0022-3565/95/2751-0325$00.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1995 by The American Society for Pharmacology and Experimental Therapeutics
JPET 275:325-333, 1995

Vol. 275, No. 1
Printed in U.S.A.

Age-Dependent Sensitivity of Rats to the Long-term Effects of the Serotonergic Neurotoxicant (±)-3,4-Methylenedioxymethamphetamine (MDMA) Correlates with the Magnitude of the MDMA-Induced Thermal Response

HARRY W. BROENING, JOHN F. BOWYER and WILLIAM SLIKKER, JR.

Interdisciplinary Toxicology Program (H.W.B., J.F.B., W.S.), University of Arkansas for Medical Sciences, Little Rock, Arkansas and Division of Neurotoxicology (H.W.B., J.F.B., W.S.), Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas

Accepted for publication June 22, 1995

ABSTRACT

The effects of developmental age on (±)-3,4-methylenedioxymethamphetamine (MDMA)-induced reductions in 5-hydroxytryptamine (5-HT) content and 5-HT reuptake sites were investigated in conjunction with the effects of developmental age on MDMA-induced thermoregulatory responses. MDMA was administered to rats at postnatal days (PND) 10, 40 and 70 in a range of ambient temperature environments (10°C, 25°C and 33°C). Animals were monitored for alterations in body temperature and sacrificed 1 week after MDMA administration. MDMA administration at PND 10 did not result in persistent reductions in 5-HT content or 5-HT reuptake sites in frontal cortex, nor could a hyperthermic response be elicited. In contrast, MDMA administration at PND 40 and PND 70 resulted in a hypothermic response in cold environments (10°C) and a hyperthermic response in warm environments (≥25°C). When hypothermia was observed after MDMA (10°C environment), long-term reductions in 5-HT content and 5-HT reuptake sites were significantly attenuated or abolished. Conversely, when a hyperthermic response was observed (25°C and 33°C environments), long-term MDMA-induced reductions in 5-HT content and 5-HT reuptake sites were significantly enhanced. Thus, thermal responses significantly correlated with MDMA-induced reductions in 5-HT content and 5-HT reuptake sites. These experiments demonstrate a role for hyperthermia in the expression of serotonergic neurotoxicity after MDMA administration.

Animal housing conditions have long been associated with the lethality of AMPH administration (Chance, 1946). The effects of group *vs.* individual housing on the lethality of AMPH are further affected by the ambient temperature at which the animals are exposed (Askew, 1962; Chance, 1947; Craig and Kupferberg, 1972; Fink and Larson, 1962; Höhn and Lasagna, 1960). Increasing the ambient temperature at which group-housed animals were exposed to AMPH enhanced increases in body temperature and lethality (Askew, 1962; Craig and Kupferberg, 1972; Fink and Larson, 1962; Höhn and Lasagna, 1960). Conversely, lowering the ambient temperature at which AMPH was administered reduced lethality and attenuated AMPH-induced hyperthermia (Askew, 1962; Fink and Larson, 1962; Höhn and Lasagna, 1960). The increases in body temperature after AMPH administration at various ambient temperatures and housing conditions were predictive of lethality in the experimental animals (Askew, 1962; Craig and Kupferberg, 1972; Zalis *et al.*, 1967).

More recently, temperature has been demonstrated to play a role in the neurotoxicity observed after exposure to the AMPH derivatives METH and MDMA (Bowyer *et al.*, 1992, 1993, 1994; Miller and O'Callaghan, 1994; Schmidt *et al.*, 1990; Farfel and Seiden, 1995a, b). Alteration of the ambient temperature at which rats are exposed to these compounds markedly affects both the thermoregulatory effects and the neurotoxicity that is observed after drug administration. Reduction of ambient temperature (≤15°C) has been demonstrated to produce a hypothermic response to MDMA or METH exposure, while in warm environments (≥25°C) a hyperthermic response is observed (Bowyer *et al.*, 1992, 1994; Gordon *et al.*, 1991; Schmidt *et al.*, 1990). If the thermoregulatory response to METH or MDMA is hypothermia, persistent reductions in DA and/or 5-HT are significantly attenuated or are not observed. Conversely, if hyperthermia is elicited, neurotoxicity is enhanced (Bowyer *et al.*, 1994;

Received for publication December 12, 1994.

ABBREVIATIONS: MDMA, (±)-3,4-methylenedioxymethamphetamine; AMPH, amphetamine; METH, (+)-methamphetamine; 5-HT, 5-hydroxytryptamine, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; DA, dopamine; PND, postnatal day; HPLC, high-performance liquid chromatography; TTR, total thermal response.

Miller and O'Callaghan, 1994; Schmidt *et al.*, 1990; Farfel and Seiden, 1995a, b). In addition, for METH exposures, a significant correlation has been demonstrated between maximal body temperature and the magnitude of persistent reductions in DA content (Bowyer *et al.*, 1994).

Developing rodents have been demonstrated to be resistant to AMPH-induced lethality (Alhava, 1972, 1973; Alhava and Klinge, 1972). Neonatal mice exhibit little or no mortality with doses of AMPH that produce 100% lethality in adult mice (Alhava, 1972). In addition, hyperthermia is not observed after AMPH administration to neonatal mice (Alhava, 1973). The lack of a hyperthermic response has been postulated to be a factor contributing to the decreased sensitivity of developing rodents to the lethal effects of amphetamines (Alhava, 1973, 1976).

Developing rodents have also been demonstrated to be resistant to the neurotoxic effects of AMPH derivatives. Parachloroamphetamine, fenfluramine and METH do not produce long-term reductions in 5-HT and/or DA content when administered to neonatal rats (Clemens *et al.*, 1978; Clineschmidt *et al.*, 1978; Lucot *et al.*, 1981; Pu and Vorhees, 1993). We have recently demonstrated a similar phenomenon in that MDMA does not produce long-term reductions in 5-HT content or 5-HT reuptake sites after neonatal administration, although 5-HT content was acutely reduced (Broening *et al.*, 1994). Considering that hyperthermia is associated with AMPH-induced lethality in adult rats, that prevention of AMPH-induced hyperthermia in adult rats reduces lethality and attenuates neurotoxicity and that neonatal rats are resistant to AMPH neurotoxicity and lethality, we propose that (1) hyperthermia exacerbates MDMA neurotoxicity and (2) that the sensitivity of the immature rat to MDMA-induced hyperthermia develops concomitantly with the sensitivity of the immature rat to the long-term effects of MDMA on 5-HT content and 5-HT reuptake sites.

In the following studies, the dose-response relationships of MDMA-induced neurochemical alterations were evaluated in the developing rat from the neonate to the adult. The effects of developmental age on the long-term (1 week) biochemical effects induced by MDMA upon the central serotonergic neurotransmitter system were evaluated and contrasted between adult and neonatal rats with a focus on the relationships between ambient temperature, body temperature and the responsiveness of developing rats to MDMA-induced neurotoxicity.

Materials and Methods

Materials. The drugs and chemicals used in this study were kindly provided by or purchased from the sources indicated: MDMA·HCl from the National Institute for Drug Abuse (Rockville, MD), HPLC reference compounds from Sigma Chemical Co. (St. Louis, MO), HPLC mobile phase solvents from Fisher Scientific (Pittsburgh, PA), [^{3}H]paroxetine from Dupont NEN^{90} (Boston, MA). All other reagents used were purchased from a variety of commercial vendors and were of ACS analytical grade. Rectal temperatures were monitored with a G-08502–14 Thermistor Thermometer with a YSI 402 or a YSI 511 flexible vinyl coated temperature probe from Cole-Parmer (Niles, IL).

