

The Role of Dopaminergic Systems in the Perinatal Sensitivity to 3,4-Methylenedioxymethamphetamine-Induced Neurotoxicity in Rats¹

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ABSTRACT

Our study was aimed at analyzing the basis for the apparent lack of perinatal sensitivity to the serotonergic neurotoxin 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). MDMA (20 mg/kg s.c.) repeatedly administered to rat dams during gestation, did not affect [³H]paroxetine-labeled serotonin (5-HT) transporter density and 5-HT content in the offspring. A single dose of MDMA was then given to pups, not exposed prenatally to MDMA, at different postnatal ages (PND14, 21, 28 and 35). Long-term significant reductions in 5-HT levels in all the brain regions examined were only found at PND35. In a different set of experiments, MDMA administered at PND21 alone or in combination with (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (R-DOI, 0.5 mg/kg s.c.), or L-3,4-dihydroxyphenylalanine (L-DOPA, 80 mg/kg s.c.), caused a significant hyperthermia in the pups. However, only L-DOPA followed

by MDMA caused a lasting reduction of 5-HT levels and 5-HT transporter density in the hippocampus and in the frontal cortex. In adult animals, no change in 5-HT levels and 5-HT transporter density in different brain regions was either found when MDMA was given to rats previously lesioned with 6-hydroxydopamine, but a significant reduction was again found in the lesioned animals receiving MDMA in combination with L-DOPA. These results appear to indicate that the hyperthermia induced by MDMA is not sufficient to produce lasting neurotoxic effects on the serotonergic system, at least at PND21, and support an important role for dopamine in the mechanism of neurotoxicity of MDMA, suggesting that an already developed dopaminergic system is necessary for the expression of the serotonergic deficits.

The administration of a single high dose or of multiple doses of MDMA ("ecstasy") to rats produces long-term alterations in different serotonergic parameters. A marked reduction in the content of 5-HT and its metabolite 5-HIAA along with a decrease in tryptophan hydroxylase activity in various brain regions has been described (Stone *et al.*, 1986; Schmidt, 1987; Schmidt and Taylor, 1987). MDMA has also been shown to reduce the number of [³H]paroxetine-labeled 5-HT transporters (Aguirre *et al.*, 1995; Battaglia *et al.*, 1987) as well as the synaptosomal uptake of [³H]5-HT (Schmidt, 1987). Furthermore, immunocytochemical evidence indicates that MDMA selectively damages fine axon terminals that arise from cell bodies located in the dorsal raphe nucleus of the midbrain which remain spared (O'Hearn *et al.*, 1988; Slikker *et al.*, 1988).

Although the precise mechanism by which MDMA selectively damages 5-HT axon terminals remains largely un-

known, studies from different laboratories have provided evidence for an important role of dopamine in MDMA-induced neurotoxicity. Stone *et al.* (1988) showed that previous dopamine depletion with the synthesis inhibitor α -methyl-p-tyrosine, or treatment with the dopamine uptake blocker GBR-12909 prevent the long-term reductions of 5-HT induced by MDMA. In an analogous fashion, if dopamine terminals are destroyed with 6-hydroxydopamine, 5-HT terminals remain spared after MDMA (Schmidt *et al.*, 1990b). It has also been shown that MDMA increases both *in vivo* dopamine release (Koch and Galloway, 1997; Nash, 1990) via a carrier-mediated mechanism (Gudelsky and Nash, 1996; Nash and Brodtkin, 1991) and dopamine synthesis (Nash *et al.*, 1990) through 5-HT₂ receptor stimulation (Huang and Nichols, 1993), although the latter effect only becomes significant during states of high serotonergic and dopaminergic transmission (Schmidt *et al.*, 1992b). Microdialysis studies have shown that blockade of striatal 5-HT₂ receptors reduces significantly the efflux of endogenous dopamine (Schmidt *et al.*, 1994) whereas the activation of these receptors with the

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ABBREVIATIONS: DOPAC, dihydroxyphenylacetic acid; E, embryonic day; HVA, homovanillic acid; 5-HT, 5-hydroxytryptamine, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; MDMA, 3,4-methylenedioxymethamphetamine; PND, postnatal day; R-DOI, (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

agonist R-DOI potentiates MDMA-induced dopamine release and serotonergic toxicity (Gudelsky *et al.*, 1994). Other studies have demonstrated that selective 5-HT₂ receptor antagonists protect against the neurotoxicity induced by MDMA (Schmidt *et al.*, 1990a,b); however, L-DOPA coadministration abolishes the protective effect of 5-HT₂ receptor antagonists (Schmidt *et al.*, 1991b, 1992a). Furthermore, pretreatment with L-DOPA potentiates the long-term serotonergic deficits induced by MDMA (Schmidt *et al.*, 1991a) and there appears to exist a linear correlation between the acute increase of extracellular dopamine and the extent of serotonergic toxicity (Nash and Nichols, 1991). It has been recently suggested that dopamine would then be transported into the 5-HT terminal where it is deaminated by MAO-B resulting in an elevated intracellular level of hydrogen peroxide that would lead to an extensive lipid peroxidation in the 5-HT terminal membrane and a subsequent neuronal degeneration (Sprague and Nichols, 1995a,b). Results from other laboratories support the hypothesis that MDMA produces acute inactivation of tryptophan hydroxylase and neurotoxicity of 5-HT neurons because of oxidative events probably associated with dopamine metabolism (Cadet *et al.*, 1995; Colado and Green, 1995; Gudelsky, 1996; Gudelsky and Yamamoto, 1994).

