

Co-administration of MDMA with Drugs That Protect Against MDMA Neurotoxicity Produces Different Effects on Body Temperature in the Rat¹

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ABSTRACT

The substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA) has been shown to be neurotoxic to serotonin (5HT) terminals in the rat, and rat body temperature (TEMP) has been shown to affect this neurotoxicity. This study looked at the effect on CORE TEMP of three drugs that protect against MDMA neurotoxicity in the rat. Male Holtzman rats were injected with a control saline (SAL) injection or with ketanserin (KET; 6 mg/kg), α -methyl-p-tyrosine (AMPT; 75 mg/kg) or fluoxetine (FLUOX; 10 mg/kg) before a 40-mg/kg MDMA or SAL injection. CORE TEMP was recorded throughout the study using a noninvasive peritoneally implanted temperature probe. Rats pretreated with KET had no change in CORE TEMP until MDMA was injected, at which time an immediate hypothermia was seen that continued for 180 minutes, with a peak low of 34.7°C. Rats treated with AMPT had no change in CORE TEMP until the MDMA was injected, at which time an immediate hypothermia was seen that continued for 240 min., with a peak low of 34.3°C. Two weeks later, brain regions were analyzed for 5-HT and 5-hydroxyindole acetic acid levels. MDMA produced

significant ($P < .05$) decreases in 5-HT and 5-hydroxyindole acetic acid levels in the frontal cortex, somatosensory cortex, striatum and hippocampus, and pretreatment with KET or AMPT prevented these depletions. When rats were given the KET/MDMA or AMPT/MDMA drug injections and warmed to prevent hypothermia, the protection against neurotoxicity was removed, which indicated that the hypothermia mediated the protective effects of KET and AMPT. In comparison with the hypothermia seen with AMPT or KET pretreatment, pretreatment with FLUOX had no effect on CORE TEMP. The rats given the FLUOX/MDMA treatment did not have different CORE TEMPs than rats given SAL/MDMA. The FLUOX pretreatment protected against MDMA-induced 5-HT and 5-hydroxyindole acetic acid depletions in the frontal cortex, somatosensory cortex, striatum and hippocampus. This study suggests that a decrease in CORE TEMP may be a mechanism of protection against MDMA neurotoxicity by some drugs but that there is also a mechanism of protection that is independent of a change in body temperature.

The amphetamine analog MDMA and other amphetamine analogs have been shown to cause neurotoxicity to 5HT nerve terminals (Commins *et al.*, 1987b; Schmidt, 1987). This MDMA neurotoxicity has been demonstrated by meeting a number of criteria. MDMA produces a decrease in 5HT and 5HIAA levels in a number of species, including rats (Battaglia *et al.*, 1988; Schmidt *et al.*, 1986), mice (O'Callaghan and Miller, 1994; Stone *et al.*, 1987), guinea pigs (Battaglia *et al.*, 1988) and monkeys (Insel *et al.*, 1989; Ricaurte *et al.*, 1992) and produces a decrease in tryptophan hydroxylase (Schmidt and Taylor, 1987; Stone *et al.*, 1988). MDMA also causes a decrease in 5HT uptake sites as measured by a decrease in

labeled paroxetine specific binding to the 5HT transporter (Battaglia *et al.*, 1988; Scheffel and Ricaurte, 1990) and a decrease in uptake as measured by synaptosomal uptake of [3H]5HT (Commins *et al.*, 1987b). Finally, there are morphological signs of degeneration as seen using Fink-Heimer staining (Commins *et al.*, 1987b), loss of axons as visualized by immunocytochemistry (O'Hearn *et al.*, 1988; Scanzello *et al.*, 1993) and an increase in silver degeneration staining and GFAP staining in the rat (Bowyer *et al.*, 1994). MDMA-induced long-term decreases in 5HT levels and 5HT uptake sites (at 12 months) and morphological changes in 5HT axons (12-18 months) have also recently been reported (Fischer *et al.*, 1995; Lew *et al.*, 1996; Sabol *et al.*, 1996). These effects individually would not be enough to indicate 5HT terminal destruction by MDMA, but when taken together, they suggest that MDMA is neurotoxic to 5HT nerve terminals (Seiden *et al.*, 1988). Recently, human studies have shown that

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ABBREVIATIONS: MDMA, methylenedioxymethamphetamine; METH, methamphetamine; KET, ketanserin; AMPT, α -methyl-p-tyrosine; FLUOX, fluoxetine; DA, dopamine; 5HT, serotonin; 5HIAA, 5-hydroxyindole acetic acid; CORE TEMP, core body temperature; SAL, saline.

recreational MDMA users experienced a decrease in 5HIAA (Ricaurte *et al.*, 1990) as measured in cerebrospinal fluid. When combined with the data from other species, these results indicate that MDMA may also be neurotoxic to humans.

In the past 10 years MDMA abuse has been increasing in the United States and Europe (Peroutka, 1987). MDMA is often taken for a euphorogenic and positive emotional feeling (Eisner, 1989). Formerly, it was often used in a therapeutic setting as an adjunct to psychotherapy (Shulgin, 1986), but in 1985 it was classified as a Schedule I drug (Steele *et al.*, 1994). It is currently a popular illegal recreational drug on college campuses (Cuomo *et al.*, 1994) and at dance parties known as "raves" (Randall, 1992; Steele *et al.*, 1994). Recently, a number of deaths due to MDMA overdoses have been reported (Dowling *et al.*, 1987; Henry, 1992). The growing use of this drug, the reports of fatalities and the awareness of MDMA as a neurotoxin have led a number of investigators to try to elucidate the mechanism of serotonergic neurotoxicity by MDMA, along with mechanisms to protect against MDMA and amphetamine-induced neurotoxicity.

Many substances have been found to protect against MDMA-induced neurotoxicity in the rat. These include NMDA antagonists such as dizocilpine (Farfel and Seiden, 1995a), DA uptake blockers (Stone *et al.*, 1988), DA antagonists (Bowyer *et al.*, 1994), AMPT (Axt and Seiden, 1990; Stone *et al.*, 1988), reserpine (Stone *et al.*, 1988), 5HT uptake inhibitors (Schmidt *et al.*, 1987) and 5HT2 antagonists (Johnson *et al.*, 1993; Schmidt *et al.*, 1994; Schmidt *et al.*, 1991). The fact that such a wide variety of drugs protect against neurotoxicity suggest that a number of systems are responsible for the protection against MDMA neurotoxicity, and it would follow that a number of systems contribute to the neurotoxic effect of MDMA as well. Indeed, Schmidt *et al.* (1992) have found evidence of 5HT/DA system interaction in MDMA and METH neurotoxicity.