Animals and housing. Female Sprague-Dawley rats were mated in the National Center for Toxicological Research rodent colony (Jefferson, AR), provided food and water *ad libitum*, and housed individually in a 22 ± 1°C environment at 50% relative humidity with a 12 h light/dark cycle (lights on at 0600 hr, off at 1800 hr). At parturition, female pups were euthanized and male pups were randomly fostered between dams to achieve a little size of eight males. Pups were identified with foot tattoos and on PND 20, were weaned from their dams and housed two per cage. Animals were housed in 46 × 24 × 20 cm polycarbonate cages (approximately 925 cm^2 floor area) with sterilized chipped hardwood bedding and stainless steel wire covers. For the housing study, group-housed animals were maintained in a modified rat cage consisting of two standard polycarbonate rat cages with a 10 × 15 cm window removed from each cage to allow free access from one side to the other (approximately 1850 cm^2 of floor area).

Temperature measurement and hyperthermia rescue treatment. Temperatures were measured every hour beginning with the time that MDMA was administered up to 10 hr after treatment. The temperature probe was inserted approximately 5 to 8 cm into the rectum for measurements taken from PND 70 and PND 40 animals and approximately 1 cm into the rectum for the PND 10 animals. If the rectal temperature of an animal exceeded 41.5°C, the animal was placed in a clean, dry polycarbonate rat cage on a bed of crushed ice until rectal temperature was reduced to approximately 39–40°C or for between 5 and 10 min. This intervention has been used with success previously to reduce mortality associated with METH-induced hyperthermia (Bowyer *et al.*, 1994).

Calculation of total thermal response. Body temperature *vs.* time data was plotted for each animal for the 8 hr experimental period. The area between each temperature *vs.* time curve and a reference temperature of 37°C was calculated using the trapezoidal rule. This value was termed the TTR and is expressed as °C × hr. It represents the magnitude and duration of each animal's temperature response and is similar to the thermal response indices used by Clark and Cumby (1975) and Miller and colleagues (1991).

Ambient temperature studies. All dosages of MDMA (calculated to correspond to the free base) were dissolved in isotonic saline and administered p.o. Doses of 0, 20 or 40 mg/kg of MDMA were given at PND 10, 40 and 70 at three different ambient temperature conditions (10°C, 25°C and 33°C) except for the PND 70 rats (10°C, 25°C and saline only at 40°C). Rats were moved into the respective ambient temperature environments 30 min before MDMA administration. MDMA was administered between 08:00 hr and 10:00 hr. Rats dosed at PND 10 were kept together as a litter for the duration of the experiment. Rats dosed at PND 40 or PND 70 were housed individually before dosing with MDMA and remained individually housed until sacrificed. Rats were maintained in the ambient temperature environments for 8 hr after MDMA administration and then returned to their home vivarium. Six rats were used at each exposure age, ambient temperature and dose of MDMA combination.

Studies on housing condition. MDMA was administered to group-housed or individually housed rats at PND 40 in doses of 0, 20 or 40 mg/kg. Group-housed rats were litters composed of eight male rats that were maintained together through dosing and sacrifice. The ambient temperature during the experiments was 21 ± 1°C. Six rats were assigned to each MDMA and housing treatment combination for a total of 32 rats (N = 6/housing condition/dose of MDMA).

Biochemical assays. Animals were euthanized between 08:00 and 10:00 hr by decapitation 1 week after MDMA administration. Brains were removed immediately, cooled in sterile physiologic saline and dissected over ice into frontal cortex, hippocampus and striatum according to the method of Glowinski and Iversen (1966). These regions were immediately frozen on dry ice and stored at −70°C for subsequent biochemical analysis. 5-HT reuptake sites were assayed by a receptor binding method adapted from the procedure of Marcusson *et al.* (1988) with [^{3}H]paroxetine. Protein content was determined by the method of Lowry *et al.* (1951) with bovine serum albumin to generate a standard curve. Monoamine content was determined by HPLC with electrochemical detection. The methods used here were described in detail previously (Broening *et al.*, 1994).

Data analysis. The effects of ambient temperature, developmental age and MDMA on neurochemical parameters were analyzed utilizing a mixed model analysis of variance by the SAS® general linear models procedure. The effects of housing condition and MDMA on neurochemical parameters were analyzed utilizing a 3 × 2 factorial design. The analysis of body temperature data was performed using these models with the addition that rectal temperature was treated as a repeated measure. *A priori* tests for the effects of MDMA treatment were conducted by constructing linear contrasts (SAS/STAT® User's Guide, 1989). P values of less than .05 were accepted as statistically significant in the presence of statistical significance ($P < .05$) in the respective main effect or interaction.

Results

Ambient temperature, developmental age and MDMA-induced alterations in rectal temperature. MDMA had variable effects on rectal temperature when administered at PND 10. MDMA exposure at PND 10 in the 10°C environment initially increased rectal temperatures when compared to the saline controls, but at later time points MDMA-treated rats exhibited lower rectal temperatures than did the saline-treated animals (see fig. 1A). The results of the experiments conducted with PND 10 rats at 10°C were difficult to interpret because rectal temperatures of the treated and control rats at the time of MDMA administration were approximately 10°C lower than the rectal temperatures of their counterparts in the 25°C and 33°C environments (see fig. 1). MDMA administration to PND 10 rats at 25°C tended to reduce rectal temperature, but significant reductions were only noted late in the time course (see fig. 1B), and then only at a single time point in the high-dose animals. MDMA exhibited minimal effects on rectal temperature when administered at PND 10 in the 33°C environment (see fig. 1C). MDMA did not elicit a hyperthermic response in the PND 10 rat even when administered in the 33°C environment. No significant changes in TTR after MDMA administration were noted in the PND 10 rats (see table 1).

MDMA administration at PND 40 and PND 70 elicited a much more consistent response in rectal temperatures in the different ambient temperature environments than did treatment at PND 10. The alterations in rectal temperature after MDMA treatment in these older rats were affected by the ambient temperature at which MDMA was administered. The general trends on rectal temperatures observed at the PND 40 and PND 70 exposure ages were for MDMA to increase rectal temperatures in the 25°C and 33°C environments and to decrease rectal temperatures in the 10°C environment (see figs. 2 and 3). These changes in rectal temperature were reflected by significant alterations in TTR after MDMA administration at the different ambient temperatures (see table 1).

The hyperthermia elicited by MDMA administration to the PND 40 and PND 70 rats in the 25°C and 33°C environments was severe enough to elicit the use of the hyperthermia rescue treatment. This intervention was applied to animals dosed at PND 40 in the 25°C environment (2/6 receiving the 40 mg/kg dose) and the 33°C environment (2/6 receiving the 20 mg/kg dose and 4/6 receiving the 40 mg/kg dose). Rats dosed at PND 70 in the 25°C environment (1/6 receiving the 20 mg/kg dose and 4/6 receiving the 40 mg/kg dose) also received this intervention.