Although there are numerous studies related to the neurochemical consequences of MDMA on the 5-HT system of adult rats, studies on the effects of MDMA administration to rats at early postnatal ages are scarce. St. Omer *et al.* (1991) found that MDMA administered during gestation had no effect on the postnatal neurochemical development of the serotonergic system of the rat. Similarly, Broening *et al.* (1994) showed that rats do not develop their sensitivity to the long-term neurochemical deficits induced by MDMA until postnatal day 40.

The first aim of our study was to assess the effect of perinatal administration of MDMA and to determine the period for the onset of susceptibility to the neurotoxic effects of MDMA in the rat pups exposed to this drug. We also tried to ascertain why at early postnatal ages MDMA does not produce, as in adult rats, a long-term reduction in brain 5-HT content and 5-HT transporter density. The study was focused on brain 5-HT₂ receptors and dopamine content, which appear to play a major role in the neurotoxicity induced by MDMA. Adult rats with a previous dopamine depletion were comparatively studied. As the hyperthermia induced by MDMA appears to contribute to its neurotoxic effects (Malberg *et al.*, 1996), the correlation of the thermal response to MDMA with the neurotoxic effect was also analyzed.

Methods

Animals and treatments. Pregnant female Wistar rats (270–290 g) were individually housed in plastic cages in a temperature controlled room ($22 \pm 1^\circ\text{C}$) and maintained on a 12-hr light-dark cycle with free access to food and water. MDMA (20 mg/kg s.c.) was given every other day to rat dams from embryonic day 6 (E6) to E20. The rat pups were killed at PND15. For the rest of the experiments with the rat pups, upon delivery (PND0), offspring in each litter was randomly culled to eight pups. At PND20, litters were weaned and male pups were housed five to a cage. Animals received saline (control group) or a single dose of MDMA (20 mg/kg s.c.) at different postnatal ages: PND14, 21, 28 and 35. In a different set of experiments, 21-day-old rats were distributed into six groups that received:

1) saline followed by saline, 2) saline followed by a single dose of MDMA (20 mg/kg s.c.), 3) L-DOPA (80 mg/kg s.c.) 15 min before saline, 4) L-DOPA 15 min before MDMA, 5) R-DOI (0.5 mg/kg s.c.) followed by saline and 6) R-DOI followed by MDMA. L-DOPA was always administered in combination with the peripheral decarboxylase inhibitor benserazide (20 mg/kg s.c.) and the doses of MDMA used refer to the hydrochloride.

Adult rats (*ca.* 3-mo-old) received desipramine-HCl (25 mg/kg i.p.) 1 hr before the injection of 6-hydroxydopamine-HBr (100 $\mu\text{g}/10 \mu\text{l}$ expressed as free base) or saline, containing ascorbic acid 0.1%, in each lateral ventricle. Lesions were performed under pentobarbital anaesthesia (50 mg/kg i.p.). Eight days later, sham- and 6-hydroxydopamine-lesioned rats, were treated with saline, MDMA, L-DOPA or L-DOPA + MDMA as above indicated.

In all cases animals were killed 7 days after the different drug treatments, their brains were rapidly removed and placed on ice. The appropriate brain regions were dissected free, frozen on dry ice and stored at -80°C until chromatographic and binding studies were performed.

Biochemical measurements. The concentrations of 5-HT, 5-HIAA, dopamine, DOPAC and HVA in the brain regions examined, were determined by high-performance liquid chromatography with electrochemical detection as previously described (Pérez-Otaño *et al.*, 1991).

5-HT transporter density. [^3H]paroxetine binding studies to the 5-HT transporter were performed according to the procedure described by Marcusson *et al.* (1988), with minor modifications. The brain regions studied were homogenized in 15 ml of ice-cold buffer (Tris-HCl 50 mM, 120 mM NaCl, 5 mM KCl, pH 7.4) and centrifuged at $48,000 \times g$ for 10 min at 4°C . The pellet was resuspended in buffer and incubated at 37°C for 10 min. After a second centrifugation in the same conditions the resultant pellet was resuspended in buffer (1.5 mg tissue/400 μl buffer). The incubation mixture contained 400 μl of tissue suspension, 200 μl of increasing concentrations of [^3H]paroxetine (0.02–0.4 nM) and 1.4 ml of incubation buffer in the absence and presence of fluoxetine 10 μM . Tubes were incubated for 60 min at 22°C . After rapid filtering through GF/C Whatman filters using a 24-well cell harvester, the filters were rinsed with $4 \times 5 \text{ ml}$ of ice cold buffer and placed in vials containing 4 ml of liquid scintillation cocktail (Biogreen3, Scharlau). All the determinations were carried out in duplicate. Data were subjected to Scatchard analysis to determine the number of binding sites (B_{max} : fmol/mg of protein) and the dissociation constant (K_d : nM).