Despite the variability in the types of drugs that protect against neurotoxicity, a common effect that has been reported is that many of these drugs cause a decrease in CORE TEMP when the drug is administered with METH or MDMA (Bowyer *et al.*, 1994; Farfel and Seiden, 1995a; Farfel and Seiden, 1995b; Miller and O'Callaghan, 1994). Farfel and Seiden (1995a) looked at the effect of dizocilpine, a drug shown to protect against METH neurotoxicity (Sonsalla *et al.*, 1989), on the CORE TEMP of the rat. When administered with METH or MDMA, dizocilpine caused a hypothermia in the rat and also protected against MDMA-induced neurotoxicity. When the rats were warmed to prevent hypothermia, there was no longer any protection against the neurotoxicity, which suggests that the drug-induced hypothermia plays a role in the protection. Bowyer *et al.* (1994) reported that when the protective agents haloperidol, diazepam and dizocilpine were administered with METH, a hypothermia in the rat and protection against neurotoxicity were seen. Both Bowyer *et al.* (1994) and Farfel and Seiden (1995b) have reported that the amount of hypothermia induced by the protective agent was positively correlated with the amount of protection against neurotoxicity, which indicates that the hypothermia induced by these drugs may be involved in protection against MDMA-induced neurotoxicity.

In addition to pharmacologically induced changes in temperature, a change in environmental temperature can affect the CORE TEMP of the rats as well as protect or exacerbate

neurotoxicity. Administration of MDMA at the low environmental temperature of 10°C decreased the animals' CORE TEMP by 2°C (Gordon *et al.*, 1991), and administration of MDMA at 15°C decreased the amount of neurotoxicity seen (Miller and O'Callaghan, 1994). Administration of METH at 4°C also reduced the effect of METH on striatal DA levels and striatal tyrosine hydroxylase activity (Ali *et al.*, 1994). Conversely, administration of MDMA at a high environmental temperature (30°C) increased the animals' body temperature by 3.2°C (Gordon *et al.*, 1991), and administration of METH at high environmental temperatures (26.5°C) exacerbates the amount of neurotoxicity as measured by a decrease in DA levels. Administration of METH at high environmental temperature also increases the animals' CORE TEMP to the point of lethality (Bowyer *et al.*, 1994). This increase or decrease in CORE TEMP as determined by environmental temperature indicates that after MDMA or METH administration, the rats thermoregulatory mechanisms may be compromised (Dafters, 1994; Gordon *et al.*, 1991). The absence of thermoregulatory ability may contribute to the amount of neurotoxicity seen when the animal is warmed and its CORE TEMP increases.

When overdose of MDMA or death due to MDMA ingestion is seen in humans, hyperthermia is a common symptom; CORE TEMPs up to 43°C have been reported (Henry, 1992; Randall, 1992). To treat MDMA overdoses, hypothermic intervention is often used *via* dantrolene, a drug that decreases CORE TEMP (Singarajah and Lavies, 1992; Watson *et al.*, 1993), and administration of cold i.v. fluids to lower the CORE TEMP of the patient who has overdosed on MDMA.

Clearly, temperature plays a role in the induction of MDMA neurotoxicity and protection against MDMA neurotoxicity. This study further investigates the interaction between CORE TEMP and neurotoxicity. We examined three drugs that are known to protect against MDMA neurotoxicity: KET, a 5HT2 receptor antagonist, AMPT, a competitive tyrosine hydroxylase inhibitor, and FLUOX, a specific 5HT uptake inhibitor, to look at their effect on CORE TEMP when administered with MDMA. We measured CORE TEMP using a biotelemetry system that provides a noninvasive CORE TEMP measurement. Preliminary experiments have determined that co-administration of KET or AMPT with MDMA produces hypothermia in the rat, whereas co-administration of FLUOX and MDMA has no effect on body temperature (Malberg *et al.*, 1994). In this study, we sought to replicate that result and determine the effect of the reversal of the hypothermia on MDMA-induced neurotoxicity. Throughout this paper, we define hyperthermia as a CORE TEMP significantly above that of the SAL/SAL group at matched times and hypothermia as a CORE TEMP significantly below that of the SAL/SAL group at matched times.

We report that pretreatment with AMPT or KET protects against MDMA-induced decreases in 5HT and 5HIAA levels, which we take to be indicative of neurotoxicity. AMPT and KET also produce a decrease in CORE TEMP (hypothermia) in the rat, when administered with an injection of MDMA. When the rats are warmed and the hypothermia is prevented, then the protection against the neurotoxicity is abolished, which indicates that the hypothermia plays a role in the protective effects of these two drugs. In contrast, pretreatment with FLUOX protects against MDMA neurotoxicity but has no effect on CORE TEMP. This study suggests

that a decrease in CORE TEMP may be a mechanism of protection in some drugs but that there is also a mechanism of protection that is independent of change in body temperature.

Materials and Methods

Animals. Male Holtzman rats weighing 250 to 300 g (Harlan, IN) were housed individually in hanging wire cages. Throughout the experiment, rats had access to food (Teklab Diet) and water *ad libitum*. Rats were maintained on a 12:12 light/dark cycle with a room temperature of 22°C to 24°C, except where indicated in "Procedure." Housing and experimental treatment of the rats were in accordance with NIH guidelines.

Materials. MDMA HCl was obtained from the National Institute on Drug Abuse; ketanserin tartrate was obtained from Janssen Life Science Products (Beerse, Belgium); AMPT methyl ester HCl was purchased from Sigma Chemical Co. (St. Louis, MO); FLUOX HCl was a gift from Eli Lilly. Ketamine and Nembutal were obtained from Abbott Laboratories (Chicago, IL).

Surgery. For rat CORE TEMP measurements, temperature-sensitive radio transmitters (Minimitter Co., Sunriver, OR) were implanted in the peritoneum of the rat. Rats were anesthetized with Nembutal (40 mg/kg) and given supplemental 1-ml injections of ketamine as needed. A midline cut was made into the peritoneum, and a sterilized transmitter was inserted into the peritoneal cavity. Rats were given a minimum of 3 days to recover from the surgeries.

Temperature measurement apparatus. The temperature-sensitive radio transmitters send out an AM radio frequency signal proportional to the rat CORE TEMP. The signal is detected by a hand-held receiver and is converted to a temperature in Celsius. This apparatus enables us to obtain CORE TEMP without handling the rat after the drug injection. Gordon (1990) reports that taking colonic temperature measurements can increase the metabolic heat production and CORE TEMP. This noninvasive method of obtaining CORE TEMP subjects the rats to less stress and gives reliable CORE TEMP measurements.

Procedure. Three separate experiments were performed. The first two experiments had five treatment groups ($n = 8$): 1) two SAL injections (SAL/SAL), 2) SAL injection followed by an MDMA injection (SAL/MDMA), 3) protective agent followed by a SAL injection (protectant/SAL), 4) protective agent followed by an MDMA injection (protectant/MDMA) and 5) protective agent followed by an MDMA injection followed by warming in order to raise the CORE TEMP (protectant/MDMA/HEAT). The third experiment consisted of only four treatment groups (SAL/SAL, SAL/MDMA, protectant/SAL and protectant/MDMA) and included no warming of the rats.