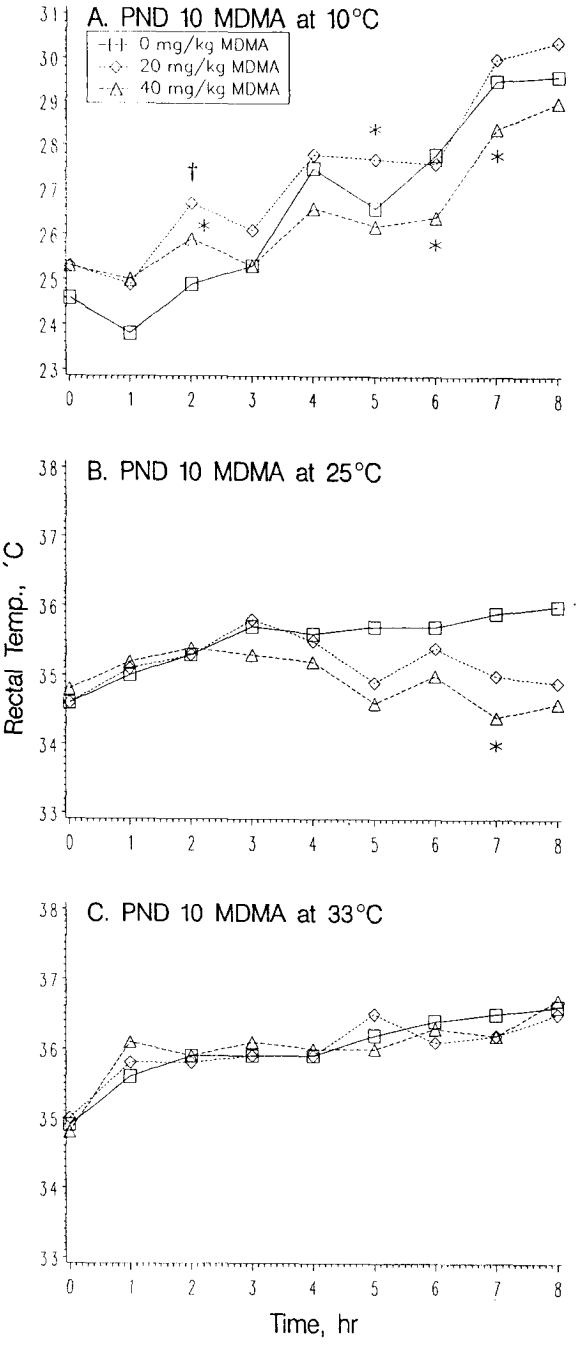

Fig. 1. The effects of MDMA administration on the rectal temperature of PND 10 rats. Rectal temperatures were monitored as described under "Materials and Methods." The solid line with open squares represents saline-treated rats, the short dashed line with open diamonds represents rats treated with 20 mg/kg MDMA and the long dashed line with open triangles represents rats treated with 40 mg/kg MDMA. The values presented are the means ($N = 6$) of rectal temperatures in °C. Error bars representing the S.E. have been omitted for the sake of clarity. *$P < .05$; †$P < .005$ *vs.* saline control.

Ambient temperature, developmental age and MDMA-induced 5-HT reductions. Concentrations of 5-HT were analyzed in frontal cortex, hippocampus and striatum 1 week after MDMA treatment. The effects of MDMA administration on 5-HT content were similar in each of the brain regions investigated. Only the data from frontal cortex will be presented here and references to the results from other

TABLE 1

TTR after MDMA administration

The values presented are means of the TTR recorded after MDMA exposure. Included is a group of PND 70 rats treated with saline at an ambient temperature of 40°C. The results are expressed in °C × hr ± S.E. $N = 6$ per cell except for PND 70 rats treated with saline at 40°C where $N = 5$.

Dosing Age (Days)	Room Temp. (°C)	Total Thermal Response (°C × hr) to MDMA (mg/kg)		
		0	20	40
10	10	−83.4 ± 3.6	−77.5 ± 2.1	−85.1 ± 2.7
10	25	−11.9 ± 0.5	−14.3 ± 0.8	−16.3 ± 1.0
10	33	−8.0 ± 0.6	−8.0 ± 0.8	−7.7 ± 0.8
40	10	4.9 ± 0.2	−0.5 ± 1.8[a]	−12.2 ± 4.9[b]
40	25	4.0 ± 0.9	14.5 ± 2.3[b]	18.5 ± 2.0[b]
40	33	7.0 ± 0.6	17.9 ± 2.6[b]	24.0 ± 2.3[b]
70	10	5.4 ± 1.0	−0.4 ± 1.2[a]	−6.5 ± 2.0[b]
70	25	7.5 ± 0.5	19.1 ± 1.8[b]	23.3 ± 1.9[b]
70	40	24.5 ± 1.4[c,d]		

[a] Significantly different from saline control, $P < .05$.
[b] Significantly different from saline control, $P < .001$.
[c] Significantly different from PND 70, 10°C saline control, $P < .001$.
[d] Significantly different from PND 70, 25°C saline control, $P < .001$.

brain regions will be made only where there was a difference in response to MDMA.

Concentrations of 5-HT were not affected by MDMA administration at PND 10. No alterations were observed in 5-HT concentrations in frontal cortex at 1 week after MDMA exposure at any of the three ambient temperatures investigated (see fig. 4A). In contrast, MDMA administration at PND 40 and PND 70 significantly reduced 5-HT content. In addition, MDMA-induced reductions in 5-HT content were significantly modulated by ambient temperature at these ages. The overall effect of ambient temperature on reductions in 5-HT content at PND 40 and PND 70 was to attenuate the effects of MDMA at cooler temperatures and exacerbate the effects of MDMA at warmer temperatures (see fig. 4).

MDMA exposure at PND 40 in the 10°C environment was without effect on 5-HT content in frontal cortex. 5-HT content was reduced only in hippocampus where 40 mg/kg decreased 5-HT concentrations by 17% (data not shown). This is in contrast to the effects of treatment at PND 40 in the 25°C environment where MDMA exposure resulted in significantly reduced 5-HT levels in all three brain regions. Increasing the ambient temperature to 33°C produced significantly greater reductions in 5-HT content in comparison to those observed after treatment at 25°C (see fig. 4B). 5-HT concentrations were reduced by more than 75% in frontal cortex after the administration of 40 mg/kg MDMA to PND 40 rats in the 33°C environment.

The effects of ambient temperature on MDMA-induced reductions in 5-HT content at PND 70 were similar in nature to the effects of ambient temperature on reductions in 5-HT content at PND 40 (see fig. 4C). The major differences between the two ages were that the 10°C environment did not offer the same protection against MDMA-induced reductions in 5-HT at PND 70 as it did at PND 40. In addition, PND 70 animals were more sensitive to the effects of MDMA exposure in the 25°C environment than were the PND 40 rats (see fig. 4).

Ambient temperature, developmental age and MDMA-induced reductions in 5-HT reuptake sites. The interaction between developmental age, ambient temperature and MDMA administration on alterations in 5-HT reuptake sites was similar to that observed with 5-HT concentrations. For MDMA administration at PND 10, no combination of MDMA or ambient temperature was observed to diminish 5-HT reuptake sites in frontal cortex (see fig. 5A). The general trend observed at PND 40 and PND 70 was that MDMA-induced reductions in 5-HT reuptake sites were enhanced as environmental temperatures were increased.

PND 40 MDMA exposure significantly reduced 5-HT reuptake sites in frontal cortex at all three environmental temperatures. 5-HT reuptake sites were reduced after

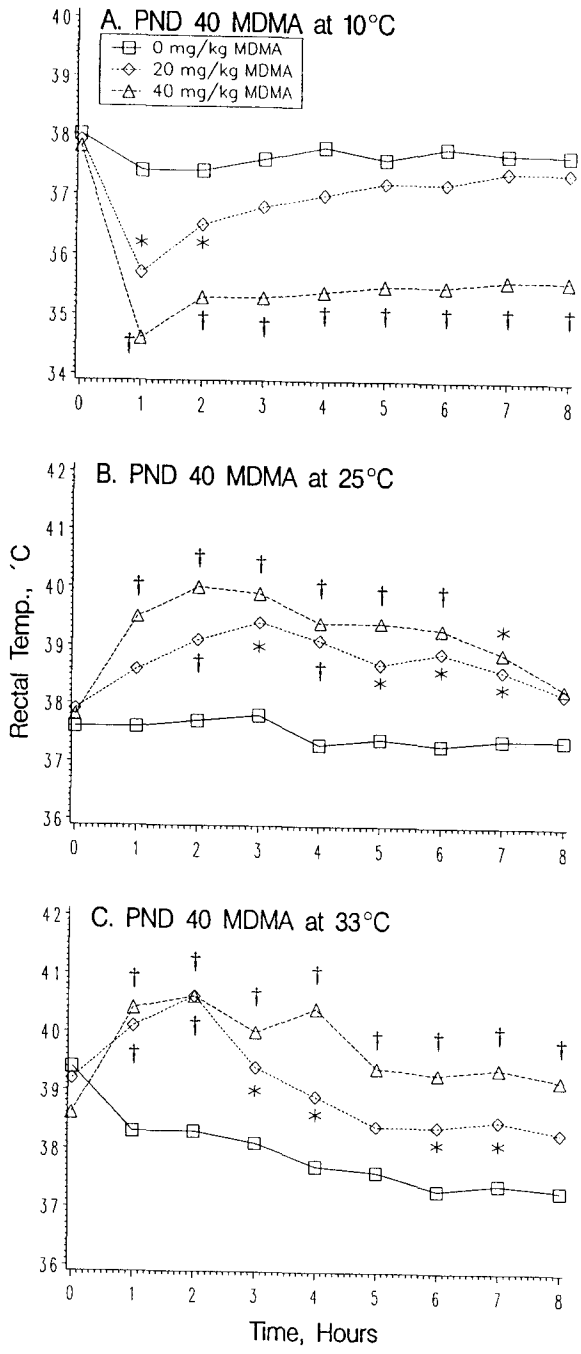

Fig. 2. The effects of MDMA administration on the rectal temperature of PND 40 rats. The lines and symbols represent the same treatment groups as those described in figure 1. The values presented are the means ($N = 6$) of rectal temperatures in °C. Error bars representing the S.E. have been omitted for the sake of clarity. *$P < .05$; †$P < .005$ *vs.* saline control.