Temperature measurements. The rectal temperature of the rats was measured at an ambient temperature of $22 \pm 1^\circ\text{C}$ with a lubricated digital thermometer probe (pb 0331, Panlab, Barcelona, Spain) inserted 3 cm into the rectum, the rat being lightly restrained by holding in the hand. Temperature was recorded at PND21, before any drug treatment (MDMA alone or in combination with L-DOPA or R-DOI) and thereafter every 30 min up to 240 min. Probes were re-inserted from time to time and maintained until the temperature stabilized. The number of animals per group are detailed in the figure legend.

Drugs. The sources of the drugs used were as follows: MDMA-HCl was either from Sigma (UK) or was a gift from the "Servicio de Restricción de Estupefacientes" (Dr. L. Domínguez, Madrid, Spain); [^3H]paroxetine (22.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA); 5-HT creatinine sulfate and 5-HIAA were from Sigma (St. Louis, MO); fluoxetine-HCl was generously donated by Eli-Lilly and Co., (Indianapolis, IN); R-DOI and desipramine-HCl were from Research Biochemicals International (Natick, MA); 6-hydroxydopamine hydrobromide was from ICN Biomedicals Inc. (Aurora, OH); L-DOPA and benserazide were from Syntex Latino (Madrid, Spain), all other chemicals were from Merck (Darmstadt, Germany).

Statistical analysis. The effect of perinatal administration of MDMA on 5-HT and 5-HIAA concentrations was compared with the saline (control) treated group using an unpaired Student's *t* test.

Analyses of the differences between multiple treatment groups consisted of analysis of variance followed by Tukey *post hoc* test. For the rectal temperature analysis, two-way analysis of variance for repeated measures was used to compare treatment groups. Single time point comparisons between groups were made using Tukey's test. Significant differences were defined at $P < .05$.

Results

Effect of perinatal administration of MDMA on 5-HT and dopamine brain levels and 5-HT transporter density. The effect on brain 5-HT content of MDMA (20 mg/kg s.c.) at different developmental ages is depicted in table 1. To assess a possible neurotoxic action of MDMA on rat progeny, the drug was first administered to rat dams every other day from E6 to E20 and the rat pups were killed at PND15. This treatment had no effect on the postnatal levels of 5-HT. Similarly, a single injection of MDMA (20 mg/kg s.c.) at PND14 and 21 did not cause any significant reduction of 5-HT. When MDMA was administered at PND28, 5-HT concentration was significantly decreased 7 days later in the hippocampus but not in any other terminal field of the serotonergic system examined (frontal cortex, striatum and hypothalamus). A significant long-term reduction of 5-HT levels in all the brain regions examined was already achieved when MDMA was given at PND35 (table 1). 5-HIAA concentration was always modified in a parallel fashion to that of 5-HT (not shown).

Dopamine concentration in the striatum and in the hypothalamus was not different from saline control values 7 days after any of the perinatal MDMA treatments used in the present study (table 1). The dopamine metabolites DOPAC and HVA were not either modified (not shown). In the frontal cortex and in the hippocampus dopamine concentration was too low for accurate quantitation.

As can be seen in table 1, 5-HT systems appear to develop earlier than the dopaminergic systems. The increase in striatal 5-HT and dopamine concentration during postnatal development, as percentage of adult levels, is illustrated in figure 1. 5-HT concentration at PND21 already reached approximately 90% of that found at PND42. However, dopa-

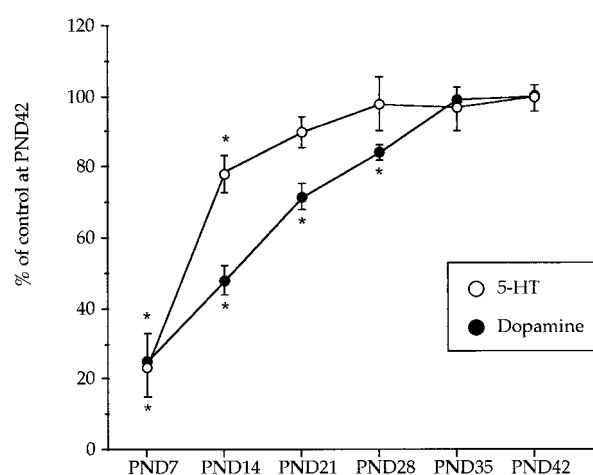


Fig. 1. Striatal concentration of 5-HT (○) and dopamine (●) during postnatal development. Animals were killed at different postnatal days (PND). All the animals received a saline injection 7 days before sacrifice. Values ($n = 10-12$) expressed as percentage of striatal monoamine concentrations at PND42. Absolute values from PND21 to PND42 are shown in table 1 (controls). 5-HT concentrations at PND7 and 14 (not shown in table 1) were: 123.7 ± 10.4 and 400.6 ± 25.9 ; dopamine levels were 2365.3 ± 183.7 and 4553.7 ± 185.6 pg/mg wet tissue for PND7 and 14 respectively. * $P < .05$ or better *vs.* PND42 values using one-way analysis of variance followed by Tukey's test.

mine concentration at PND28 was still significantly lower than at PND42.