After recovery from the temperature implant surgery, base-line CORE TEMP measurements were made for all rats at 900, 1200, 1500, 1800 and 2100 h. On the next day (INJECTION DAY), one base-line CORE TEMP measurement was taken before the start of the drug injections, and then CORE TEMP was measured once an hour until the MDMA injection. After the MDMA injection, CORE TEMP was recorded every 15 min for 4 h; in experiments 1 and 2, the rats were also warmed during the 4 h. For the rest of the experiment, CORE TEMP was measured once an hour for at least another 4 h. The day after the injections, CORE TEMP was recorded in all rats at 900, 1200, 1500, 1800 and 2100 h.

In experiment 1, the protective agent KET (6 mg/ml, 1 ml/kg i.p.; dissolved in distilled deionized water) or SAL (1 ml/kg) was injected 60 min before an MDMA [40 mg/kg (free base), 1 ml/kg s.c. dissolved in SAL] or SAL injection. This dose of KET was selected for its ability to produce behavioral effects (Marek *et al.*, 1989). Furthermore, a lower dose of KET (3 mg/kg) has been shown to protect against MDMA-induced neurotoxicity (10 mg/kg) (Nash *et al.*, 1988), but because a larger dose of MDMA (40 mg/kg) was used in this experiment, we selected the dose of 6 mg/kg KET. In experiment 2, the

protective agent AMPT (75 mg/ml, 1 ml/kg i.p., dissolved in SAL) or SAL was injected 5 and 1 h before an MDMA or SAL injection. In experiment 3, the protective agent FLUOX (10 mg/kg, 1 ml/kg, dissolved in distilled deionized water) or SAL was injected 15 min before an MDMA or SAL injection.

Warming procedure. Rats were given the AMPT/MDMA or KET/MDMA drug combinations in the procedure described above. After the MDMA injection, rats were placed in a plastic cage with a wire top, and a heat lamp was positioned over the cage. The rats were then warmed to attain a CORE TEMP of 38.5°C to 39.5°C. If the CORE TEMP rose above 40.3°C, the heat lamp was turned off and the rats were moved to hanging wire cages where more ventilation would prevent overheating that might lead to lethality. Rats were placed on an ice chest for additional cooling if necessary. The warming procedure continued for 4 h (240 min), at which time rats were returned to their home cages and the experiment was continued.

Neurochemical analysis. One week after the injections, rats were sacrificed by decapitation, and their brains were dissected as described by Heffner *et al.* (1980). Tissue samples containing the frontal cortex, somatosensory (frontal-parietal) cortex, striatum and hippocampus were stored in liquid nitrogen until the time of analysis for each region. 5HT and 5HIAA levels were determined for each region using high-performance liquid chromatography (HPLC) as described in Kotake *et al.* (1985).

Data analysis. For the neurochemical analysis, differences in 5HT and 5HIAA transmitter concentrations among treatment groups were determined by ANOVA followed by Bonferroni *post-hoc* comparisons (Instat for McIntosh, GraphPad software). For the CORE TEMP analysis, two-way ANOVA for repeated measures was used to compare treatment groups. Single time-point comparisons between groups were made using a Tukey *post-hoc* test. $P < .05$ was considered to indicate statistical significance.

Results

KET Pretreatment and MDMA

KET-induced hypothermia prevents MDMA-engendered 5HT and 5HIAA neurotoxicity; furthermore, warming the rats to prevent hypothermia reverses this protection. Analysis indicated that this experiment had a significant treatment effect ($F = 45.2$, $P < .00001$). *Post-hoc* tests indicate that the SAL/MDMA group showed a significant increase in CORE TEMP ($F = 36.95$, $P < .001$) 240 min after the MDMA injection when compared to the SAL/SAL group. This hyperthermia continued until 600 min after the MDMA injection (fig. 1).

From -60 min (when the KET was injected) to 0 min, the KET/MDMA group showed no difference in CORE TEMP from any of the treatment groups. The KET/MDMA group showed a significant hypothermia of 36.5°C, 1°C below baseline ($F = 18.85$, $P < .001$), 15 min after the MDMA injection, which was the first time-point when CORE TEMP was measured. This hypothermia reached a peak low of 34.7°C (2.7°C below base line) at 105 min ($F = 33.4$, $P < .001$), and the hypothermia continued for 180 min after the MDMA injection. At 180 min, the KET/MDMA group returned to baseline values that did not differ from the SAL/SAL group until 420 min (7 h). At this time-point, the KET/MDMA group showed hyperthermia ($F = 7.88$, $P < .001$) when compared with the SAL/SAL group. There was no significant effect of KET on CORE TEMP when the KET/SAL group was compared with the SAL/SAL group throughout the testing interval. The SAL/SAL group had an average temperature of 37.5°C (S.E.M. = 0.05) throughout the entire testing interval.

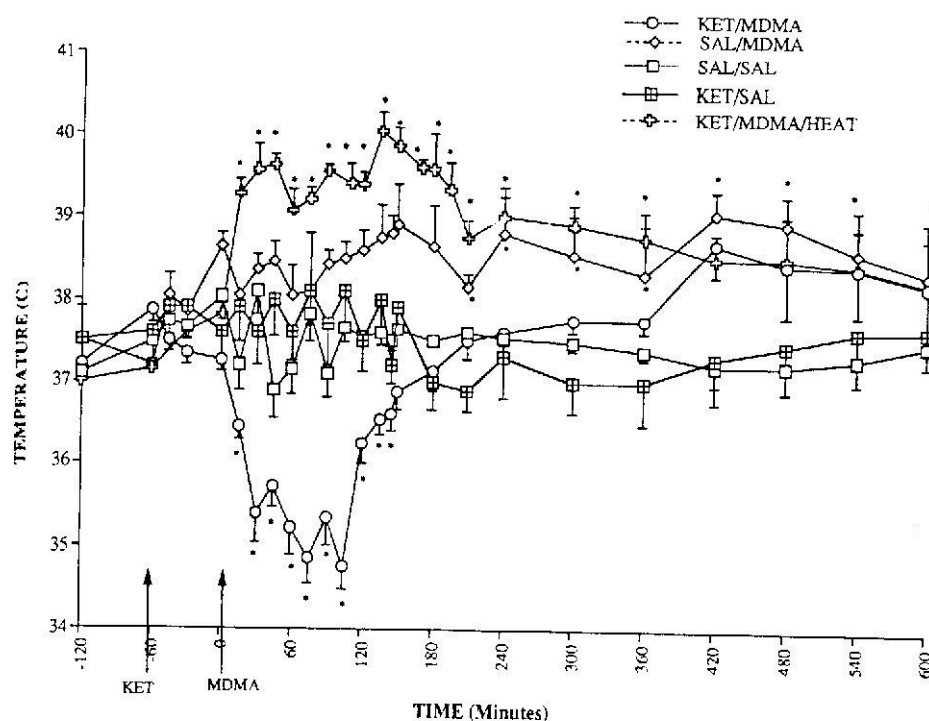


Fig. 1. Effects of KET and MDMA on CORE TEMP (°C) in the rat. Injection times are indicated by arrows. The KET/MDMA injections produce a hypothermia in the rat, which is prevented by heating the rats (KET/MDMA/HEAT). *Significantly different from SAL/SAL ($P < .05$).