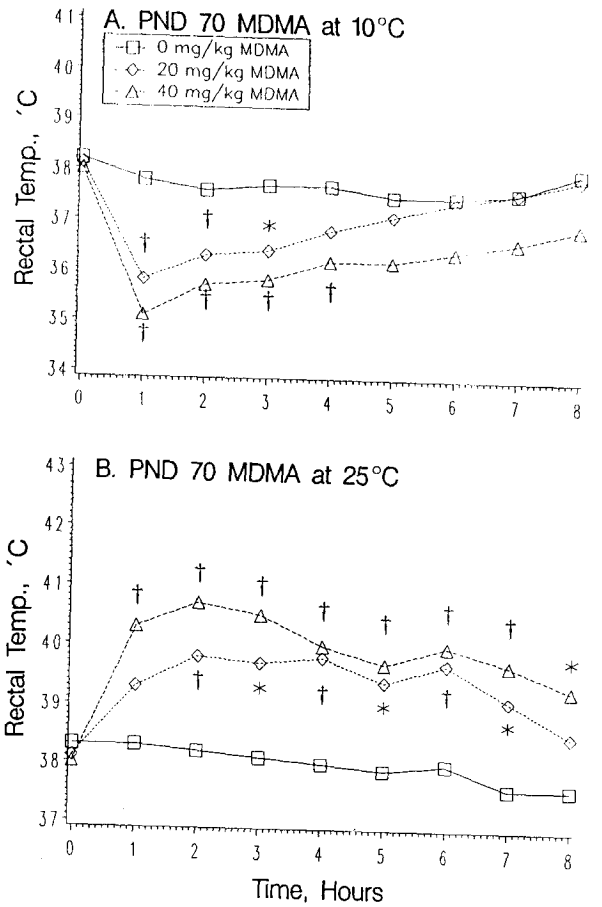

Fig. 3. The effects of MDMA administration on the rectal temperature of PND 70 rats. The lines and symbols represent the same treatment groups as those described in figure 1. The values presented are the means ($N = 6$) of rectal temperatures in °C. Error bars representing the S.E. have been omitted for the sake of clarity. *$P < .05$; †$P < .005$ *vs.* saline control.

MDMA exposure in the 10°C environment at PND 40 but only at the 40 mg/kg dose level (see fig. 5B). As was observed for 5-HT concentrations, increasing ambient temperature to 25°C and 33°C significantly enhanced MDMA-induced reductions in 5-HT reuptake sites in frontal cortex when compared with treatment in the 10°C environment. At PND 40, 40 mg/kg MDMA reduced 5-HT reuptake sites by only 21% in the 10°C environment, whereas in the 25°C and 33°C environments, 40 mg/kg MDMA reduced 5-HT reuptake sites by 47% and 73%, respectively.

MDMA administration to PND 70 rats in both the 10°C and the 25°C environments also diminished 5-HT reuptake sites in frontal cortex (see fig. 5C). Only the 40 mg/kg dose of MDMA significantly reduced 5-HT reuptake sites at PND 70 in the 10°C environment, but 5-HT reuptake sites were significantly diminished at both the 20 and the 40 mg/kg dose levels after administration in the 25°C environment. As was noted at PND 40, reductions in 5-HT reuptake sites after PND 70 MDMA exposure were significantly enhanced by the increase in ambient temperature from 10°C to 25°C.

MDMA administration was not observed to significantly alter the dissociation constants for [^{3}H]paroxetine binding to the 5-HT reuptake site at any combination of exposure age, ambient temperature and dose of MDMA (data not shown).

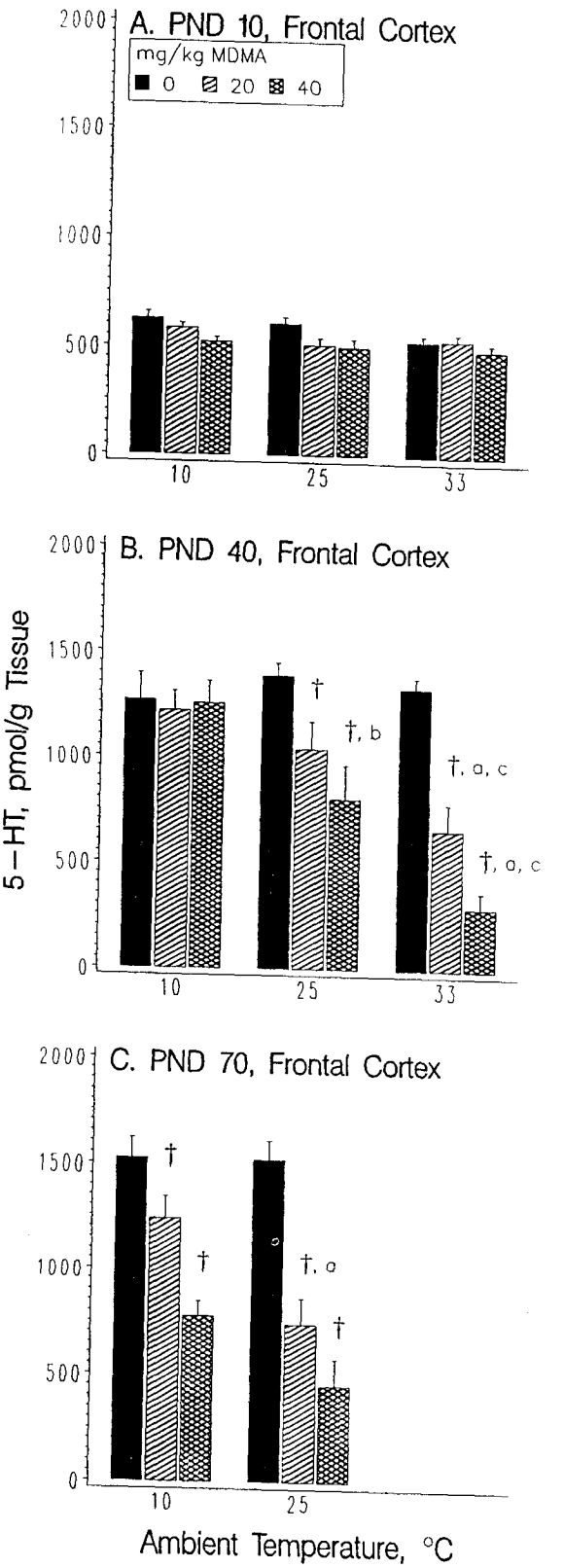

Fig. 4. The effects of ambient temperature on MDMA-induced reductions in 5-HT concentrations in frontal cortex. MDMA was administered at PND 10, PND 40 or PND 70 in doses of 0 (solid bar), 20 (right-hatched bar) or 40 mg/kg (cross-hatched bar) at the ambient temperatures indicated. Results are expressed in picomoles of 5-HT per gram of tissue and each bar represents the mean of six rats ± S.E. *$P < .05$; †$P < .005$ *vs.* saline control. A, $P < .01$ *vs.* 10°C value; B, $P < .05$ *vs.* 10°C value; C, $P < .05$ *vs.* 25°C value.