Saturation binding experiments of [3 H]paroxetine to the 5-HT transporter in the frontal cortex indicated that MDMA (20 mg/kg s.c.) administered repeatedly during gestation or as a single dose at different postnatal ages up to PND28, did not cause any significant change in the density of 5-HT transporters (data not shown). However, when the drug was injected at PND35, 5-HT transporter density was significantly decreased in the frontal cortex 7 days later. B_{max} values (means $\pm$ S.E., $n = 5$) for control and MDMA-treated rats were: 360.8 ± 22.8 and 228.6 ± 19.8 fmol/mg protein respectively ($P < .05$).

Effect of combined treatments of MDMA and R-DOI or L-DOPA on brain 5-HT levels and 5-HT transporter

TABLE 1

Effect of perinatal administration of MDMA on 5-HT and dopamine content in different rat brain regions

Days		Frontal Cortex		Striatum		Hippocampus	Hypothalamus	
Administration	Death	5-HT	5-HT	Dopamine	5-HT	5-HT	5-HT	Dopamine
E6 to E20	PND15							
Control		278.3 $\pm$ 18.1	336.5 $\pm$ 33.8	4595.1 $\pm$ 190.6	315.9 $\pm$ 11.5	764.3 $\pm$ 67.7	226.5 $\pm$ 16.7	
MDMA		266.2 $\pm$ 10.1	362.7 $\pm$ 24.3	4512.3 $\pm$ 329.7	318.8 $\pm$ 37.4	710.6 $\pm$ 33.9	239.0 $\pm$ 16.9	
PND14	PND21							
Control		284.9 $\pm$ 18.0	467.7 $\pm$ 21.2	6788.4 $\pm$ 250.1	336.7 $\pm$ 9.0	934.3 $\pm$ 69.9	399.6 $\pm$ 11.5	
MDMA		305.2 $\pm$ 12.9	466.0 $\pm$ 17.8	6912.9 $\pm$ 385.9	370.0 $\pm$ 9.2	935.4 $\pm$ 66.8	387.3 $\pm$ 19.4	
PND21	PND28							
Control		388.9 $\pm$ 20.9	509.5 $\pm$ 39.5	7983.9 $\pm$ 176.3	381.0 $\pm$ 32.4	930.4 $\pm$ 57.1	412.1 $\pm$ 21.4	
MDMA		422.5 $\pm$ 40.1	463.1 $\pm$ 25.5	7792.4 $\pm$ 339.4	379.2 $\pm$ 19.1	864.4 $\pm$ 30.1	393.8 $\pm$ 16.0	
PND28	PND35							
Control		441.7 $\pm$ 38.5	505.5 $\pm$ 34.2	9408.0 $\pm$ 347.9	426.6 $\pm$ 33.5	993.5 $\pm$ 64.3	444.2 $\pm$ 22.4	
MDMA		381.3 $\pm$ 43.7	446.5 $\pm$ 14.1	9696.3 $\pm$ 315.8	310.6 $\pm$ 28.0*	848.2 $\pm$ 72.3	416.0 $\pm$ 21.1	
PND35	PND42							
Control		415.4 $\pm$ 15.9	521.2 $\pm$ 21.9	9497.6 $\pm$ 306.8	497.4 $\pm$ 12.8	1180.4 $\pm$ 54.9	424.0 $\pm$ 19.4	
MDMA		343.4 $\pm$ 19.8*	428.1 $\pm$ 28.8*	9351.6 $\pm$ 257.7	297.2 $\pm$ 26.2**	873.5 $\pm$ 32.8*	434.1 $\pm$ 31.9	

MDMA (20 mg/kg s.c.) was initially given every other day to rat dams from embryonic day 6 (E6) to E20 and the pups were killed at postnatal day 15 (PND15). In a second set of experiments, a single dose of MDMA (20 mg/kg s.c.) was administered to the pups at different postnatal ages and the animals were killed 7 days later. Control group received saline in all cases. Values are means $\pm$ S.E. in pg/mg of wet tissue from 10 to 12 rats.

* $P < .05$, * $P < .01$ *vs.* the corresponding control group using Student's *t* test.

density in rat pups. The combined administration of R-DOI and MDMA at PND21 did not result in altered levels of 5-HT 7 days later. However, the administration of L-DOPA (80 mg/kg s.c.) 15 min before a single injection of MDMA (20 mg/kg s.c.) already caused at PND21 a significant long-term reduction of 5-HT levels in the frontal cortex and in the hippocampus. Although the concentration of 5-HT tended to be lower in the striatum and in the hypothalamus, the reduction did not reach statistical significance (fig. 2).

The administration of MDMA, alone or in combination with R-DOI at PND21, did not cause any significant change in the number of [3 H]paroxetine-labeled 5-HT uptake sites. At this same age, L-DOPA given 15 min before a single dose of MDMA also produced a significant decrease in the density of these sites ($P < .05$), both in the frontal cortex (by approximately 35%) and in the hippocampus (by approximately 30%). Results are shown in figure 3.

