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Environment-, Drug- and Stress-Induced Alterations in Body Temperature Affect the Neurotoxicity of Substituted Amphetamines in the C57BL/6J Mouse

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ABSTRACT

In the companion paper we demonstrated that d-methamphetamine (d-METH), d-methylenedioxyamphetamine (d-MDA) and d-methylenedioxymethamphetamine (d-MDMA), but not d-fenfluramine (d-FEN), appear to damage dopaminergic projections to the striatum of the mouse. An elevation in core temperature also was associated with exposure to d-METH, d-MDA and d-MDMA, whereas exposure to d-FEN lowered core temperature. Given these findings, we examined the effects of temperature on substituted amphetamine (AMP)-induced neurotoxicity in the C57BL/6J mouse. Levels of striatal dopamine (DA) and glial fibrillary acidic protein (GFAP) were taken as indicators of neurotoxicity. Alterations in ambient temperature, pretreatment with drugs reported to cause hypothermia in the mouse and hypothermia induced by restraint stress were used to affect AMP-induced neurotoxicity. Mice received d-METH (10 mg/kg), d-MDA (20 mg/kg) or d-MDMA (20 mg/kg) every 2 hr for a total of four s.c. injections. All three AMPs increased core temperature and caused large (>75%) decreases in striatal dopamine and large (>300%) increases in striatal glial fibrillary acidic protein 72 hr after the last injection. Lowering ambient temperature from 22°C to 15°C blocked (d-MDA and d-MDMA) or severely attenuated (d-METH) these effects. Pretreatment with MK-801 lowered core temperature and blocked AMP-induced neurotoxicity; elevation of ambient temperature during this regimen elevated core temperature and markedly attenuated the neuroprotective effects of MK-801. Pretreatment with MK-801 also lowered core temperature in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice but did not block 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. Elevation of ambient temperature during treatment with d-FEN did not result in evidence of neurotoxicity. Pretreatment with drugs that caused hypothermia (ethanol, pentobarbital, diethyldithiocarbamate and d-FEN) blocked or attenuated d-MDMA-induced neurotoxicity. MK-801 and ethanol caused the greatest hypothermia and provided complete protection against d-MDMA-induced neurotoxicity. Likewise, restraining mice during dosing with d-MDMA resulted in severe hypothermia and completely blocked d-MDMA-induced neurotoxicity. These data suggest that the neurotoxic effects of AMPs in the mouse are sensitive to changes in body temperature.

In an accompanying paper (O'Callaghan and Miller, 1994), we demonstrated that the AMPs d-METH, d-MDA and d-MDMA preferentially damage the striatum of the C57BL/6J mouse as evidenced by silver degeneration staining and enhanced expression of GFAP, a biochemical indicator of gliosis. Dopaminergic projections to the striatum appeared to be the damaged targets, because the concentration of DA and tyrosine hydroxylase were decreased for at least 3 weeks after dosing. In contrast to these actions, another AMP, d-FEN, had no effect on any of these measures, suggesting that it did not cause neural damage. Another action of d-FEN that clearly differentiates its profile of activity from that of the other AMPs is its effect on body temperature. D-METH, d-MDA and d-MDMA cause large, and in some instances lethal, elevations in core temperature in the rat (Bowyer *et al.*, 1992, 1993, 1994; Gordon *et al.*, 1991; D. B. Miller and J. P. O'Callaghan, unpublished observations). In contrast, d-FEN, at typical laboratory temperatures of 20 to 23°C, lowers rat core temperature by several degrees (Miller *et al.*, 1991). Data presented in the accompanying paper (O'Callaghan and Miller, 1994) confirm that the thermoregulatory actions of all four of these AMPs in the mouse appear to be quite similar to those obtained in the rat. Although lethal hyperthermia was not observed in the mouse at dosages of d-METH, d-MDA and d-MDMA that caused striatal damage, these AMPs caused a sustained elevation in core temperature, whereas d-FEN caused a marked

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ABBREVIATIONS: DA, dopamine; GFAP, glial fibrillary acidic protein; AMP, substituted amphetamine; d-MDMA, d-methylenedioxymethamphetamine; d-METH, d-methamphetamine; d-MDA, d-methylenedioxyamphetamine; d-FEN, d-fenfluramine; DDC, diethyldithiocarbamate; PB, pentobarbital; ETOH, ethanol; COC, cocaine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; HPA, hypothalamic-pituitary-adrenal; EEA, excitatory amino acid.