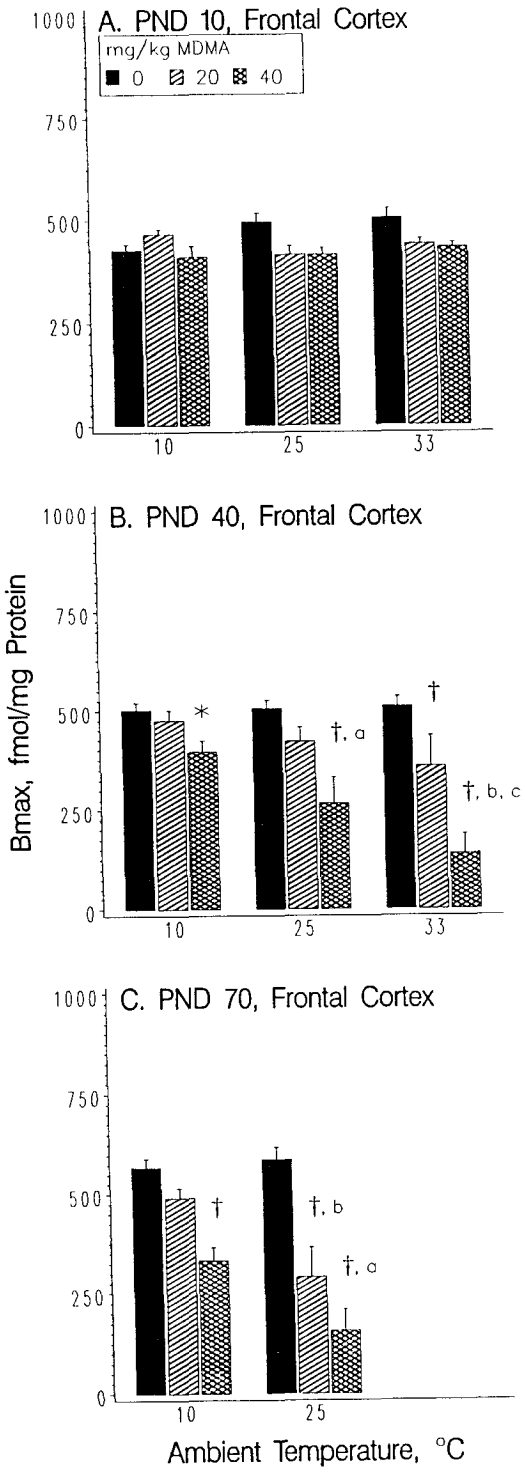

Fig. 5. The effects of ambient temperature on MDMA-induced reductions in 5-HT reuptake sites in frontal cortex. MDMA was administered at PND 10, PND 40 or PND 70 in doses of 0 (solid bar), 20 (right-hatched bar) or 40 mg/kg (cross-hatched bar) at the ambient temperatures indicated. Results are expressed in femtomoles per milligram of protein and each bar represents the mean of six rats ± S.E. *$P < .05$; †$P < .005$ *vs.* saline control. A, $P < .05$ *vs.* 10°C value; B, $P < .01$ *vs.* 10°C value; C, $P < .05$ *vs.* 25°C value.

Effects of hyperthermia on 5-HT content in saline-treated rats. To control for the effects of hyperthermia and the hyperthermia rescue treatment, saline was administered to PND 70 rats in a 40°C environment. Rats treated in this manner exhibited severe hyperthermia (rectal temperature ≥ 41.5°C) and were given the rescue treatment (4/6 receiving 1 ml/kg saline, one of these died). The TTR these hyperthermic control rats achieved was significantly greater than that observed in rats treated with saline at PND 70 in the 10°C and 25°C environments (see table 1). However, these hyperthermic control rats (1609 ± 66 pmol 5-HT/g tissue) exhibited no reductions in 5-HT content in frontal cortex in comparison to rats treated with saline at PND 70 in the 10°C (1524 ± 93 pmol 5-HT/g tissue) and 25°C (1520 ± 86 pmol 5-HT/g tissue) environments. Additionally, 5-HT content was not altered in the hippocampal or striatal regions of these hyperthermic control rats (data not shown). Individual values of 5-HT content in the frontal cortices of the hyperthermic control rats are plotted as a function of TTR in figure 6C (open stars).

Housing condition and MDMA-induced serotonergic alterations. MDMA administration (40 mg/kg) significantly increased TTR only in the individually housed rats (see table 2). However, MDMA administration (40 mg/kg) significantly reduced 5-HT content and 5-HT reuptake sites in frontal cortex in both the group-housed and the individually housed rats (see table 3). Striatal and hippocampal 5-HT content was also reduced in these animals by 40 mg/kg MDMA (data not shown). No significant differences were noted between the group-housed and the individually housed rats in the responses of 5-HT content, 5-HT reuptake sites or TTR to MDMA treatment.

Correlation between TTR and MDMA-induced serotonergic alterations. Figure 6 contains scatter plots of individual values of 5-HT content from frontal cortex plotted against TTR. A regression analysis was performed for each age group for the 0, 20 and 40 mg/kg doses of MDMA over the three ambient temperatures. R^2 values are provided in table 4 for each regression line to provide a measure of the total variance in 5-HT content that is accounted for by TTR after MDMA exposure. R^2 values are low for MDMA exposures at PND 10 indicating that temperature has little effect on variations in 5-HT content or 5-HT reuptake sites after MDMA treatment at this age. In contrast, R^2 values from rats treated with MDMA at PND 40 and PND 70 show that a significant fraction of the variance in 5-HT content can be correlated to TTR. A similar correlation was obtained for reductions in 5-HT reuptake sites (data not shown).

Discussion

These experiments demonstrate that developmental age modulates both the thermoregulatory responses and the neurotoxicity that results from MDMA administration. Furthermore, these experiments show that developmental ages at which a hyperthermic response could not be elicited after MDMA (PND 10) did not exhibit long-term reductions in 5-HT content or 5-HT reuptake sites. This suggests that the development of the thermoregulatory response to MDMA is linked to the age-related changes in neurotoxic sensitivity to this compound.

These experiments confirm the findings of others who have demonstrated that thermoregulatory phenomena can modulate MDMA neurotoxicity (Miller and O'Callaghan, 1994; Schmidt *et al.*, 1990; Farfel and Seiden, 1995a). Alterations in ambient temperature modified the ability of MDMA to