Effect of combined treatment of MDMA and L-DOPA on brain 5-HT levels and 5-HT transporter density in adult rats lesioned with 6-hydroxydopamine. After treatment with 6-hydroxydopamine, the depletion of dopamine was approximately 85% in the striatum and 50% in the hypothalamus. Administration of MDMA (20 mg/kg s.c.) or L-DOPA (80 mg/kg s.c.) to 6-hydroxydopamine-lesioned rats did not produce any change in the concentration of 5-HT (not shown). However, the combination of L-DOPA with MDMA caused a 40 to 60% reduction of 5-HT content in the frontal

cortex, hippocampus, striatum and hypothalamus. For example, the 5-HT concentration (pg/mg wet tissue) in the frontal cortex and hippocampus of lesioned rats receiving only MDMA was 418.8 ± 32.2 and 364.8 ± 26.0 , respectively (means $\pm$ S.E., $n = 6$). In rats receiving the combined treatment of MDMA and L-DOPA the corresponding values were 159.0 ± 19.8 and 130.0 ± 9.0 ($P < .05$).

5-HT transporter density (fmol/mg of protein) of 6-hydroxydopamine-lesioned rats was 452.1 ± 23.3 and 268.2 ± 19.4 (means $\pm$ S.E., $n = 6$) in the frontal cortex and in the hippocampus respectively. These values were almost identical to those of the corresponding sham controls. MDMA treatment did not produce any change when administered to 6-hydroxydopamine lesioned rats. By contrast, the combination of L-DOPA and MDMA significantly decreased ($P < .05$) in the lesioned rats 5-HT transporter density by 40 and 50% in the frontal cortex and in the hippocampus respectively.

MDMA induced hyperthermia. MDMA administered at PND21 caused a significant raise of core temperature that lasted approximately 4 hr. When temperature in the MDMA-treated group returned to control values (at 240-min time point), no other temperature measurement was taken in the rest of the groups. The highest raise in temperature for the MDMA treated group (ca. 2°C), was obtained during the first 90 min after drug administration.

L-DOPA administration produced a mild hypothermia (ca. -1.6°C at 120-min time point), although R-DOI had no effect on