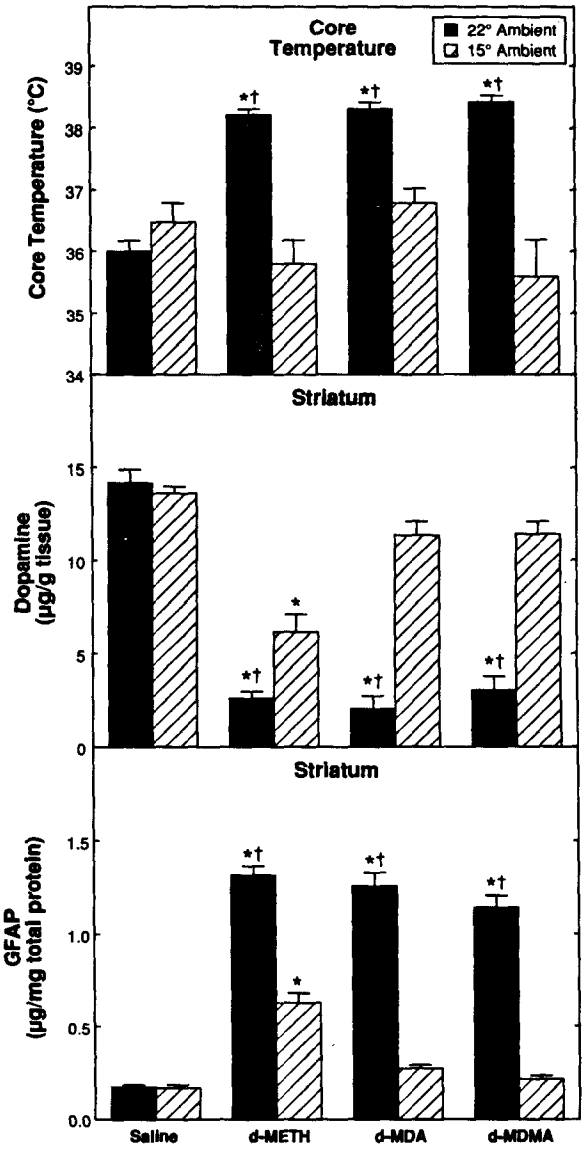

Fig. 1. The influence of lowered ambient temperature on the alterations in core temperature, striatal DA and striatal GFAP induced by d-METH (10 mg/kg × 4), d-MDA (20 mg/kg × 4) and d-MDMA (20 mg/kg × 4). Core temperature measurements were made 1 hr after the fourth injection of d-MDA, d-MDMA or d-METH. Striatal samples were obtained 72 hr after treatment with AMPs. Each value represents the mean ± S.E.M. for six mice. * Significantly different from saline at same ambient temperature. † Significantly different from same treatment group kept at 15°C.