In the KET/MDMA/HEAT group, the goal was to prevent the hypothermia seen in the KET/MDMA group by manually raising the CORE TEMP of the rats immediately after the MDMA injection. The experiment attempted to maintain CORE TEMP between 38.5°C and 39.5°C, although some variability did occur, with an average CORE TEMP of 39.3°C $\pm$ 0.5°C. At the beginning of the manual warming procedure, 15 min after the MDMA injection, the KET/

MDMA/HEAT group had a higher CORE TEMP than the SAL/SAL group ($F = 18.85$, $P < .001$). The increase in CORE TEMP in the KET/MDMA/HEAT group was seen throughout the manual warming period and until hour 10, which was 6 h after the manual warming was discontinued. The hyperthermia seen in the KET/MDMA/HEAT group after the manual warming was discontinued was not significantly different from that in the SAL/MDMA group at those time-points.

TABLE 1

5-HT and 5-HIAA levels of rats given the indicated treatment regimens. All animals were sacrificed 14 days after injection. All tissue values are expressed in nanograms per milligram of wet tissue weight. SEM values are indicated in parentheses.

KET/MDMA Experiment			AMPT/MDMA Experiment			FLUOX/MDMA Experiment		
Group	5-HT	5-HIAA	Group	5-HT	5-HIAA	Group	5-HT	5-HIAA
Frontal Cortex								
KET/MDMA	0.31 (.03)	0.24 (.02)	AMPT/MDMA	0.26 (.01)	0.15 (.01)	FLUOX/MDMA	0.27 (.03)	0.14 (.02)
SAL/SAL	0.33 (.02)	0.21 (.03)	SAL/SAL	0.26 (.02)	0.14 (.01)	SAL/SAL	0.28 (.02)	0.15 (.02)
KET/SAL	0.33 (.02)	0.19 (.02)	AMPT/SAL	0.25 (.02)	0.16 (.01)	FLUOX/SAL	0.30 (.02)	0.13 (.02)
SAL/MDMA	0.21 (.02)*	0.22 (.02)	SAL/MDMA	0.15 (.02)*	0.08 (.01)*	SAL/MDMA	0.15 (.01)*	0.08 (.02)*
KET/MDMA/HEAT	0.24 (.02)*	0.17 (.03)	AMPT/MDMA/HEAT	0.18 (.02)*	0.07 (.01)*			
Somatosensory cortex								
KET/MDMA	0.16 (.01)	0.09 (.01)	AMPT/MDMA	0.16 (.02)	0.11 (.01)	FLUOX/MDMA	0.17 (.02)	0.11 (.01)
SAL/SAL	0.17 (.01)	0.10 (.02)	SAL/SAL	0.16 (.01)	0.10 (.01)	SAL/SAL	0.16 (.01)	0.12 (.02)
KET/SAL	0.15 (.01)	0.10 (.01)	AMPT/SAL	0.14 (.01)	0.11 (.02)	FLUOX/SAL	0.19 (.02)	0.13 (.01)
KET/MDMA/HEAT	0.09 (.01)*	0.05 (.02)*	SAL/MDMA	0.09 (.02)*	0.07 (.01)	SAL/MDMA	0.07 (.01)*	0.07 (.02)*
KET/MDMA/HEAT	0.07 (.01)*	0.05 (.01)*	AMPT/MDMA/HEAT	0.11 (.01)*	0.06 (.01)			
Striatum								
KET/MDMA	0.25 (.02)	0.22 (.03)	AMPT/MDMA	0.19 (.01)	0.32 (.03)	FLUOX/MDMA	0.30 (.04)	0.33 (.05)
SAL/SAL	0.27 (.03)	0.27 (.02)	SAL/SAL	0.22 (.02)	0.37 (.04)	SAL/SAL	0.25 (.03)	0.31 (.08)
KET/SAL	0.28 (.03)	0.26 (.05)	AMPT/SAL	0.21 (.02)	0.30 (.02)	FLUOX/SAL	0.23 (.02)	0.30 (.06)
SAL/MDMA	0.14 (.01)*	0.16 (.01)*	SAL/MDMA	0.15 (.02)*	0.18 (.06)*	SAL/MDMA	0.14 (.02)*	0.21 (.05)
KET/MDMA/HEAT	0.19 (.02)*	0.14 (.02)*	AMPT/MDMA/HEAT	0.15 (.01)*	0.20 (.03)*			
Hippocampus								
KET/MDMA	0.33 (.03)	0.22 (.02)	AMPT/MDMA	0.29 (.02)	0.23 (.03)	FLUOX/MDMA	0.32 (.02)	0.27 (.09)
SAL/SAL	0.32 (.02)	0.24 (.05)	SAL/SAL	0.30 (.02)	0.22 (.02)	SAL/SAL	0.34 (.04)	0.25 (.07)
KET/SAL	0.30 (.03)	0.23 (.02)	AMPT/SAL	0.27 (.02)	0.20 (.01)	FLUOX/SAL	0.38 (.03)	0.23 (.05)
SAL/MDMA	0.16 (.01)*	0.14 (.03)*	SAL/MDMA	0.16 (.02)*	0.13 (.01)*	SAL/MDMA	0.17 (.02)*	0.11 (.06)*
KET/MDMA/HEAT	0.19 (.02)*	0.13 (.02)*	AMPT/MDMA/HEAT	0.19 (.01)*	0.15 (.02)*			

* $P < .05$ compared to SAL/SAL group

HPLC analysis of the brain regions (table 1) indicate that there was a significant effect of treatment on 5HT levels in the frontal cortex ($F = 6.71$, $P < .001$) and on 5HT and 5HIAA levels in the somatosensory cortex ($F = 14.2$, $P < .0001$ for 5HT; $F = 6.49$, $P < .001$ for 5HIAA), striatum ($F = 14.84$, $P < .001$; $F = 13.95$, $P < .0001$) and hippocampus ($F = 17.43$, $P < .0001$; $F = 17.43$, $P < .0001$). *Post-hoc* tests indicate that rats given a 40-mg/kg MDMA injection (SAL/MDMA group) had significantly ($P < .05$) decreased levels of 5HT in the frontal cortex (69% of SAL/SAL), somatosensory cortex (54%), striatum (50%) and hippocampus (50%). 5HIAA levels in the SAL/MDMA group were also decreased in the somatosensory cortex (50%), striatum (60%) and hippocampus (58%). The KET/MDMA group had 5HT and 5HIAA levels that were not significantly different from those of the SAL/SAL group, which indicates that KET pretreatment protected against MDMA-induced 5HT and 5HIAA depletions. There were no differences in the transmitter levels of KET/SAL and SAL/SAL groups in any of the regions, which indicates that KET alone had no effect on 5HT or 5HIAA levels.