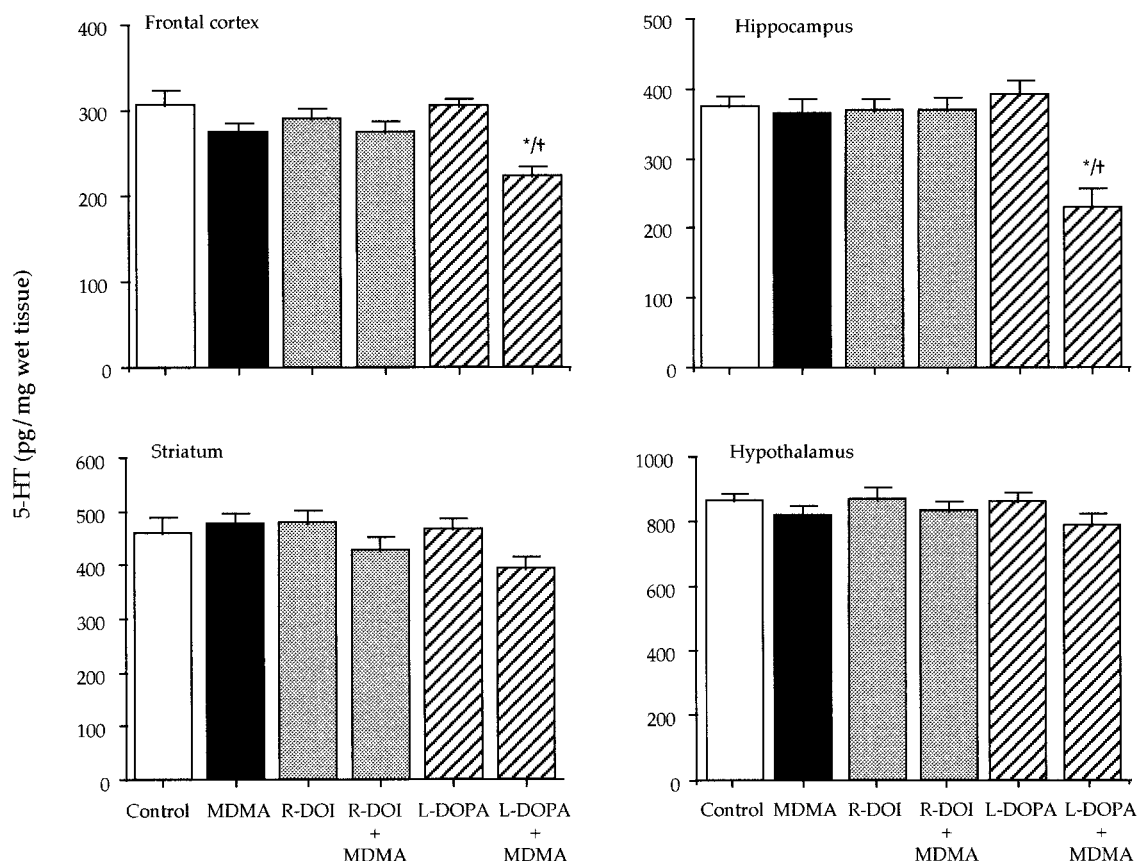


Fig. 2. Effect of combined treatments of MDMA with R-DOI or L-DOPA on 5-HT concentration in different rat brain regions. Rats received on PND21 two injections, spaced 15 min apart, and were killed 7 days later. Control: saline + saline; MDMA: saline + MDMA (20 mg/kg s.c.); R-DOI: R-DOI (0.5 mg/kg s.c.) + saline; R-DOI + MDMA: R-DOI (0.5 mg/kg s.c.) + MDMA (20 mg/kg s.c.); L-DOPA: L-DOPA (80 mg/kg s.c.) + saline; L-DOPA + MDMA: L-DOPA (80 mg/kg s.c.) + MDMA (20 mg/kg s.c.). Values are means $\pm$ S.E. from 8 to 10 rats. Data were analyzed by one-way analysis of variance followed by Tukey's test. * $P < .05$ vs. control; † $P < .05$ vs. MDMA.

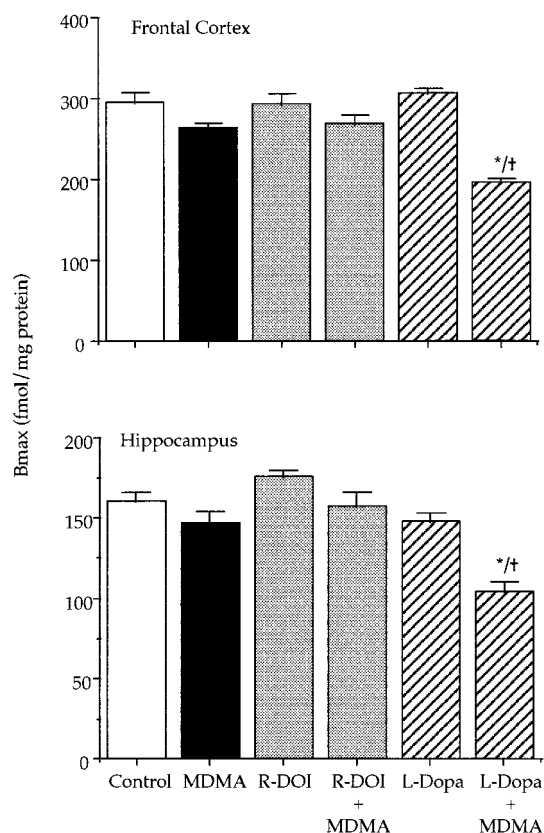


Fig. 3. Effects of MDMA (20 mg/kg s.c.) administered alone or in combination with R-DOI (0.5 mg/kg s.c.) or L-DOPA (80 mg/kg s.c.) on the density of 5-HT transporters (B_{max}) in the frontal cortex and in the hippocampus of the rat. Drug treatments, doses and days of administration and death are described in the legend to figure 2. Values represent the means $\pm$ S.E. in fmol/mg prot; $n = 6$ in all groups. Data were analyzed by one-way analysis of variance followed by Tukey's test. * $P < .05$ vs. control group. † $P < .05$ vs. MDMA group.

core temperature of the rat pups when compared to controls. The combination of R-DOI or L-DOPA with MDMA, induced in both cases a significant hyperthermia that lasted beyond the period of measurement (240 min). The highest raise in core temperature was obtained for both groups during the first 90 min (ca. 3° and 2.5°C, respectively) as happened with the MDMA-treated group. The combination of R-DOI and MDMA resulted in temperatures combination significantly higher than MDMA alone at all time points ($P < .05$). Significant differences between L-DOPA + MDMA and MDMA-treated groups were obtained only beyond the 120-min time point after injection ($P < .05$). Results are depicted in figure 4.

Discussion

Our results indicate that MDMA, administered repeatedly to rat dams during gestation, did not apparently produce any change in the development of the serotonergic system of the offspring. In agreement with previous studies, rat pups were also resistant to the neurotoxic effects of a single dose of MDMA over the first 4 wk of life and it seems that the time for the onset of susceptibility is placed between PND28 and PND35. However, MDMA induced neurotoxicity at an earlier postnatal age in rats pretreated concomitantly with the dopamine precursor L-DOPA. MDMA was not either neurotoxic in adult rats with a previous dopaminergic lesion but the neurotoxicity was rein-