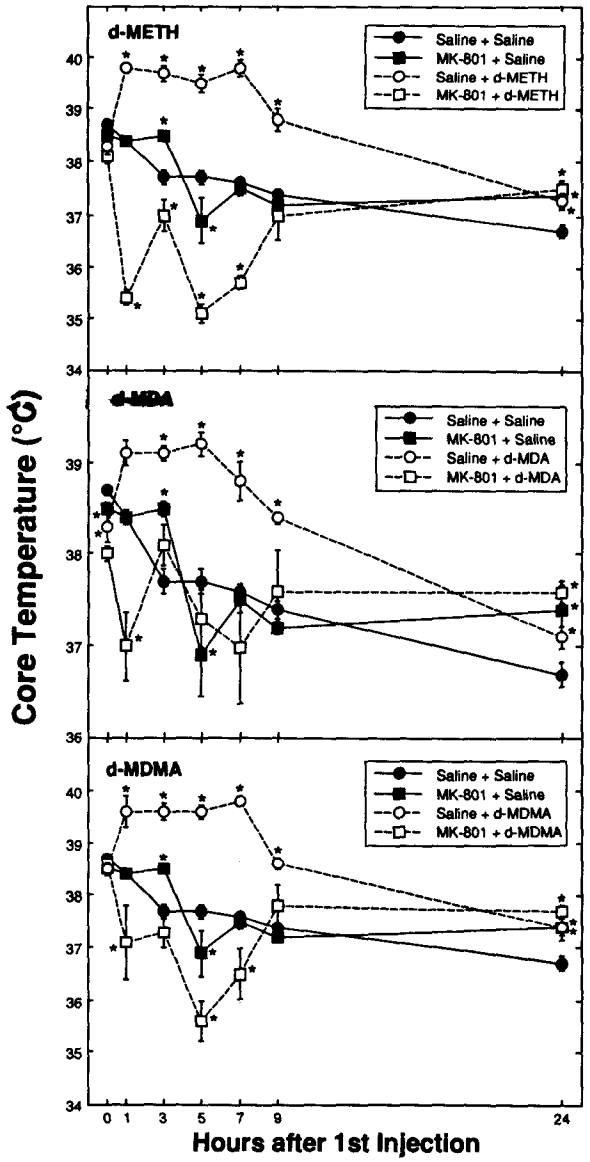

Fig. 2. Time course of the effects of MK-801 (1.0 mg/kg before AMP injections 1 and 3) on the alterations in core temperature induced by d-METH (10 mg/kg × 4), d-MDA (20 mg/kg × 4) and d-MDMA (20 mg/kg × 4). Each value represents the mean ± S.E.M. for six mice; the S.E.M. is not shown if it is smaller than the radius of the point. All groups were dosed and tested at the same time but are plotted in separate panels for ease of viewing; the core temperature values for the groups receiving saline and MK-801 are replotted in each panel. Core temperature measurements were made 0.5 hr after assignment to a new cage (base line or 0) and 1, 3, 5, 7, 9 and 24 hr after the first AMP injection. For the measurements at 1, 3, 5 and 7 hr, this reading also represents a reading 1 hr after AMP injections 1, 2, 3 and 4, respectively. * Significantly different from saline + saline.

reduction in core temperature (see fig. 9, O'Callaghan and Miller, 1994). These data suggest that core temperature may affect the neurotoxicity of AMPs in the mouse as well as in the rat. Indeed, as in the rat (see Bowyer *et al.*, 1992, 1993), housing and handling conditions that alter body temperature have long been known to affect amphetamine-induced lethality in the mouse (Hohn and Lasagna, 1960; Askew, 1962), a phenomenon that may also influence the neurotoxic effects of this compound and its congeners in this species. In light of these observations, the purpose of the present investigation was to examine the role of body (core) temperature in the neurotoxic effects of AMPs in the C57BL/6J mouse.

A variety of approaches were used to alter core temperature in control and AMP-treated mice. We attempted to cause hyper- and hypothermia by altering ambient temperature, by administering pharmacological agents reported to cause hypothermia, and by inducing stress via physical restraint. The data indicate that manipulations that lower core temperature in AMP-treated mice also attenuate or block the neurotoxic effects of these compounds. Protection against AMP-induced neurotoxicity could be effected by conditions as disparate as restraint, a 15°C ambient temperature and coadministration of

