3}H]MDMA was significantly altered by pretreatment with paroxetine (10 mg/kg), but not by pretreatment with 6-nitroquipazine (10 mg/kg) (a very potent 5-HT uptake inhibitor) (Vaatstra et al , 1981, Hashimoto and Goromaru, 1990e, f), GBR 12909 (10 mg/kg) (a dopamine uptake inhibitor) (Van der Zee et al , 1980) and desipramine (10 mg/kg) (a norepinephrine uptake inhibitor). The distributions of radioactivity in the spleen were decreased significantly by pretreatment with GBR 12909 and desipramine Furthermore, the radioactivity in the brain was not altered by pretreatment with other 5-HT uptake inhibitors fluoxetine (10 mg/kg) and clomipramine (10 mg/kg), as shown in table 5 (exp 1)

Table 5 (exp 2) shows the effects of several phenethylamine derivatives (MDA, MDMA, PCA, PEA) (see fig 1) on the distribution of radioactivity in the blood and brain after injection of [^{3}H]MDMA The distribution of radioactivity in the brain 60 min after i v administration of [^{3}H]MDMA was increased significantly by pretreatment with MDA (15 mg/kg) and MDMA (15 mg/kg), but not by pretreatment with PCA (15 mg/kg) or PEA (15 mg/kg) These results suggest that the 3,4-methylenedioxyphenyl group may play an important role in the alteration of radioactivity in the brain after injection of [^{3}H]MDMA

The effects of several derivatives (see fig. 1) including the piperonyl group on the distribution of [^{3}H]MDMA in mice are shown in table 5 (exp 3 and exp 4) and table 6 The radioactivity in the brain 60 min after i v administration of [^{3}H]MDMA was significantly increased by pretreatment with 1-piperonylpiperazine (20 mg/kg), N,α-dimethylpiperonylamine (a derivative shorting side-chain of MDMA) (20 mg/kg) Furthermore, the distribution of brain radioactivity was increased significantly by coadministration of piperony-

TABLE 6

Dose effects of 1-piperonylpiperazine on distribution of radioactivity in mice 60 min after i v administration of [^{3}H]MDMA

Vehicle (10 ml/kg) or drugs were administered i p to mice 5 min before i v administration of [^{3}H]MDMA The distributions of radioactivity in mice 60 min after injection of [^{3}H]MDMA were measured as described in Materials and methods The values are the mean ± S D of three mice

	% Dose/g tissue			
	Blood	Heart	Lung	Brain
Vehicle	0 50 ± 0 14	0 61 ± 0 12	0 93 ± 0 26	0 63 ± 0 11
2 5 mg/kg	0 44 ± 0 06	0 61 ± 0 09	1 15 ± 0 31	0 78 ± 0 21
5 0 mg/kg	0 39 ± 0 02	0 69 ± 0 02	1 27 ± 0 17	0 94 ± 0 09
10 mg/kg	0 44 ± 0 01	1 22 ± 0 09 [a]	2 33 ± 0 26 [a]	1 88 ± 0 20 [a]
20 mg/kg	0 46 ± 0 08	1 32 ± 0 16 [a]	2 41 ± 0 32 [a]	2 28 ± 0 12 [a]
40 mg/kg	0 44 ± 0 10	1 28 ± 0 09 [a]	2 20 ± 0 39 [a]	2 09 ± 0 04 [a]

[a] $P < 0.01$ when compared with vehicle group

lamine and pargyline, but not by pretreatment with piperonylamine (20 mg/kg) alone or pargyline (100 mg/kg) alone (table 5, exp 3) These results suggest that the increase in brain radioactivity by pretreatment with piperonylamine plus pargyline might be due to piperonylamine itself, which exists in the mouse brain, since piperonylamine seems to be metabolized by monoamine oxidase. However, the distributions of radioactivity in the brain 60 min after injection of [^{3}H]MDMA were not altered by pretreatment with piperonylacetone (20 mg/kg), piperonyl butoxide (20 mg/kg) or piperonyl isobutyrate (20 mg/kg), as shown in table 5 (exp 4) Furthermore, piperonyl butoxide (1 g/kg) did not affect the distribution of brain radioactivity (data not shown) In addition, the distribution of radioactivity in the heart, lung and brain 60 min after i v. administration of [^{3}H]MDMA was increased significantly by pretreatment with 1-piperonylpiperazine (2 5, 5, 10, 20 and 40 mg/kg, i p., 5 min), in a dose-dependent manner (table 6). The data indicate that the basic compounds including the piperonyl group could increase the radioactivity in the brain after injection of [^{3}H]MDMA.