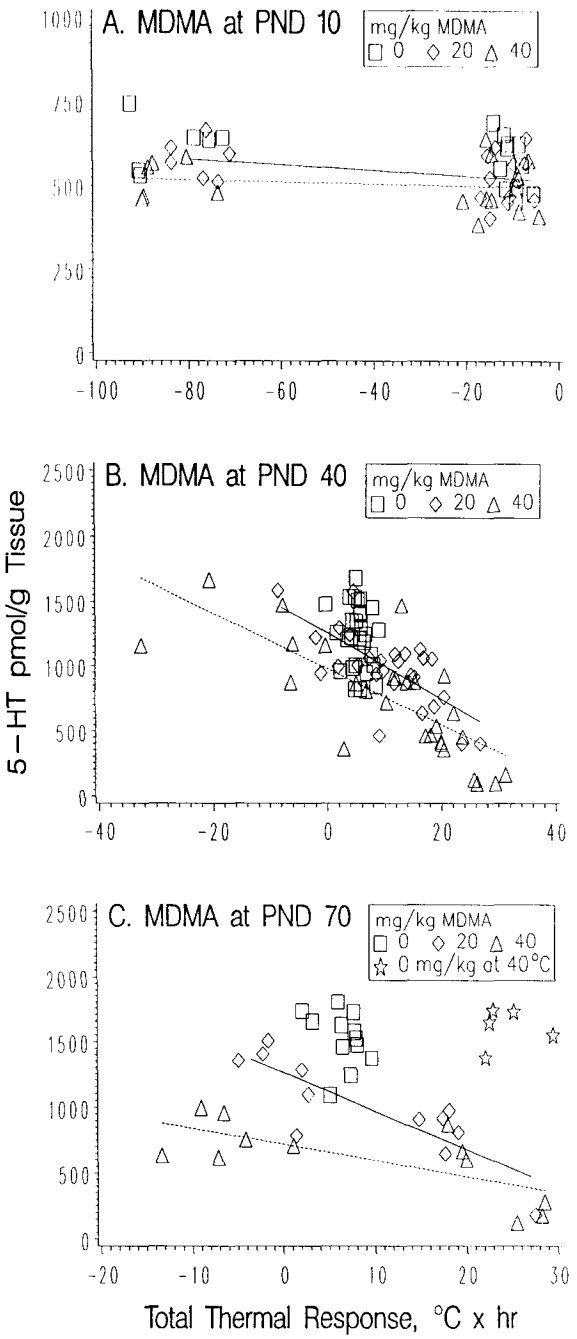

Fig. 6. The correlation between reductions in 5-HT content in frontal cortex and TTR after MDMA administration. Open squares represent saline-treated control rats, open diamonds represent rats administered 20 mg/kg MDMA and open triangles represent rats administered 40 mg/kg MDMA. The solid line represents a regression line for 20 mg/kg MDMA through the observations from each of the three ambient temperatures. The dashed line represents a regression line for 40 mg/kg MDMA through the observations from each of the three ambient temperatures. Rats from the housing study were included in the PND 40 plot. The open stars on the PND 70 plot represent rats treated with saline in a 40°C environment. A regression line for the saline-treated rats has been omitted for purposes of clarity. 5-HT content is expressed in picomoles per gram of tissue and TTR is expressed in °C × hr.

cause reductions in 5-HT content and 5-HT reuptake sites in older rats (≥PND 40) by altering the thermal response to MDMA. In general, a cool environment (10°C) attenuated MDMA-induced reductions in 5-HT content and 5-HT reuptake sites, whereas warm environments (≥25°C) enhanced reductions in these endpoints. A correlation was observed to exist between the extent of reductions in 5-HT content and 5-HT reuptake sites and the temperature response to MDMA in rats PND 40 and older (see fig. 6). These observations demonstrate a role for hyperthermia in MDMA neurotoxicity and show that thermal responses are predictive, in part, of the severity of reductions in 5-HT content and 5-HT reuptake sites after MDMA administration.

It is important to note that hyperthermia alone does not alter neurochemical indices of serotonergic function. Rats administered saline at PND 70 in a 40°C environment exhibited increases in TTR equivalent to those observed in PND 70 rats treated with 40 mg/kg MDMA in the 25°C environment (see table 1 and fig. 6). In spite of this, these rats exhibited no alterations in 5-HT content in comparison with the saline-treated PND 70 rats in either the 10°C or the 25°C environments. Thus, hyperthermia *per se* does not produce long-term

TABLE 2

The effects of housing condition on TTR after MDMA administration

The data presented in this table are means of the TTR observed after MDMA administration. TTR is expressed as °C × hr ± S.E. $N = 6$ animals per cell. GRP and IND refer to group-housed and individually housed animals, respectively.

Housing Condition	Total Thermal Response (°C × hr) to MDMA (mg/kg)		
	0	20	40
GRP	8.0 ± 0.9	12.1 ± 1.8	13.7 ± 1.9
IND	4.6 ± 1.0	8.4 ± 1.4	12.1 ± 3.5[a]

[a] $P < .05$ *versus* the individually housed saline control.

TABLE 3

Effects of housing condition on MDMA-induced reductions in 5-HT content and 5-HT reuptake sites in frontal cortex

5-HT content means are reported as picomoles per gram of tissue ± S.E. and 5-HT reuptake site (B_{max}) means are reported as femtomoles per milligram of protein ± S.E. $N = 6$ animals per cell. GRP and IND refer to group-housed and individually housed animals, respectively.

	Housing Condition	MDMA (mg/kg)		
		0	20	40
5-HT	GRP	1084 ± 81	983 ± 34	700 ± 87[a]
	IND	1067 ± 80	1059 ± 58	649 ± 144[a]
B_{max}	GRP	503 ± 29	494 ± 12	367 ± 44[b]
	IND	511 ± 18	498 ± 16	368 ± 79[b]

[a] Significantly different from saline control, $P < .01$.
[b] Significantly different from saline control, $P < .05$.

TABLE 4

Regression parameters for correlations between 5-HT content and TTR after MDMA administration

This table contains linear regression parameters for the correlations of TTR *versus* reductions in 5-HT content after MDMA administration. The graphical representations of these correlations are presented in figure 6. Correlations for the PND 40 group contain data from the housing experiments.

Dosing Age (Days)	MDMA (mg/kg)	Slope	R^2	P Value	N
10	0	−0.782 ± 0.518	.125	.150	18
	20	−0.840 ± 0.527	.137	.130	18
	40	−0.298 ± 0.492	.022	.554	18
40	0	−25.2 ± 18.7	.061	.190	30
	20	−25.5 ± 4.8	.500	.001	30
	40	−21.4 ± 3.6	.560	.001	30
70	0	−26.6 ± 29.6	.075	.390	12
	20	−29.1 ± 5.9	.710	.001	12
	40	−12.3 ± 4.0	.486	.012	12

alterations in biomarkers of serotonergic function. Instead, hyperthermia in combination with events occurring during the acute phase of MDMA intoxication facilitates serotonergic neurotoxicity.

The contribution of housing condition to MDMA neurotoxicity was examined in PND 40 rats primarily because PND 10 rats are group-housed with their dams when administered MDMA, whereas all other age groups were housed individually before dosing. In these experiments, housing condition was not observed to affect MDMA neurotoxicity (see table 2). The explanation for this may lie in the observation that there were no differences between the two housing conditions in their thermal responses to MDMA (see table 3). This is in contrast to studies on AMPH toxicity in which housing condition affected both temperature responses and lethality (Askew, 1962; Chance, 1946; Fink and Larson, 1962; Höhn and Lasagna, 1960).

The idea that the age-related sensitivity of rats to MDMA neurotoxicity is linked to the development of temperature regulation is intriguing. Hyperthermia clearly potentiates MDMA neurotoxicity at PND 40 or older. Thus, the development of the hyperthermic response to MDMA can play a significant role in neurotoxicity. However, because high doses of MDMA can produce neurotoxicity without inducing hyperthermia in adult rats, the maturation process results in an increased susceptibility that cannot be explained by changes in thermoregulation alone.

The development of temperature regulation is closely tied to the development of monoaminergic neurotransmitter systems. Both dopamine and serotonin are involved in thermoregulation in the adult rat (Jacob and Girault, 1979; Cox, 1979). Rats do not fully develop homeothermic capabilities until between PND 17 to PND 21 (Brody, 1943; Conklin and Heggeness, 1971). The dopaminergic and serotonergic neurotransmitter systems undergo synaptogenesis and terminal field innervation before this (Bruinink *et al.*, 1983; Herregodts *et al.*, 1990; Kirksey and Slotkin, 1979; Lidov and Molliver, 1982; Nomura *et al.*, 1976; Voorn *et al.*, 1988). The co-development of MDMA-induced hyperthermia with MDMA neurotoxic sensitivity may be related simply because both phenomena are dependent on a well-developed neurotransmitter system.