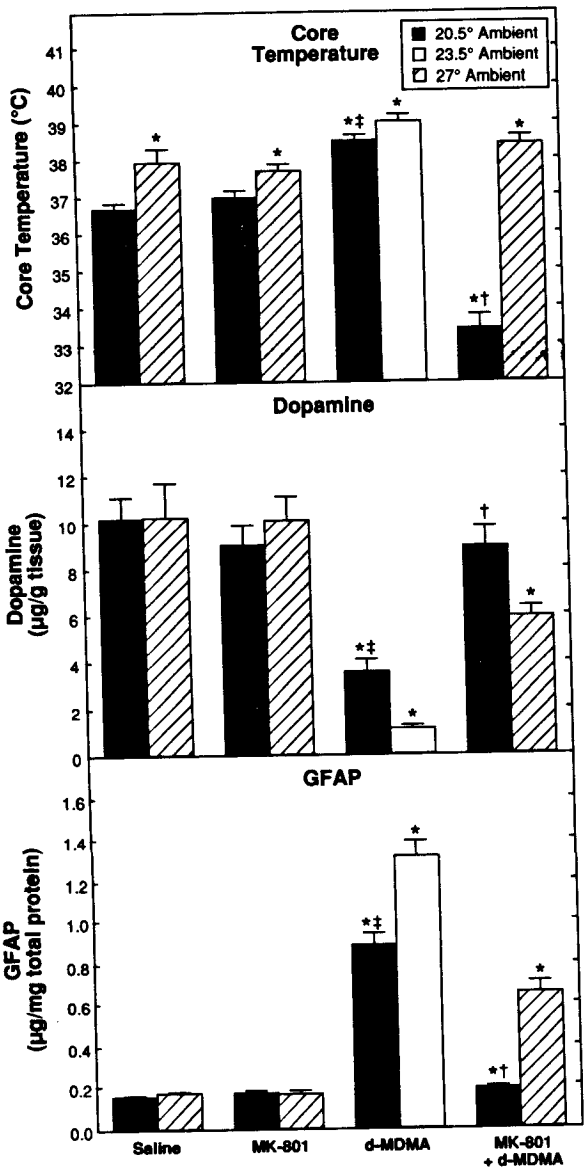

Fig. 3. The influence of elevated ambient temperature on the ability of MK-801 (1.0 mg/kg before d-MDMA injections 1 and 3) to block the alterations in core temperature, striatal DA and striatal GFAP induced by d-MDMA (20 mg/kg × 4). Each value represents the mean ± S.E.M. for six mice. Core temperature measurements were made 1 hr after the fourth d-MDMA injection. Striatal samples were obtained 72 hr after treatment with d-MDMA. * Significantly different from saline kept at 20.5°C. ‡ Significantly different from the same treatment group kept at 23.5°C. † Significantly different from the same treatment group kept at 27°C.