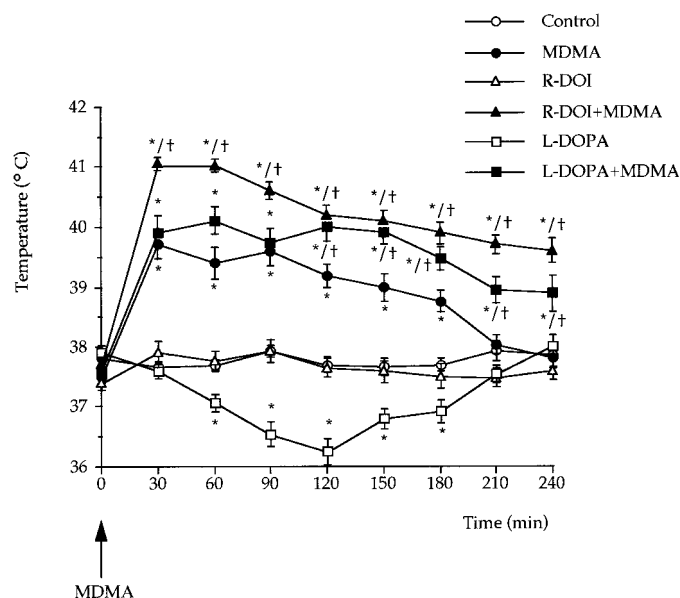


Fig. 4. Rectal temperature of rats at PND21 after the administration of: a, saline followed by saline (○); b, saline followed by a single dose of MDMA (20 mg/kg s.c.) (●); c, L-3,4-dihydroxyphenylalanine (L-DOPA, 80 mg/kg s.c.) 15 min before saline (□); d, L-DOPA 15 min before MDMA (■); e, (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (R-DOI, 0.5 mg/kg s.c.) followed by saline (△) and f, R-DOI followed by MDMA (▲). Values are means $\pm$ S.E. ($n = 7-10$). * $P < .05$ or better vs. control group (saline + saline). † $P < .05$ vs. saline/MDMA group.

stated in the lesioned animals when MDMA was given in combination with L-DOPA.

By using the MAO-B inhibitor, 1-deprenyl, Sprague and Nichols (1995b) prevented MDMA-induced neurotoxicity and blocked the formation of thiobarbituric reactive substances, often viewed as an index of enhanced lipid peroxidation. According to their results and other numerous studies focused on the mechanism of MDMA-induced neurotoxicity, these authors suggested that a possible sequence of events leading to 5-HT axon terminal degeneration after MDMA could be the following: 1) acute 5-HT depletion from serotonergic neurons (Sprague *et al.*, 1994) and acute increase in dopamine release (Nash, 1990; Nash and Brodtkin, 1991). 2) Stimulation of 5-HT_{2A} receptors, by the released 5-HT, responsible for an increased dopamine synthesis necessary to support MDMA-induced transmitter efflux (Huang and Nichols, 1993; Koch and Galloway, 1997; Nash *et al.*, 1990; Schmidt *et al.*, 1992a). 3) Transport by the 5-HT carrier of extracellular dopamine into the depleted serotonergic terminal (Faraj *et al.*, 1994; Schmidt and Lovenberg, 1985). 4) Deamination of dopamine by MAO-B inside the 5-HT terminal, generating not only DOPAC but also hydrogen peroxide and possibly other oxidative species responsible for lipid peroxidation and final axon degeneration (Sprague and Nichols, 1995b).

Serotonergic and dopaminergic neurotransmitter systems undergo a significant maturation during the postnatal development of the central nervous system. As MDMA-induced 5-HT and dopamine release is carrier mediated (Koch and Galloway, 1997; Nash and Brodtkin, 1991; Schmidt, 1987), their respective uptake systems may not be present on 5-HT and dopamine axons; however, this seems unlikely as it has been demonstrated that both uptake systems exhibit a rapid proliferation from PND0 to PND14 (Kirksey and Slotkin, 1979). 5-HT₂ receptors seem to play also an important role in the maintenance of MDMA-induced dopamine release and serotonergic toxicity.

It has been shown that 5-HT₂ receptor agonists potentiate MDMA-induced dopamine release and serotonergic neurotoxicity in adult rats (Gudelsky *et al.*, 1994) although blockade of these receptors prevents the long-term deficits produced by MDMA (Schmidt *et al.*, 1990a,b). The lack of sensitivity of rat pups to the long-term brain 5-HT depletion may then indicate that 5-HT₂ receptors are not entirely functional at early postnatal ages. Yet, it is known that, in the rat, cells expressing 5-HT₂ receptors reach a peak at about the end of the second postnatal week and this is followed by a regressive process until approximately PND28 (Bruinink *et al.*, 1983; Morilak and Ciaranello, 1993; Roth *et al.*, 1991). Consistent with this ontogeny, we found in our study that rats treated at PND21 with the selective 5-HT₂ receptor agonist, R-DOI (0.5 mg/kg s.c.), showed a high number of head twitches (not shown), an effect mediated by 5-HT₂ receptor stimulation (*e.g.*, Zifa and Fillion, 1992), suggesting that 5-HT₂ receptors were already functional at this age. Despite this, the combination of R-DOI and MDMA at PND21 neither produced a significant decrease in 5-HT transporter density in the frontal cortex and in the hippocampus nor caused a significant deficit of 5-HT and 5-HIAA in any of the brain regions examined. In consequence, the lack of neurotoxicity after MDMA exposure, at least at PND21, is not apparently due to low 5-HT₂ receptor density or functioning.

It has been reported (Johnson and Nichols, 1991) that the combination of the nonneurotoxic serotonin releaser 5-methoxy-6-methyl-2-aminoindan with the nonvesicular dopamine releaser, amphetamine, produces long-term serotonergic deficits similar to those induced by MDMA. However, increased dopamine synthesis and release after the combination of the selective 5-HT₂ receptor agonist R-DOI (at the same dose used in our study) with amphetamine is not sufficient to induce serotonergic deficits (Huang and Nichols, 1993). These authors suggested that it is probably necessary a previous 5-HT depletion from the neuron to render the serotonergic terminal vulnerable to the toxic action of dopamine, as increased dopamine synthesis and efflux cannot explain by itself the neurotoxic effects induced by MDMA. Since acute MDMA already depletes 5-HT from the terminal at the age of 10 days (Broening *et al.*, 1994) and 5-HT₂ receptors seem to be functional at PND21, there must be other reason to explain why the long-term neurotoxic effects of MDMA are not observed until a time between PND28 and PND35.