The KET/MDMA/HEAT rats had 5HT and 5HIAA levels (table 1) that were significantly ($P < .05$) lower than those of the SAL/SAL group in the frontal cortex (80% 5HT depletion), somatosensory cortex (41% 5HT; 50% 5HIAA depletion), hippocampus (59% 5HT; 54% 5HIAA) and striatum (69% 5HT; 51% 5HIAA). In addition, the KET/MDMA/HEAT group had 5HT and 5HIAA levels that were not significantly different from those of the SAL/MDMA group. These data indicate that the protection against 5HT and 5HIAA deple-

tions afforded by the KET pretreatment was removed when the rats were warmed and hypothermia was prevented.

AMPT Pretreatment and MDMA

AMPT-induced hypothermia prevents MDMA-engendered 5HT and 5HIAA neurotoxicity, and prevention of hypothermia by heating the rats reverses this protection. Analysis indicated that this experiment had a significant treatment effect ($F = 33.05$, $P < .00001$). *Post-hoc* tests indicate that the SAL/MDMA group displayed a hypothermia ($F = 16.32$, $P < .001$) of 1°C below the SAL/SAL group 45 min after the MDMA injection. This hypothermia continued until 180 min, when it returned to base-line values. This brief MDMA-induced hypothermia was also seen by Dafters (1994). At 300 min after the MDMA injection, the SAL/MDMA group displayed a hyperthermia compared to that of the SAL/SAL group ($F = 10.772$, $P < .001$), which continued throughout the experiment (fig. 2).

From -300 min, when the first AMPT injection was given, to 0 min, the AMPT/MDMA group showed no difference in CORE TEMP compared with the other treatment groups. After the MDMA injection at time 0 , the AMPT/MDMA group showed a CORE TEMP 1.1°C below base line (36.4°C) at the first CORE TEMP measurement ($F = 9.62$, $P < .001$). The AMPT/MDMA group had a significantly lower CORE TEMP than the SAL/MDMA group ($F = 15.57$, $P < .001$). This hypothermia seen in the AMPT/MDMA group reached a peak low of 34.3°C (3.2°C below base line) at 90 min ($F = 78.15$, $P < .001$), and the hypothermia continued for 240 min. After

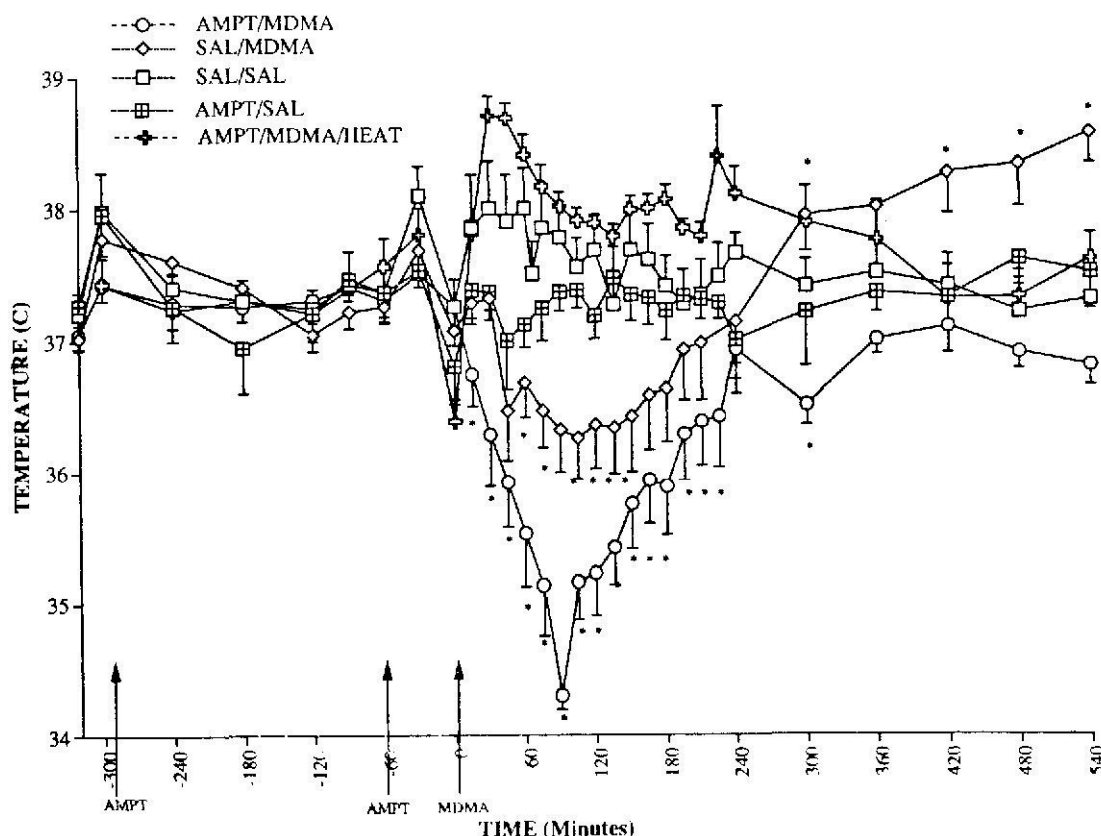


Fig. 2. Effects of AMPT and MDMA on CORE TEMP ($^{\circ}\text{C}$) in the rat. Injection times are indicated by arrows. The AMPT/MDMA injections produce hypothermia in the rat, which is prevented by heating the rats (AMPT/MDMA/HEAT). *Significantly different from SAL/SAL ($P < .05$).

240 min, the AMPT/MDMA group was not different from the SAL/SAL group throughout the rest of the testing interval, except at 300 min, when the AMPT/MDMA group was hypothermic compared with the SAL/SAL group. The AMPT/SAL and SAL/SAL groups were significantly different only at 30 to 60 minutes after the MDMA injection. At all other time-points, AMPT/SAL and SAL/SAL groups were not significantly different from each other, which indicates that AMPT did not have a large effect on CORE TEMP. The SAL/SAL group had an average temperature of 37.5°C (S.E.M. = 0.05) throughout the testing interval.

The AMPT/MDMA/HEAT rats were manually warmed to prevent the hypothermia seen in the AMPT/MDMA group. The AMPT/MDMA/HEAT rats had an average CORE TEMP of 38.1°C ± 0.3°C, with a range of 37.8°C to 38.7°C. This treatment group more closely followed the CORE TEMPs of the SAL/SAL group; both during the warming and after the warming was discontinued, the AMPT/MDMA/HEAT group was not significantly different from the SAL/SAL group.