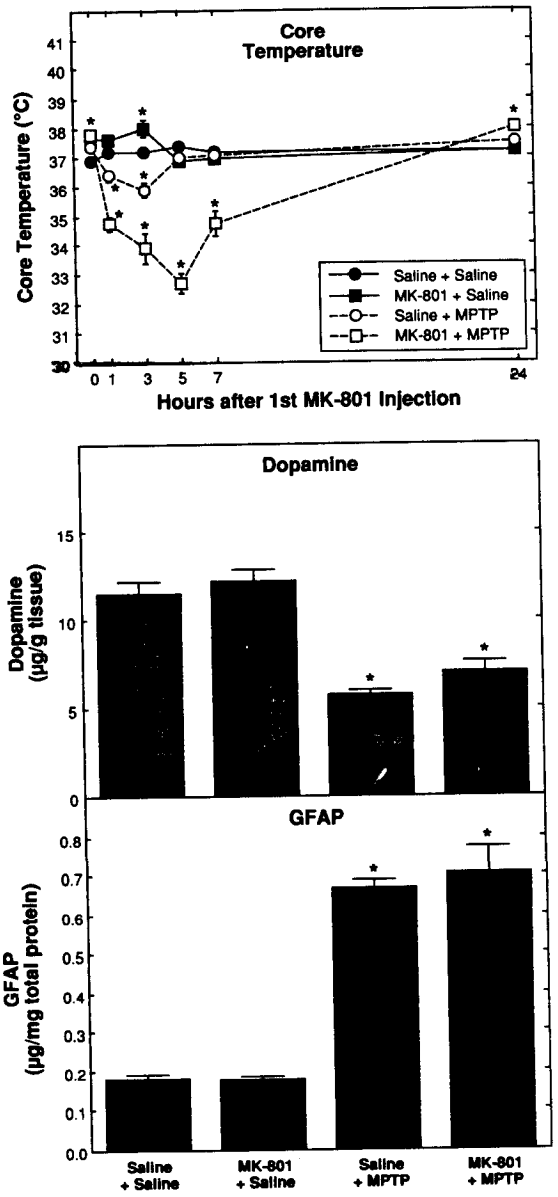

Fig. 4. Effects of pretreatment with MK-801 (1.0 mg/kg 0.5 hr before MPTP and 3 hr after MPTP) on the alterations in core temperature, striatal DA and striatal GFAP induced by a single injection of MPTP (12.5 mg/kg). Each value represents the mean ± S.E.M. for six mice; the S.E.M. is not shown if it is smaller than the radius of the point. Core temperature measurements were made 0.5 hr after assignment to a new cage (base line or 0) and 1, 3, 5, 7 and 24 hr after the MPTP injection. Striatal samples were obtained 72 hr after treatment with MPTP. * Significantly different from saline + saline.

hypothermia-inducing drugs, including PB, ETOH, MK-801 and d-FEN. Our data suggest that the neurotoxic effects of d-METH, d-MDA and d-MDMA are linked in some way to their actions on body temperature.

Materials and Methods

Materials. The following drugs and chemicals were kindly provided by or obtained from the sources indicated: d-METH, and high-performance liquid chromatography (HPLC) standards (Sigma Chemical Co., St. Louis, MO), d-MK-801, (Research Biochemicals, Inc., Natick, MA), MPTP (Aldrich, Milwaukee, WI), d-MDA and d-MDMA (Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD), d-FEN (Les Laboratoires Servier, Fleury-les-Aubrais, France), BCA protein assay reagent and bovine serum albumin (Pierce Chemical Co., Rockford, IL), reagents used for HPLC were of HPLC grade (Burdick and Jackson, Muskegon, MI). The materials used in the GFAP assay have been described in detail in O'Callaghan (1991). All other reagents were of at least analytical grade and were obtained from a variety of commercial sources.

Animals. Female C57BL/6J mice 4 to 6 weeks of age were purchased from Jackson Labs (Bar Harbor, ME) and maintained in a colony certified by the American Association for Accreditation of Laboratory Animal Care. Upon receipt, the mice were housed in groups of ten in a

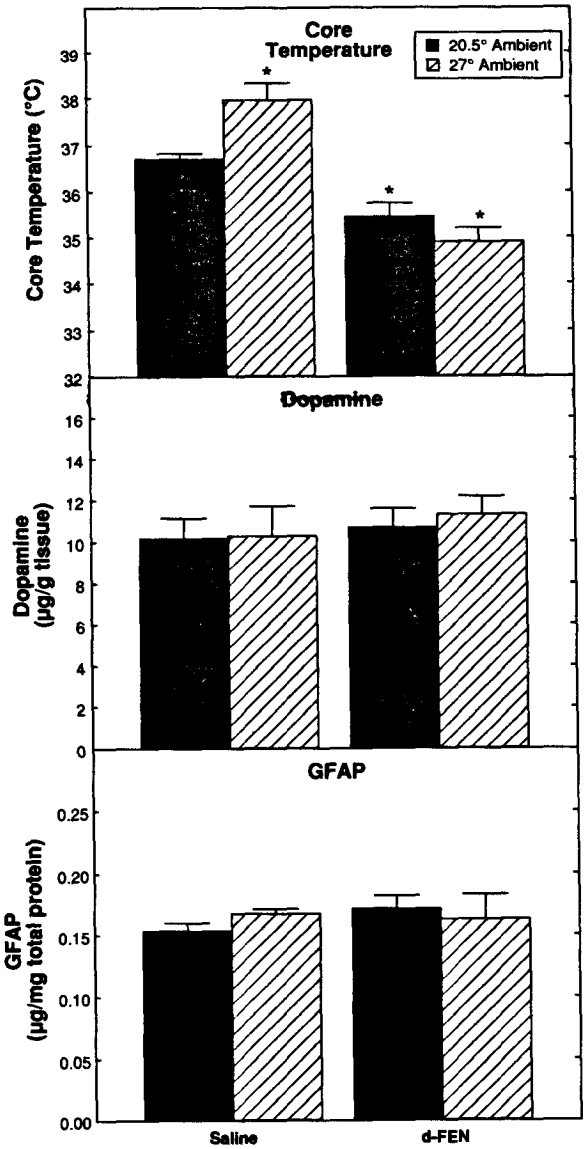

Fig. 5. The influence of elevated ambient temperature on the effects of d-FEN (25.0 mg/kg × 4) on core temperature, striatal DA and striatal GFAP. Each value represents the mean ± S.E.M. for six mice. Core temperature measurements were made 1 hr after the fourth injection of d-FEN. Striatal samples were obtained 72 hr after treatment with d-FEN. * Significantly different from the group given saline and maintained at 20.5°C.