It was noted that MDMA administration to PND 70 rats in a cool environment attenuated but did not prevent reductions in 5-HT content. Similar observations were noted in 5-HT reuptake sites in that reductions in PND 40 and PND 70 rats treated with 40 mg/kg MDMA at 10°C were attenuated but not prevented. Thus, although administering MDMA in a cool environment prevented hyperthermia and significantly attenuated neurotoxicity, it did not completely protect 5-HT and 5-HT reuptake sites. The observation that induction of hypothermia did not fully protect against MDMA-induced reductions in 5-HT content and 5-HT reuptake sites may be interpreted in one of two ways. First, at least two mechanisms may exist whereby MDMA exposure can produce long-term alterations in serotonergic endpoints, one that functions in a temperature-dependent manner and one that functions independently of temperature. Additional evidence for this is obtained from the housing studies. Individually housed PND 40 rats treated with 40 mg/kg MDMA at 21°C exhibited 39% and 28% reductions in 5-HT content and 5-HT reuptake sites, respectively, despite the lack of a robust increase in TTR (see tables 2 and 3). Alternatively, it may be that higher doses of MDMA can overcome the protection afforded by a cool environment. MDMA is not as effective at reducing 5-HT content and 5-HT reuptake sites in the PND 40 rats at a given ambient temperature in comparison to the PND 70 rats. Thus, a cool environment may afford greater protection against MDMA-induced neurotoxicity at PND 40 *vs.* PND 70 simply because PND 40 rats are less sensitive to MDMA.

Hyperthermia may interact with MDMA to promote neurotoxicity in a variety of ways. 5-HT reuptake transporter function and the neurotransmitter release caused by AMPH or METH have been characterized as temperature dependent (Bowyer *et al.*, 1993; Fischer and Cho, 1979; Stauderman and Jones, 1985; Shaskan and Snyder, 1970). Interaction of MDMA with the 5-HT reuptake transporter and MDMA-induced release of serotonin have been implicated in MDMA neurotoxicity (Gough *et al.*, 1991; Rudnick and Wall, 1992; Schmidt, 1987, 1994). Thus, increases in body temperature after MDMA treatment may increase serotonin efflux resulting in enhanced neurotoxicity. Additionally, there is evidence suggesting that oxidative stress is involved in MDMA neurotoxicity (Stone *et al.*, 1989a, b). If so, then hyperthermia may simply accelerate the production of reactive oxygen species or free radicals potentiating MDMA neurotoxicity.

Two potential mechanisms through which MDMA may produce long-term changes in the serotonergic neurotransmitter system independently of alterations in body temperature are represented by gene regulation and metabolism. Recent findings suggest that MDMA exposure can alter biomarkers of serotonergic function at the molecular level. Both MDMA and fenfluramine can down-regulate messenger RNA for the serotonin reuptake transporter in the raphe nuclei in the rat (Rattray *et al.*, 1993, 1994). Alternatively, it has been proposed that MDMA can be metabolized to a reactive intermediate. The metabolism of MDMA has been extensively investigated and the chemical structure of MDMA lends itself to conversion to several compounds with structural and toxicological similarities to 6-hydroxydopamine and 5,7-dihydroxytryptamine (Hiramatsu *et al.*, 1990; Johnson *et al.*, 1992; Lim and Foltz, 1988, 1991).

In summary, the persistent effects of MDMA on the serotonergic neurotransmitter system in the rat are modulated by developmental age and ambient temperature. The development of sensitivity to MDMA neurotoxicity was observed to coincide with the development of the hyperthermic response to MDMA administration. In rats PND 40 and older, thermal responses to MDMA significantly correlated with subsequent reductions in 5-HT content and 5-HT reuptake sites. The observation that reductions in 5-HT content and 5-HT reuptake sites are noted in the absence of a hyperthermic response suggests that several mechanisms may be responsible for the persistent alterations in serotonergic endpoints observed after MDMA administration or alternatively, that large doses of MDMA can overcome the protective effect of cool environments.

References

ALHAVA, E.: Amphetamine toxicity in adult and developing mice. Acta Pharmacol. Toxicol. **31:** 387–400, 1972.

ALHAVA, E.: Modification by I-DOPA methylester (H 19/61) of amphetamine-induced brain catecholamine changes, thermal responses and toxicity in developing mice. Acta Pharmacol. Toxicol. **33:** 330–347, 1973.

Aghava, E.: Effects of catecholamine receptor blocking agents on toxicity and thermal responses induced by amphetamine isomers in adult and developing mice. Acta Pharmacol. Toxicol. **38:** 161–173, 1976.

Aghava, E. and Klinge, E.: Age and brain catecholamine content as factors influencing amphetamine toxicity in mice. Acta Pharmacol. Toxicol. **31:** 401–411, 1972.

Askew, B. M.: Hyperpyrexia as a contributory factor in the toxicity of amphetamine to aggregated mice. Br. J. Pharmacol. **19:** 245–257, 1962.

Bowyer, J. F., Davies, D. L., Schmued, L., Broening, H. W., Newport, G. D., Slikker, W., Jr. and Holson, R. R.: Further studies of the role of hyperthermia in methamphetamine neurotoxicity. J. Pharmacol. Exp. Ther. **268:** 1571–1580, 1994.

Bowyer, J. F., Gough, B., Slikker, W., Jr., Lipe, G. W., Newport, G. D. and Holson, R. R.: Effects of a cold environment on methamphetamine-induced dopamine release in the caudate putamen of female rats. Pharmacol. Biochem. Behav. **44:** 87–98, 1993.

Bowyer, J. F., Tank, A. W., Newport, G. D., Slikker, W., Jr., Ali, S. F. and Holson, R. R.: The influence of environmental temperature on the transient effects of methamphetamine on dopamine levels and dopamine release in rat striatum. J. Pharmacol. Exp. Ther. **260:** 817–824, 1992.

Brody, E. B.: Development of homeothermy in suckling rats. Am. J. Physiol. **139:** 230–232, 1943.

Broening, H. W., Bacon, L. and Slikker, W., Jr.: Age modulates the long-term but not the acute effects of the serotonergic neurotoxicant 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. **271:** 285–293, 1994.

Bruinink, A., Lichtensteiger, W. and Schlumpf, M.: Pre- and postnatal ontogeny and characterization of dopaminergic D_2, serotonergic S_2 and spirodecanone binding sites in rat forebrain. J. Neurochem. **40:** 1227–1236, 1983.

Chance, M. R. A.: Aggregation as a factor influencing the toxicity of sympathomimetic amines in mice. J. Pharmacol. Exp. Ther. **87:** 214–219, 1946.

Chance, M. R. A.: Factors influencing the toxicity of sympathomimetic amines to solitary mice. J. Pharmacol. Exp. Ther. **89:** 289–296, 1947.

Clark, W. G. and Cumby, H. R.: The antipyretic effect of indomethacin. J. Physiol. **248:** 625–638, 1975.

Clemens, J. A., Fuller, R. W., Perry, K. W. and Sawyer, B. D.: Effects of *p*-chloroamphetamine on brain serotonin in immature rats. Commun. Psychopharmacol. **2:** 11–16, 1978.

Clineschmidt, B. V., Zacchei, A. G., Totaro, J. A., Pflueger, A. B., McGuffin, J. C. and Wishousky, T. I.: Fenfluramine and brain serotonin. Ann. N.Y. Acad. Sci. **305:** 222–241, 1978.

Conklin, P. and Heggeness, F. W.: Maturation of temperature homeostasis in the rat. Am. J. Physiol. **220:** 333–336, 1971.

Cox, B.: Dopamine. *In* Body Temperature: Regulation, Drug Effects, and Therapeutic Implications, ed. by P. Lomax and E. Schonbaum, pp. 231–255, Marcel Dekker, Inc., New York, 1979.

Craig, A. L. and Kupferberg, H. J.: Hyperthermia in *d*-amphetamine toxicity in aggregated mice of different strains. J. Pharmacol. Exp. Ther. **180:** 616–624, 1972.

Farfel, G. M. and Seiden, L. S.: Role of hypothermia in the mechanism of protection against serotonergic toxicity. 1. Experiments using 3,4-methylenedioxymethamphetamine, dizocilpine, CGS 19755 and NBQX. J. Pharmacol. Exp. Ther. **272:** 860–867, 1995a.