HPLC analysis of the brain regions (table 1) indicates that there was a significant effect of treatment on 5HT levels in the somatosensory cortex ($F = 7.83$, $P < .0001$), and an effect on both 5HT and 5HIAA levels in the frontal cortex ($F = 5.67$, $P < .005$ for 5HT; $F = 5.83$, $P < .01$ for 5HIAA), striatum ($F = 8.65$, $P < .0001$; $F = 10.02$, $P < .0001$) and hippocampus ($F = 10.3$, $P < .0001$; $F = 17.442$, $P < .0001$). *Post-hoc* tests indicate that rats given 40 mg/kg MDMA (SAL/MDMA group) had significantly decreased levels of 5HT in the frontal cortex (57% of SAL/SAL), somatosensory cortex (56%), striatum (65%) and hippocampus (53%). SAL/MDMA-treated rats also had depletions of 5HIAA in the frontal cortex (57%), striatum (48%) and hippocampus (59%). The AMPT/MDMA group had 5HT and 5HIAA levels that were not significantly different from those of the SAL/SAL group, which indicates that AMPT pretreatment protected against MDMA-induced depletions. There was no difference in 5HT and 5HIAA levels between the AMPT/SAL and SAL/SAL groups in the regions examined.

As can be seen in table 1, rats that were warmed (AMPT/MDMA/HEAT) do not show protection against MDMA-induced neurotoxicity. In these regions, the 5HT levels of AMPT/MDMA/HEAT rats are significantly below those of the AMPT/MDMA and SAL/SAL rats in the somatosensory cortex (68% of SAL/SAL 5HT), frontal cortex (69% 5HT; 50% 5HIAA), striatum (68% 5HT; 54% 5HIAA) and hippocampus (63% 5HT; 68% 5HIAA). The AMPT/MDMA/HEAT group had 5HT and 5HIAA levels that were not significantly different from those of the SAL/MDMA group, which indicates that the protection afforded by the AMPT pretreatment was removed when the rats were warmed and hypothermia was prevented.

FLUOX Pretreatment and MDMA

Pretreatment with FLUOX has no effect on CORE TEMP but completely protects against MDMA-induced neurotoxicity. Analysis indicates that this experiment had a significant treatment effect ($F = 31.3$, $P < .00001$). *Post-hoc* tests indicate that from -60 min to 135 min, there was no difference in CORE TEMP among the four treatment groups (FLUOX/MDMA, FLUOX/SAL, SAL/MDMA and SAL/SAL). At 210 min after the MDMA injection, the SAL/MDMA ($F = 3.79$, $P < .05$) and FLUOX/MDMA ($F = 5.82$, $P < .01$) groups

exhibited hyperthermia compared to base line. The SAL/MDMA group exhibited hyperthermia compared to base line throughout the rest of the testing session. The FLUOX/MDMA group exhibited hyperthermia until 540 min (9 h) after the MDMA injection, when the FLUOX/MDMA group returned to base-line CORE TEMP values. At all time-points in the experiment, there was no significant difference between the FLUOX/SAL and SAL/SAL groups, and the SAL/SAL group had an average CORE TEMP of 37.6°C (S.E.M. = 0.07) throughout the testing day. This is a different response than was seen with the KET and AMPT groups, FLUOX having no effect on CORE TEMP in either the FLUOX/SAL or the FLUOX/MDMA group (fig. 3).

HPLC analysis of the brain regions (table 1) indicates that there was a significant effect of treatment on 5HT levels in the striatum ($F = 11.94$, $P < .0001$) and on 5HT and 5HIAA levels in the frontal cortex ($F = 7.176$, $P < .001$ for 5HT; $F = 5.82$, $P < .001$ for 5HIAA), somatosensory cortex ($F = 7.36$, $P < .001$; $F = 3.75$, $P < .001$) and hippocampus ($F = 6.91$, $P < .005$; $F = 6.16$, $P < .005$). *Post-hoc* tests show that rats given an injection of MDMA (SAL/MDMA group) have depletions of 5HT in the frontal cortex (53% SAL/SAL), somatosensory cortex (43%), striatum (54%) and hippocampus (48%). 5HIAA depletions were also seen in the frontal cortex (53%), somatosensory cortex (57%) and hippocampus (44%). The FLUOX/MDMA group showed no significant change from the SAL/SAL group, which indicates that FLUOX pretreatment protected against MDMA-induced 5HT and 5HIAA depletions. There was no difference in 5HT and 5HIAA levels between the FLUOX/SAL and SAL/SAL groups in the regions examined. These data indicate that FLUOX pretreatment had no effect on CORE TEMP and that protection against MDMA-induced 5HT neurotoxicity can occur even with animals that exhibit hyperthermia.

Discussion

These results indicate that pretreatment with AMPT or KET prevents MDMA-induced 5HT and 5HIAA depletions by producing hypothermia in the rat. We report that prevention of the AMPT- and KET-induced hypothermia eliminates the protection against MDMA-induced neurotoxicity, which indicates that the hypothermia plays a role in the protection. In contrast, pretreatment with FLUOX, a specific 5HT uptake inhibitor (Wong *et al.*, 1983) has no effect on CORE TEMP; the FLUOX/MDMA group displays a hyperthermia similar to that seen in rats treated only with MDMA (SAL/MDMA). However, the FLUOX/MDMA group showed protection against MDMA neurotoxicity even with the hyperthermia. These findings further extend the interaction of neurotoxicity and CORE TEMP and agree with past studies showing that some drugs that prevent MDMA (Ali *et al.*, 1994; Farfel and Seiden, 1995a; Miller and O'Callaghan, 1994) and METH-induced neurotoxicity (Bowyer *et al.*, 1994; Farfel and Seiden, 1995b) exert their protective effects by inducing hypothermia or preventing hyperthermia. Notably, this study also indicates that the mechanism of protection by FLUOX does not depend on an induction of hypothermia but protects *via* another mechanism.

Our findings show that prevention of the hypothermia induced by the AMPT/MDMA or KET/MDMA combination reverses the protection against neurotoxicity. This reversal of