temperature-controlled (21 ± 1°C) and humidity-controlled (50 ± 10%) colony room maintained under filtered positive pressure ventilation on a 12-hr light/12-hr dark cycle beginning at 0600 EDT. The plastic tub cages (46 cm × 25 cm × 15 cm) used for housing were filled to a depth of approximately 4 cm with heat-treated pine shavings as bedding. Food (Purina rat/mouse chow) and water were available *ad libitum*. Upon arrival, animals were habituated to the animal quarters for a period of no less than 2 weeks before use.

Dosing. Preliminary observations indicated that administration of d-MDA and d-MDMA at the dosage and regimen selected for this study caused significant lethality if mice were housed ten to twelve per group in bedded cages (46 cm long × 25 cm wide × 15 cm high). Consequently, unless specified otherwise, all experiments were conducted with mice housed in groups of six. On the day of dosing, six mice, each chosen randomly from a different original housing cage (ten to twelve per cage), were moved to a new cage of the same size. After assignment to a new cage, mice were weighed, and approximately 0.5 hr later base-line temperature was taken. At 0.5 hr after the base-line temperature measurement, mice received their first of four s.c. injections of an AMP; the injections were given 2 hr apart. All AMPs were administered as the d-form, and the dosage per injection is expressed as the base. Mice received saline, d-METH (10 mg/kg), d-MDA (20 mg/kg) or d-MDMA (20 mg/kg). The first AMP injection was always given between 10:00 and 11:00 A.M. to minimize circadian influences on toxicity. In the experiment evaluating the effects of lowered ambient temperature, mice were individually housed in small plastic cages (22 cm long × 14 cm wide × 15 cm high) to prevent huddling. In the experiment that evaluated the effect of temperature on MPTP-induced neurotoxicity, MPTP was given as a single s.c. injection at 11:00 A.M. utilizing a dosage (12.5 mg/kg as the base) previously shown to damage dopaminergic projections to mouse striatum (O'Callaghan *et al.*, 1990). In the experiments that evaluated the effects of drug pretreatments, they were given s.c., 0.5 hr before the first and third AMP injections. The compounds used as pretreatments were PB (50 mg/kg as the base), COC (100 mg/kg as the salt), DDC (400 mg/kg as the salt), ETOH (3 gm/kg), d-FEN (25 mg/kg as the base) and d-MK-801 (1.0 mg/kg as the base). Dosages of these pretreatments were selected on the basis of preliminary experiments (D. B. Miller and J. P. O'Callaghan, unpublished data) or reports in the literature concerning the hypothermic effects of these agents in mice.

Restraint. When restraint was used to alter temperature, mice were placed in the restrainers approximately 0.5 hr before the first AMP injection (d-MDMA in this case). Plastic centrifuge tubes (50 ml) were adapted for use as restrainers that snuggly restrained the mice and prevented them from turning from front to back, although they could rotate from a supine to a prone position. The tubes had a large hole at the rostral end to provide for unimpeded breathing. A grid at the caudal end prevented escape but allowed air exchange and protrusion of the tail. Restrainers were placed on toweling to facilitate absorption of urine and feces. The body of the tube also contained numerous small holes to facilitate air and fluid exchange. A base-line temperature measurement was taken immediately before placement in the restrainer. At the time a d-MDMA injection was to be given, the mice were removed from the restrainer, a temperature measurement was obtained and the injection was then given. The mice remained in the restrainer for 0.5 hr after the final d-MDMA injection. After removal from the restrainer, temperature was measured and the mice were housed in a group (six per cage) until collection of tissue for assay; other mice in the cage had received the same treatment. A final temperature measurement was made approximately 24 hr after the first d-MDMA injection.