Fig 2 shows the HPLC analysis of radioactivity in the mouse brain after i v administration of [^{3}H]-MDMA The radioactivity in the mouse brain 1 min after i v administration of [^{3}H]MDMA was due to unmetabolized [^{3}H]MDMA However, the radioactivity in the brain 60 min after injection of [^{3}H]MDMA was due to unmetabolized [^{3}H]MDMA and radioactive

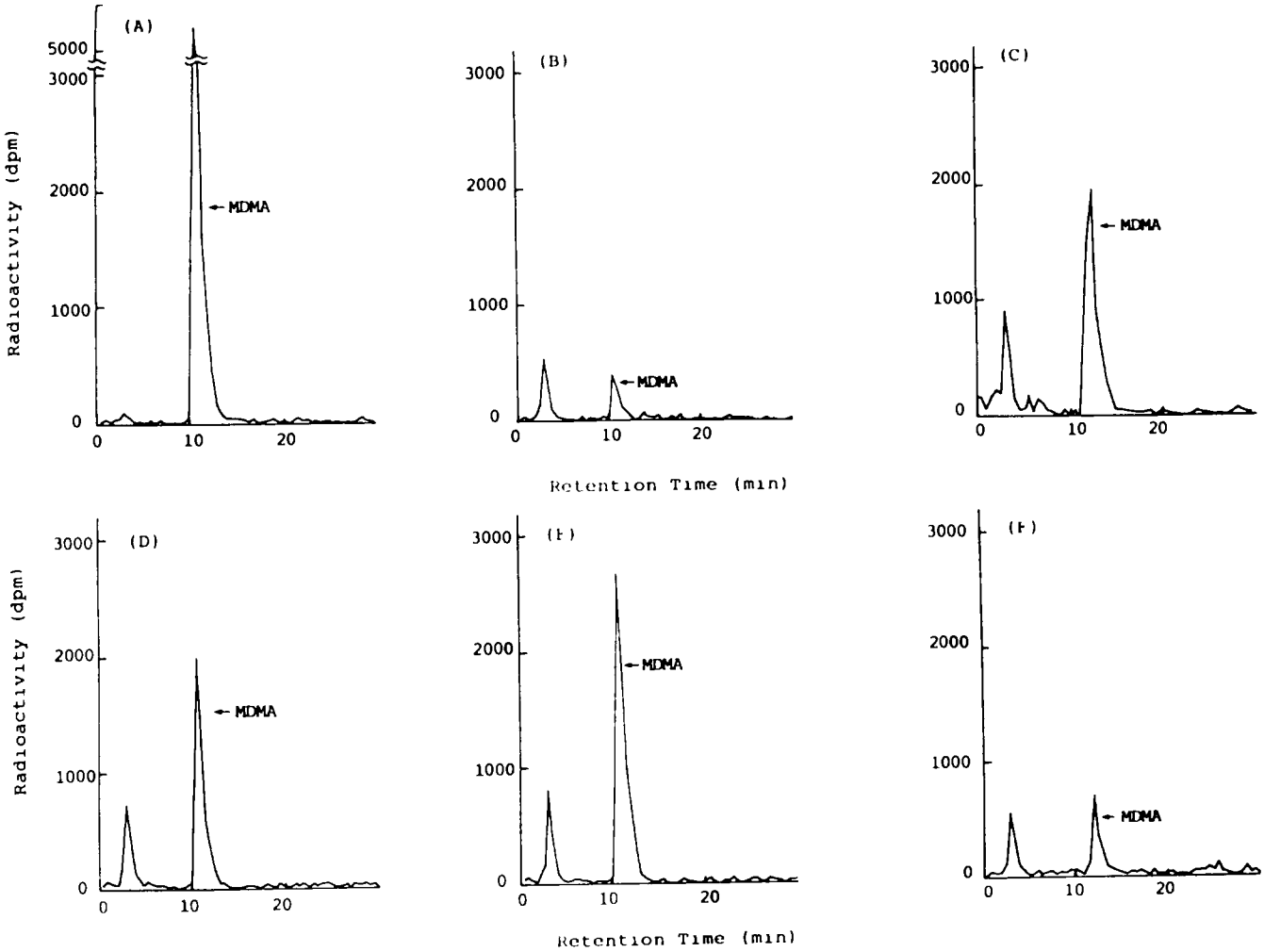

Fig 2 HPLC analysis of radioactive materials in the mouse brain after i v administration of [^{3}H]MDMA The radioactive materials in the brain were analyzed as described in Materials and methods Retention time of MDMA was 11 min A HPLC chart of the brain radioactivity 1 min after injection of [^{3}H]MDMA B HPLC chart of brain radioactivity 60 min after injection of [^{3}H]MDMA C HPLC chart of brain radioactivity 60 min after injection of [^{3}H]MDMA into the mouse pretreated with paroxetine (10 mg/kg, i p 5 min) D HPLC chart of brain radioactivity 60 min after injection of [^{3}H]MDMA into the mouse pretreated with 1-piperonylpiperazine (20 mg/kg, i p 5 min) E HPLC chart of brain radioactivity 60 min after injection of [^{3}H]MDMA into the mouse pretreated with MDMA (15 mg/kg, i p 5 min) F HPLC chart of brain radioactivity 60 min after injection of [^{3}H]MDMA into the mouse pretreated with 6-nitroquipazine (10 mg/kg, i p 5 min)