Farfel, G. M. and Seiden, L. S.: Role of hypothermia in the mechanism of protection against serotonergic toxicity. 2. Experiments with methamphetamine, *p*-chloroamphetamine, fenfluramine, dizocilpine and dextromethorphan. J. Pharmacol. Exp. Ther. **272:** 868–875, 1995b.

Fink, G. B. and Larson, R. E.: Some determinates of amphetamine toxicity in aggregated mice. J. Pharmacol. Exp. Ther. **137:** 361–364, 1962.

Fischer, J. F. and Cho, A. K.: Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. J. Pharmacol. Exp. Ther. **208:** 203–209, 1979.

Glowinski, J. and Iversen, L. L.: Regional studies of catecholamines in the rat brain. 1. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J. Neurochem. **13:** 655–669, 1966.

Gordon, C. J., Watkinson, W. P., O'Callaghan, J. P. and Miller, D. B.: Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. Pharmacol. Biochem. Behav. **38:** 339–344, 1991.

Gough, B., Ali, S. F., Slikker, W., Jr. and Holson, R. R.: Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. Pharmacol. Biochem. Behav. **39:** 619–623, 1991.

Herregodts, P., Velkeniers, B., Ebinger, G., Michiotte, Y., Vanhaelst, L. and Hooghe-Peters, E.: Development of monoamine neurotransmitters in fetal and postnatal rat brain: analysis by HPLC with electrochemical detection. J. Neurochem. **55:** 774–779, 1990.

Hiramatsu, M., Kumagai, Y., Unger, S. E. and Cho, A. K.: Metabolism of methylenedioxymethamphetamine: formation of dihydroxymethamphetamine and a quinone identified as its glutathione adduct. J. Pharmacol. Exp. Ther. **254:** 521–527, 1990.

Höhn, R. and Lasagna, L.: Effects of aggregation and temperature on amphetamine toxicity in mice. Psychopharmacologia **1:** 210–220, 1960.

Jacob, J. J. and Girault, J.-M. T.: 5-Hydroxytryptamine. *In* Body Temperature: Regulation, Drug Effects, and Therapeutic Implications, ed. by P. Lomax and E. Schonbaum, pp. 183–230, Marcel Dekker, Inc., New York, 1979.

Johnson, M., Elayan, I., Hanson, G. R., Foltz, R. L., Gibb, J. W. and Lim, H. K.: Effects of 3,4-dihydroxymethamphetamine and 2,4,5-trihydroxymethamphetamine, two metabolites of 3,4-methylenedioxymethamphetamine, on central serotonergic and dopaminergic systems. J. Pharmacol. Exp. Ther. **261:** 447–453, 1992.

Kirksey, D. F. and Slotkin, T. A.: Concomitant development of [^{3}H]-dopamine and [^{3}H]-5-hydroxytryptamine uptake systems in rat brain regions. Br. J. Pharmacol. **67:** 387–391, 1979.

Lidov, H. G. W., and Molliver, M. E.: An immunohistochemical study of serotonin neuron development in the rat: Ascending pathways and terminal fields. Brain Res. Bull. **8:** 389–430, 1982.

Lim, H. K. and Foltz, R. L.: *In vivo* and *in vitro* metabolism of 3,4-(methylenedioxy)methamphetamine in the rat: identification of metabolites using an ion trap detector. Chem. Res. Toxicol. **1:** 370–378, 1988.

Lim, H. K. and Foltz, R. L.: Ion trap tandem mass spectrometric evidence for the metabolism of 3,4-(methylenedioxy)methamphetamine to the potent neurotoxins 2,4,5-trihydroxymethamphetamine and 2,4,5-trihydroxyamphetamine. Chem. Res. Toxicol. **4:** 626–632, 1991.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

Lucot, J. B., Horwitz, J. and Seiden, L. S.: The effects of *p*-chloroamphetamine administration on locomotor activity and serotonin in neonatal and adult rats. J. Pharmacol. Exp. Ther. **217:** 738–744, 1981.

Marcusson, J. O., Bergström, M., Eriksson, K. and Ross, S. B.: Characterization of [^{3}H]paroxetine binding in rat brain. J. Neurochem. **50:** 1783–1790, 1988.

Miller, D. B. and O'Callaghan, J. P.: Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. J. Pharmacol. Exp. Ther. **270:** 752–760, 1994.

Miller, E. K., Raese, J. D. and Morrison-Bogorad, M.: Expression of heat shock protein 70 and heat shock cognate 70 messenger RNA's in rat cortex and cerebellum after heat shock or amphetamine treatment. J. Neurochem. **56:** 2060–2071, 1991.

Nomura, Y., Naitoh, F. and Segawa, T.: Regional changes in monoamine content and uptake of the rat brain during postnatal development. Brain Res. **101:** 305–315, 1976.

Pu, C. and Vorhees, C. V.: Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocyte reaction in the rat striatum. Dev. Brain Res. **72:** 325–328, 1993.

Rattray, M., Wotherspoon, G., Savery, D., Marden, C., Lopez-Costa, J. J., Bendotti, C. and Priestley, J. V.: Fenfluramine and MDMA down-regulate serotonin transporter messenger RNA in subdivisions of the rat dorsal raphe complex (Abstract). Soc. Neurosci. Abs. **19:** 206.6, 1993.

Rattray, M., Wotherspoon, G., Savery, D., Baldessari, S., Marden, C., Priestley, J. V. and Bendotti, C.: Chronic D-fenfluramine decreases serotonin transporter messenger RNA expression in dorsal raphe nucleus. Eur. J. Pharmacol. Mol. Pharmacol. **268:** 439–442, 1994.

Rudnick, G. and Wall, S. C.: The molecular mechanism of "ecstasy" [3,4-methylenedioxymethamphetamine (MDMA)]: Serotonin transporters are targets for MDMA-induced serotonin release. Proc. Natl. Acad. Sci. U.S.A. **89:** 1817–1821, 1992.

SAS/STAT User's Guide, Version 6, SAS Institute Inc. Cary, NC, 1989.

Schmidt, C. J.: Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. **240:** 1–7, 1987.

Schmidt, C. J.: Neurochemistry of ring-substituted amphetamine analogs. *In* Amphetamine and Its Analogs: Pharmacology, Toxicology and Abuse, ed. by A. K. Cho and D. S. Segal, pp. 151–175, Academic Press, San Diego, 1994.

Schmidt, C. J., Black, C. K., Abbate, G. M. and Taylor, V. L.: Methylenedioxymethamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT_2 receptors. Brain Res. **529:** 85–90, 1990.

Shaskan, E. G. and Snyder, S. H.: Kinetics of serotonin accumulation into slices from rat brain: relationship to catecholamine uptake. J. Pharmacol. Exp. Ther. **175:** 404–418, 1970.

Stauderman, K. A. and Jones, D. J.: Characterization of sodium-dependent, high-affinity serotonin uptake in rat spinal cord synaptosomes. Brain Res. **330:** 11–20, 1985.

Stone, D. M., Hanson, G. R. and Gibb, J. W.: *In vitro* reactivation of rat cortical tryptophan hydroxylase following *in vivo* inactivation by methylenedioxymethamphetamine. J. Neurochem. **53:** 572–581, 1989a.

Stone, D. M., Johnson, M., Hanson, G. R. and Gibb, J. W.: Acute inactivation of tryptophan hydroxylase by amphetamine analogs involves the oxidation of sulfhydryl sites. Eur. J. Pharmacol. Mol. Pharmacol. **172:** 93–97, 1989b.

Voorn, P., Kalsbeek, A., Jorritsma-Byham, B. and Groenewegen, H. J.: The pre- and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat. Neuroscience **25:** 857–887, 1988.

Zalis, E. G., Lundberg, G. D. and Knutson, R. A.: The pathophysiology of acute amphetamine poisoning with pathologic correlation. J. Pharmacol. Exp. Ther. **158:** 115–127, 1967.

Send reprint requests to: William Slikker, Jr., Division of Neurotoxicology, HFT-132, National Center for Toxicological Research, 3900NCTR Road, Jefferson, Arkansas 72079-3039.