Temperature measurement. Rectal temperature was recorded with a Bat-10 thermometer coupled to a RET-3 mouse rectal probe (Physitemp, Inc., Clinton, NJ) lubricated with mineral oil. Preliminary observations indicated that mice held by the tail on a flat surface or on the edge of the cage exhibited a significant degree of struggling, particularly in mice receiving AMPs. This, in turn, caused difficulty in obtaining reliable temperature measurements. To avoid these problems, mice were placed under a "Quonset hut"-shaped piece of foam that was approximately the length of the mouse and that was blocked at the front end. Mice were held by the tail while the temperature probe was inserted to a premarked depth of 2.8 cm. This method minimized handling and, in conjunction with the use of a fast-rise time of the rectal probe, made it possible to obtain reliable measurements of rectal temperature in less than 60 s per mouse. Temperature sampling times varied from experiment to experiment. In the three experiments in which ambient temperature was manipulated, core temperature was monitored 1 hr after the last AMP injection. In the other experiments, core temperature was monitored throughout the dosing period. See the figure legends for exact details.

Brain dissection and tissue preparation. All tissue was obtained 72 hr after the final AMP injection, a time point at which GFAP elevations and DA decreases are maximal for d-METH, d-MDA and d-MDMA (see O'Callaghan and Miller, 1994, for the complete time