metabolite(s). Furthermore, the amounts of radioactive metabolite(s) in the brain 60 min after injection of [^{3}H]MDMA were decreased by pretreatment with paroxetine (10 mg/kg), 1-piperonylpiperazine (20 mg/kg) and MDMA (15 mg/kg), but not by pretreatment with 6-nitroquipazine (10 mg/kg). The data suggest that the increases in brain radioactivity by pretreatment with paroxetine, 1-piperonylpiperazine and MDMA might be, in part, due to inhibition of the metabolism of [^{3}H]MDMA to hydrophilic metabolite(s) in the brain Furthermore, fig 3 shows the HPLC analysis of the radioactivity in the heart, lung, liver and brain 60 min after i v administration of [^{3}H]MDMA It was found that the radioactivity in the liver 60 min after injection of [^{3}H]MDMA was almost due to the radioactive metabolite(s) of [^{3}H]MDMA, indicating that [^{3}H]MDMA was metabolized rapidly in the liver. Moreover, it was found that 1-piperonylpiperazine could prevent, at least in part, the metabolism of [^{3}H]MDMA to hydrophilic metabolites in the peripheral organs (heart, lung, liver) and brain

4. Discussion

The major finding of the present study is that the distribution of radioactivity in the mouse brain after i v administration of [^{3}H]MDMA was altered significantly by pretreatment with paroxetine or basic compounds (MDA, MDMA, 1-piperonylpiperazine and N,α-dimethylpiperonylamine) including the piperonyl group Furthermore, the present results indicate that paroxetine-induced alteration of brain radioactivity after injection of [^{3}H]MDMA was not due to the inhibitory effect of 5-HT uptake by paroxetine, since other 5-HT uptake inhibitors (6-nitroquipazine, fluoxetine, clomipramine) had no effect. As shown in fig. 1, paroxetine, MDA, MDMA, 1-piperonylpiperazine, N,α-dimethyl-