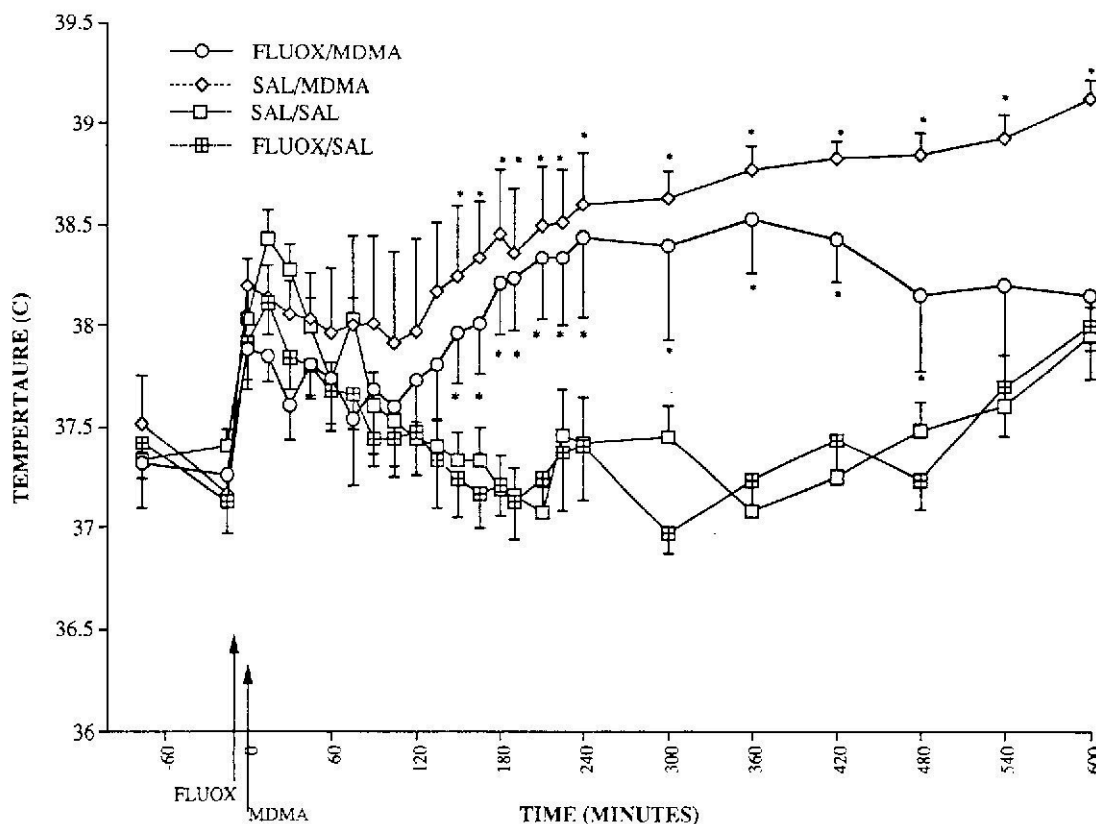


Fig. 3. Effects of FLUOX and MDMA on CORE TEMP (°C) in the rat. Injection times are indicated by arrows. FLUOX has no significant effect on temperature. *Significantly different from SAL/SAL ($P < .05$).

protection was accomplished by heating the rats. In the KET experiment, the KET/MDMA/HEAT rats were warmed until they were hyperthermic compared with the control (SAL/SAL) group (see fig. 1); in the AMPT experiment, the AMPT/MDMA/HEAT rats were warmed until they reached control (SAL/SAL) levels but were not hyperthermic compared with the control (SAL/SAL) group (see fig. 2). The AMPT/MDMA/HEAT rats were within a normal CORE TEMP range (Gordon, 1993) of 37.5°C to 38.0°C. Both the KET/MDMA/HEAT and AMPT/MDMA/HEAT groups showed a reversal of protection against MDMA-induced neurotoxicity. Given that heating METH-treated rats increases neurotoxicity (Bowyer *et al.*, 1994), the argument could be made that the warming of the KET/MDMA/HEAT and AMPT/MDMA/HEAT rats may itself have caused the neurotoxicity; that is, the decrease in 5HT and 5HIAA levels seen in the HEAT groups might not result from a reversal of KET- or AMPT-induced protection but from a warming-induced neurotoxicity. The fact that the AMPT/MDMA/HEAT rats exhibited a loss of protection against neurotoxicity, but were not hyperthermic, provides evidence against the argument for heating- or hyperthermia-induced neurotoxicity. The data from the HEATED groups indicate that it is prevention of the drug-induced hypothermia that removes the KET and AMPT protection against MDMA-induced neurotoxicity.

KET (Nash, 1990) and other 5HT₂ antagonists such as ritanserin (Johnson *et al.*, 1993) and MDL 11,939 (Schmidt *et al.*, 1990a) have been shown to prevent both MDMA- and METH-induced serotonergic neurotoxicity. The effect of KET on neurotoxicity and CORE TEMP has also been previously investigated. Schmidt *et al.* (1990b) reported that for a 10-

mg/kg dose of MDMA, pretreatment with MDL 11,939, a 5HT₂ receptor antagonist, prevented MDMA-induced hyperthermia and protected against neurotoxicity. However, when higher doses of MDMA were given (20–30 mg/kg), there was no attenuation of the hyperthermia, although there was protection against neurotoxicity. Nash *et al.* (1988) also observed that pretreatment with a low dose of KET (3 mg/kg) attenuated the hyperthermia induced by MDMA (10 mg/kg). In the Nash *et al.* (1988) and Schmidt *et al.* (1990b) studies, no hypothermia was seen with the 5HT₂ antagonist/MDMA combination, which is inconsistent with our findings. However, the differences between their results and ours may be due to the methods by which CORE TEMP was measured; Schmidt *et al.* (1990b) measured CORE TEMP once an hour for 3 h, and Nash *et al.* (1988) measured CORE TEMP once during the experiment using colonic temperature measurements. It may be that these groups did have a hypothermic response but that this hypothermia was masked by a stress-induced increase in CORE TEMP due to the colonic temperature measurement (Gordon, 1990). It may also be that a brief hypothermia was not detected in these studies because of the infrequency of CORE TEMP measurement. In addition, the Schmidt *et al.* (1990b) study reported base-line CORE TEMPs of 34°C to 35°C, which are 2°C lower than our base-line CORE TEMPs and are similar to the lowest CORE TEMPs attained by our AMPT/MDMA and KET/MDMA rats. Therefore, the number of differences between the studies may account for the differences in the results. It has been hypothesized (Huang and Nichols, 1993; Johnson *et al.*, 1993; Schmidt *et al.*, 1990a) that KET's protective mechanism comes from the 5HT₂ antagonist interfering with stimulation

of DA synthesis and DA release, which in turn blocks the 5HT neurotoxicity seen with MDMA. Our study indicates that it is the lowering of CORE TEMP that mediates KET's protective effects.

AMPT has also been shown to have protective effects against METH (Schmidt *et al.*, 1985) and MDMA (Axt and Seiden, 1990; Stone *et al.*, 1988). AMPT is an inhibitor of tyrosine hydroxylase, which is the rate-limiting enzyme for DA synthesis. The observations that AMPT, the DA uptake inhibitor GBR 12909 and haloperidol (Stone *et al.*, 1988) all protect against neurotoxicity imply that the DA system mediates some of the protective effects of these drugs. AMPT's interference with the DA system is hypothesized to underlie the protection, for it has been suggested that an intact DA system is necessary for MDMA-induced serotonergic neurotoxicity (Stone *et al.*, 1988; Axt and Seiden, 1990). However, because we eliminated the protection by preventing the AMPT-induced hypothermia, we believe our data question a direct role for the DA system in MDMA-induced neurotoxicity and protection by AMPT.