Numerous authors have suggested that MDMA-induced neurotoxicity depends on its ability to release dopamine through a carrier-mediated mechanism (*e.g.*, White *et al.*, 1996). Furthermore, there appears to exist a linear correlation between the acute increase of extracellular dopamine and the extent of serotonergic toxicity induced by MDMA (Nash and Nichols, 1991). Even though studies on the functional ontogeny of dopaminergic neurons have demonstrated that they are able to initiate or conduct action potentials at approximately PND8 (Cheronis *et al.*, 1979; Erinoff and Heller, 1978), adult levels of striatal dopamine are not reached until approximately PND30 (Nomura *et al.*, 1976). Another possibility to explain the lack of sensitivity to MDMA in newborn rats is that extracellular dopamine concentrations are not high enough to get into the serotonergic terminal, producing the oxidative oxygen species responsible for the neurotoxicity. Supporting this hypothesis, we found that the combined treatment with the dopamine precursor, L-DOPA, and MDMA at PND21 significantly reduced the concentrations of 5-HT and 5-HIAA in the frontal cortex and

in the hippocampus and also decreased significantly the number of [³H]paroxetine-labeled 5-HT transporters in these two brain regions. It could be also supposed that norepinephrine and not dopamine was involved in 5-HT depletion, as norepinephrine concentration is higher in the hippocampus than in the striatum whereas the striatum, having the highest dopamine concentration, was less affected than the hippocampus. However, it has been suggested (Schmidt *et al.*, 1991a), that such considerations only become relevant by assuming a terminal-terminal interaction between the 5-HT and dopaminergic systems, a structural aspect still unresolved at the time of analyzing the mechanism(s) of action of MDMA. Further, desipramine should prevent the uptake of 6-hydroxydopamine into the noradrenergic nerve terminal (*e.g.*, review by Kostrzewa and Jacobowitz, 1974), so a partial reduction of 5-HT content in animals previously lesioned with 6-hydroxydopamine should be expected if norepinephrine were involved in MDMA-induced neurotoxicity. Decreased 5-HT concentrations and 5-HT transporter density were only found when MDMA was given to 6-hydroxydopamine-lesioned rats in combination with L-DOPA. These results support an important role for dopamine in the mechanism of neurotoxicity induced by MDMA. It should be noted that Perry *et al.* (1995), found lasting serotonergic deficits after p-chloroamphetamine in adult rats neonatally treated with 6-hydroxydopamine. Although the neurotoxicity induced by p-chloroamphetamine is apparently similar to that seen after MDMA, the mechanisms are not at all identical and drugs such as dizocilpine or deprenyl are able to prevent the long-term neurotoxicity of MDMA but not that of p-chloroamphetamine (Colado and Green, 1994; Sprague *et al.*, 1996).

The serotonergic deficits found in PND21 rats after the combined treatment of L-DOPA and MDMA were not as dramatic as those found in adult animals. Colado *et al.* (1997), using the same rat strain and a higher MDMA dose (40 mg/kg), did not find increased formation of thiobarbituric acid reacting substances in neonates (PND7–10). These authors suggested that the lack of sensitivity of neonates to the neurotoxic effects of MDMA and other related amphetamines could be due to a much higher capacity of neonates to scavenge free radicals. This possibility, along with the much lower intracellular concentration of dopamine in immature rats, could be determinant for the lack of neurotoxicity of MDMA at early postnatal ages.

It has been also suggested that the sensitivity of the immature rat to MDMA-induced hyperthermia develops concomitantly with the sensitivity of the immature rat to the long-term neurotoxic effects of MDMA on the serotonergic system (Broening *et al.*, 1995). As indicated (see "Methods"), temperature was measured by reinserting the probes from time to time. Although it is known that colonic temperature of rats can be slightly raised by handling (Gordon, 1990), this factor should not probably exert an influence on the results as experimental conditions were identical for all animal groups. MDMA caused a significant and long-lasting hyperthermia when administered at PND21 without producing 1 wk later any significant deficit in 5-HT content or in 5-HT transporter density. Furthermore, the combination of R-DOI and MDMA produced in the rat pups a similar or slightly higher hyperthermia than the combination of L-DOPA and MDMA. However, only the latter treatment was able to induce a lasting reduction in the density of 5-HT transporters and 5-HT content in the frontal cortex and in the hippocampus. According to our results, it appears that the hyper-

thermia induced by MDMA is not sufficient to produce the lasting neurotoxic effects on the serotonergic system at least in the 21-day-old rats used in these experiments.

In summary, our results appear to indicate that the lack of neurotoxicity to MDMA exposure at early postnatal ages is probably due to an insufficient concentration of dopamine in the rat brain, and argue for a certain threshold of dopamine release to observe a serotonergic deficit. It is consequently proposed that an already developed dopaminergic system is critical for the long-term expression of serotonergic neurotoxicity in rats by MDMA.

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