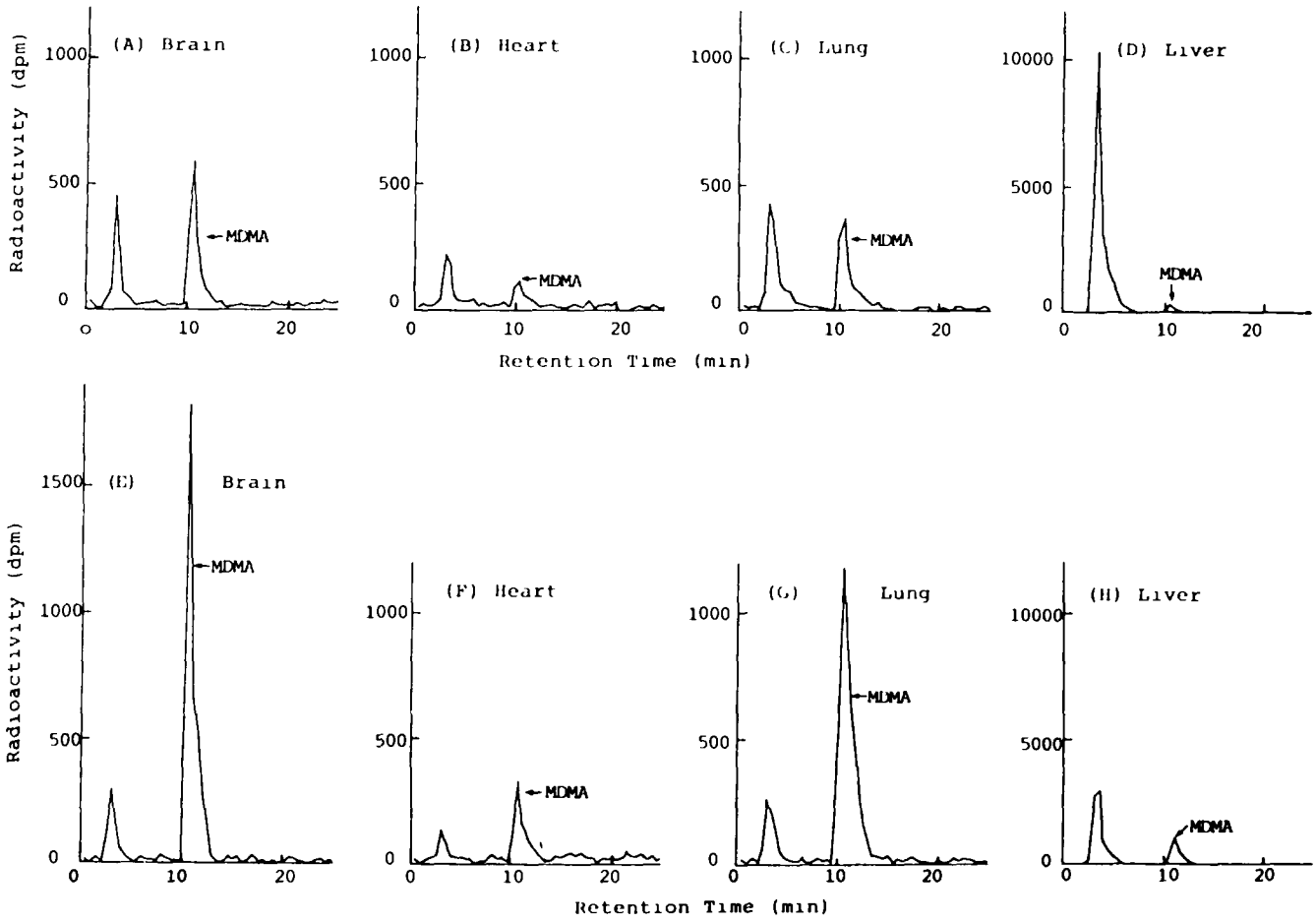

Fig 3 HPLC analysis of radioactive materials in the mouse brain, heart, lung and liver after i v administration of [^{3}H]MDMA The radioactive materials in the tissue were analyzed as described in Materials and methods Retention time of MDMA was 11 min A–D HPLC chart of radioactivity in the brain, heart, lung and liver 60 min after injection of [^{3}H]MDMA E–H HPLC chart of radioactivity in the brain, heart, lung and liver 60 min after injection of [^{3}H]MDMA into the mouse pretreated with 1-piperonylpiperazine (20 mg/kg, i p 5 min)

piperonylamine and piperonylamine possess the 3,4-methylenedioxyphenyl group We reported previously that the time course of radioactivity in the mouse brain after i v administration of [^{3}H]paroxetine was altered by pretreatment with MDA or MDMA (Hashimoto and Goromaru, 1990b,c) Taken as a whole, the present data suggest that a mechanism for the 3,4-methylenedioxyphenyl group that rapidly alters the disposition of [^{3}H]MDMA might exist in the mouse brain