KET and AMPT exert their primary effects on the 5HT and DA systems, respectively. It is apparent that the induction of hypothermia is the mechanism by which both mediate neuroprotection. The finding that both KET and AMPT induce hypothermia when combined with MDMA is supportive of a small body of literature showing that other drugs that protect against neurotoxicity have no effect on body temperature alone when administered but result in hypothermia when paired with METH or MDMA (Ali *et al.*, 1994; Bowyer *et al.*, 1994; Bowyer *et al.*, 1993; Farfel and Seiden, 1995a; Farfel and Seiden, 1995b; Miller and O'Callaghan, 1994). Moreover, some drugs protect against METH- and MDMA-induced neurotoxicity and prevent the METH- or MDMA-induced hyperthermia but do not induce hypothermia when administered with METH or MDMA (Bowyer *et al.*, 1994; Albers and Sonsalla, 1995). It has not yet been determined whether the hypothermia mediates the protection in all the drugs that induce hypothermia, because only a few studies besides this one have examined the effect of a reversal or prevention of the hypothermia (Bowyer *et al.*, 1994; Farfel and Seiden, 1995a; Miller and O'Callaghan, 1994). However, the data in these studies suggest that for at least some drugs that protect against neurotoxicity, the hypothermia induced by these drugs in conjunction with MDMA or METH plays an important role in protection against neurotoxicity.

Albers and Sonsalla (1995) found that in mice, reserpine did not protect against METH-induced neurotoxicity in spite of reserpine-induced hypothermia. On the basis of these findings Albers and Sonsalla suggest that hyperthermia *per se* may not mediate neurotoxicity (Albers and Sonsalla, 1995), because reserpine-induced hypothermia did not protect against METH-induced neurotoxicity. This finding is apparently in contrast to other studies in which METH- and MDMA-induced neurotoxicity is prevented by administration of drugs that produce hypothermia or by a cold environment (Bowyer *et al.*, 1993; Farfel and Seiden, 1995a,b; Miller and O'Callaghan, 1994). In addition, studies have reported a protective effect of reserpine against MDMA-induced neurotoxicity (Stone *et al.*, 1988; Schmidt *et al.*, 1990c), and Wagner *et al.* (1983) have reported that reserpine enhances METH-induced neurotoxicity in rats.

We also present evidence that hypothermia or a change in

CORE TEMP is not necessary for all drugs to exert their protective effects; indeed, hyperthermia may be observed with drugs that protect against neurotoxicity. Figure 3 shows that in comparison with AMPT and KET experiments, FLUOX does not induce hypothermia and indeed seems to have little effect on CORE TEMP. The two treatment groups given MDMA (FLUOX/MDMA and SAL/MDMA) have similar CORE TEMP profiles in that both groups display a delayed hyperthermia. This indicates that FLUOX protected against MDMA-induced neurotoxicity even though there was a hyperthermia seen in the FLUOX/MDMA group.

FLUOX selectively blocks the 5HT uptake transporter (Wong *et al.*, 1983) and may prevent the uptake of MDMA into the nerve terminal. FLUOX may also prevent endogenously produced neurotoxins such as 5,6-DHT from entering the cell or prevent MDMA-induced 5HT efflux (Rudnick and Wall, 1992). In either case, FLUOX may be blocking the uptake of MDMA or of putative neurotoxic substances into the cell. This blockade may be its primary protective mechanism against MDMA-induced 5HT neurotoxicity, in comparison with drugs that use hypothermia as a protectant. We therefore suggest that one mechanism involved in protection against MDMA-induced neurotoxicity is an induction of hypothermia and that a second possible mechanism is the blockade of the 5HT transporter with a drug such as FLUOX, with no dependence on a hypothermia.

It has been reported that administration of low doses of MDMA (2.5–10 mg/kg) induces hyperthermia shortly (1 h) after the injection in rats (Dafters, 1994; Nash *et al.*, 1988). We report a hyperthermia that begins 3 to 4 h after the injection of 40 mg/kg MDMA. This delayed hyperthermia is in contrast to the more rapid onset (within 1 h) of hyperthermia observed after METH administration (Farfel and Seiden, 1995a; Bowyer *et al.*, 1994). The differences between our study and those of Dafters (1994) and Nash *et al.*, (1988) may be related to differences in dose of MDMA or in frequency and method of measurement of CORE TEMP. No other study that we know of has measured the effect of high doses of MDMA (40 mg/kg) on CORE TEMP or behavior from 4 to 10 h after MDMA administration. It has been demonstrated that there is a positive correlation between METH-induced hyperthermia (peak body temperature) and the amount of neurotoxicity seen (Bowyer *et al.*, 1994). The FLUOX/MDMA group displays a hyperthermia similar to that of the SAL/MDMA group even though the FLUOX/MDMA group had protection against neurotoxicity. This study suggests that induction of hyperthermia by MDMA is not a sufficient explanation for neurotoxicity.

The function of hypothermia as a neuroprotectant is not well understood. Hypothermia is regarded as a protectant not only against amphetamine neurotoxicity but also against other types of CNS damage. When concussive brain injury is seen in the rat, post-traumatic brain hypothermia significantly reduces the amount of necrotic neurons as well as the contusion volume (Dietrich *et al.*, 1994). It has also been demonstrated that hypothermia has an effect on excitatory amino acids; animals that were hypothermic during ischemia had lower concentrations of hippocampal glutamate and glycine than animals that were not hypothermic (Illievich *et al.*, 1994). Hypothermia also slows down hypoxia-induced calcium accumulation in gerbil hippocampal slices (Mitani *et al.*, 1991). Both of these processes, activation of excitatory amino

acids and calcium accumulation, have also been implicated in METH- and MDMA-induced neurotoxicity (Sonsalla *et al.*, 1989). In addition, protection against ischemia by dizocilpine may be due to the fact that it induces hypothermia to protect against cerebral ischemia (Buchan and Pulsinelli, 1990; Corbett *et al.*, 1990). These data indicate that hypothermia may exert its effect by slowing down cellular processes that might otherwise contribute to neurotoxicity or other types of brain injury such as ischemia.

Our results indicate that several protective drugs induce hypothermia when administered in combination with MDMA. The function of the drug-induced hypothermia would seem to play the same protective role as a cold environment, because both the drug-induced and the environmentally produced hypothermia protect against drug-induced neurotoxicity. This hypothermia would slow down all processes, possibly slowing down the chemical reactions that lead to neurotoxicity. For example, a cold environment decreases the amount of amphetamine-induced DA release in the caudate of rats and protects against subsequent neurotoxicity (Bowyer *et al.*, 1993). It may be that the hypothermia induced by the protective agents in this study slows down all reactions that lead to neurotoxicity, including MDMA-induced 5HT release and oxidation of 5HT into a neurotoxin (Commins *et al.*, 1987a).

This paper provides evidence that in rats pretreated with AMPT or KET, hypothermia mediates the protection against MDMA-induced neurotoxicity. This conclusion is supported by data showing that prevention of the hypothermia by heating the rats restores the neurotoxic effect of MDMA. In rats pretreated with FLUOX the hyperthermia was not significantly different from that in the SAL/MDMA group, but the FLUOX pretreatment protected against MDMA neurotoxicity. This difference in the effect of these drugs on CORE TEMP and subsequent neurotoxicity may be accounted for by the fact that FLUOX works directly on the 5HT transporter, possibly preventing MDMA-induced transmitter release. This paper supports a dichotomy between drugs that protect against MDMA-induced neurotoxicity by altering CORE TEMP and drugs that protect by mechanisms independent of CORE TEMP.

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