HPLC analyses indicate that the radioactivity in the mouse brain 60 min after i v administration of [^{3}H]MDMA was due to unmetabolized [^{3}H]MDMA and radioactive metabolite(s), whereas the radioactivity in the brain 1 min after injection of [^{3}H]MDMA was due to unchanged [^{3}H]MDMA Thus, it was found that the amount of radioactive metabolite(s) in the brain 60 min after injection of [^{3}H]MDMA was approximately 50% of total brain radioactivity The data show that [^{3}H]MDMA is either metabolized to radioactive metabolite(s) in the mouse brain or radioactive metabolite(s) of [^{3}H]MDMA formed in the periphery enter into the brain The latter appear to be unlikely since radioactive metabolite(s), which are usually hydrophilic, should not cross the blood-brain barrier In addition, it is well known that MDMA is metabolized into MDA, indicating that the N-methyl group of MDMA is cleaved off in the periphery (Lim and Foltz, 1988, Fitzgerald et al , 1989) It is likely that [^{3}H]MDMA is metabolized to non-radioactive MDA and a radioactive methyl group by N-demethylation in the periphery, since tritium is labeled at the N-methyl group of MDMA Probably, a radioactive methyl group which was cleaved off in the periphery would be rapidly metabolized and eliminated Furthermore, the distribution of radioactivity in the mice 60 min after injection of [^{3}H]MDMA was not altered by pretreatment with piperonyl butoxide (a well known inhibitor of N-demethylase activity) (Dahl and Brezinski, 1985), suggesting that radioactive metabolite(s) in the brain might not be metabolite(s) formed by N-demethylation of [^{3}H]MDMA Further studies of the disposition and metabolism of [^{3}H]MDMA using ^{3}H labeled at the phenyl ring would be very interesting Although the chemical structure of radioactive metabolite(s) of [^{3}H]MDMA have not yet been identified, it has been suggested that the major radioactive metabolites of [^{3}H]MDMA might be [^{3}H]4-hydroxy-3-methoxymethamphetamine, [^{3}H]3-hydroxy-4-methoxymethamphetamine and [^{3}H]3,4-dihydroxymethamphetamine (Lim and Foltz, 1988, Hiramatsu et al , 1990, Yousif et al , 1990)

The most interesting finding of the present study is that metabolism of [^{3}H]MDMA to radioactive metabolite(s) in the mouse brain was, in part, blocked by pretreatment with paroxetine, 1-piperonylpiperazine and MDMA Furthermore, it was also shown that metabolism of [^{3}H]MDMA to radioactive metabolite(s) in the heart, lung and liver was, in part, prevented by pretreatment with 1-piperonylpiperazine Also, it has been reported that paroxetine is initially oxidized to an unstable catechol intermediate (Haddock et al , 1989). The drugs studied in the present study may inhibit a metabolic pathway of MDMA (e g , O oxidation of 3,4-methylenedioxy function on the phenyl ring) These results suggest that a specific mechanism for the 3,4-methylenedioxyphenyl group which rapidly alters the disposition and/or metabolism of [^{3}H]MDMA may exist in the brain and peripheral organs (heart, lung, liver)

It has been reported that marked species differences in the MDMA-induced neurotoxicity are found in mice and rats (Stone et al , 1987a, Logan et al , 1988, Peroutka, 1988, Battaglia et al , 1988), indicating that mice are less susceptible to the neurotoxic effects of MDMA These investigators attribute the altered sensitivity to metabolic differences between the two species Moreover, in contrast to systemic administration, direct intracerebral injection of MDMA failed to produce neurotoxicity to the 5-HT neurons (Schmidt and Taylor, 1988, Molliver et al , 1989) Thus, it is suggested that an active metabolite of MDMA in the periphery may be responsible for eliciting the neurotoxic effects, although there has been no direct demonstration of a neurotoxic metabolite of MDMA Molliver et al (1989) suggest that the formation of a peripheral metabolite of MDMA may be an essential step in the MDMA-induced neurotoxicity Very recently, we have observed that MDMA-induced neurotoxicity of 5-HT nerve terminals in the rat brain was antagonized significantly by coadministration of 1-piperonylpiperazine (Hashimoto et al , 1992) In addition, it has been shown that the disposition and metabolism of [^{3}H]MDMA in the brain and periphery organs (heart, lung, liver) of rats are significantly altered by pretreatment with 1-piperonylpiperazine (Hashimoto, unpublished) However, it is unclear whether 1-piperonylpiperazine prevents the MDMA-induced neurotoxicity by inhibiting the metabolism of MDMA to an active agent It was also found that 1-piperonylpiperazine had very weak inhibitory effects on 5-HT uptake into rat brain synaptosomes (Hashimoto and Goromaru, 1992b) Thus, it is possible that 1-piperonylpiperazine prevents the MDMA-induced neurotoxicity by effects other than inhibition of 5-HT uptake Further studies on this are now in progress

In summary, the distribution of radioactivity in the mouse brain after i v administration of [^{3}H]MDMA was significantly increased by pretreatment with paroxetine or basic compounds including the piperonyl group Furthermore, the alteration of radioactivity in the brain and periphery organs (heart, lung, liver) produced by these drugs might be, in part, due to an

alteration of metabolism of [^{3}H]MDMA in the mice The present results suggest that a specific mechanism for the 3,4-methylenedioxyphenyl group which rapidly alters the disposition and metabolism of [^{3}H]MDMA may exist in the brain and periphery organs of mouse

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