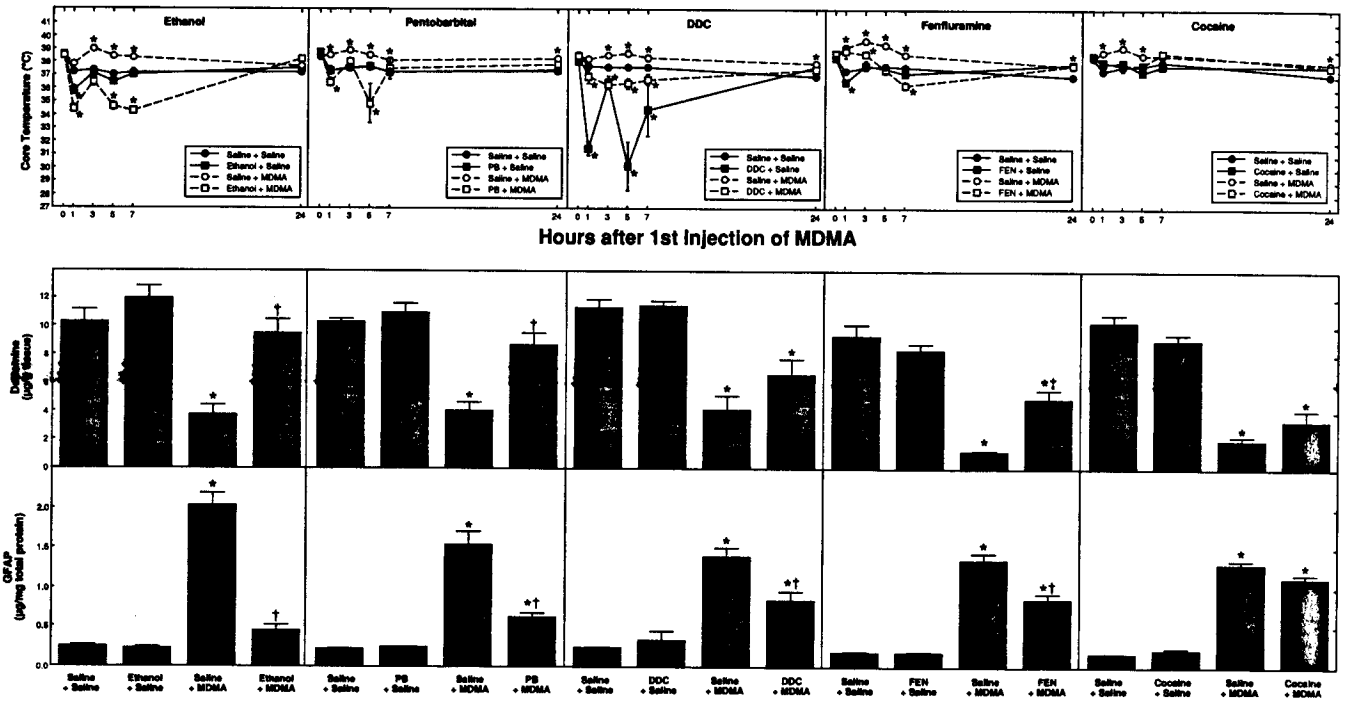

Fig. 6. The effects of pretreatment with ETOH (3 g/kg), PB (50 mg/kg), DDC (400 mg/kg), d-FEN (25 mg/kg) and COC (100 mg/kg) on the alterations in core temperature, striatal DA and striatal GFAP induced by d-MDMA (20 mg/kg × 4). All pretreatments were administered before d-MDMA injections 1 and 3. Striatal samples were obtained 72 hr after the fourth injection of d-MDMA. Each value represents the mean ± S.E.M. for six mice; the S.E.M. is not shown if it is smaller than the radius of the point. * Significantly different from saline + saline. † Significantly different from saline + d-MDMA.

course). Immediately after decapitation, whole brains were removed from the skull with the aid of blunt curved forceps. Striatum was dissected free-hand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI) using a pair of fine curved forceps (Roboz, Washington, D.C.). Striatum from the left side of the brain was weighed, frozen on dry ice and stored at −70°C for subsequent analysis of DA by HPLC. Striatum from the right side was weighed, homogenized with an ultrasonic probe (model XL-2005, Heat Systems, Farmingdale, NY) in 10 vols. of hot (90–95°C) 1% SDS and stored frozen at −70°C before immunoassay of GFAP.

GFAP immunoassay, protein assay, DA analysis. GFAP was assayed according to modifications (O'Callaghan and Miller, 1994) of a previously described sandwich ELISA (O'Callaghan, 1991). Total protein concentration was determined by the method of Smith *et al.* (1985). DA was analyzed by HPLC with electrochemical detection. For complete details regarding these assays, see the accompanying paper (O'Callaghan and Miller, 1994).

Statistics. The Statistical Analysis System (SAS Institute Inc., 1986) was used for data analyses. Individual variables were evaluated by analysis of variance followed by Duncan's Multiple Range Test for mean comparisons. The alpha level used to determine significance was .05 in all cases.

Results

Effects of lowered ambient temperature on core temperature and AMP-induced neurotoxicity. Mice administered d-METH, d-MDA or d-MDMA at an ambient temperature of 22°C showed significant elevations in core temperature 1 hr after the last injection compared to the temperatures for vehicle controls (fig. 1, top panel). In contrast, lowering ambient temperature to 15°C did not alter core temperature in any treatment group relative to saline-treated mice housed at 22°C. Mice administered d-METH, d-MDA or d-MDMA at an ambient temperature of 22°C showed evidence of striatal neurotoxicity: a large (>75%) decrease in DA and a large (>300%) increase in GFAP (fig. 1, middle and bottom panels). When the AMPs were administered at an ambient temperature of 15°C, the neurotoxic effects of d-MDA and d-MDMA were completely blocked and the neurotoxic effects of d-METH were markedly attenuated (fig. 1, middle and bottom panels).

Effect of MK-801 on core temperature in AMP-treated mice. Both lowered ambient temperature (fig. 1) and MK-801 (Sonsalla *et al.*, 1989, 1991; O'Callaghan and Miller, 1994) appear to protect against AMP neurotoxicity. Because the neuroprotective action of MK-801 has been linked to its ability to lower core temperature (Buchan and Pulsinelli, 1990), we examined the effects of MK-801 on core temperature in AMP-treated mice (fig. 2). Compared to saline controls, all three AMPs caused significant elevations (to approximately 40°C) in core temperature that were evident within 1 hr of the first dose, and the core temperature remained significantly elevated throughout the duration of dosing (fig. 2). Core temperature declined to approximately 39°C by 1 hr after the final AMP injection (in agreement with the data in fig. 1), but it was still elevated above saline controls 24 hr after dosing. The temperature of mice treated with saline showed a consistent decrease across the period of testing. MK-801 caused a decrease in core temperature over the period of testing that, in general, followed the decline observed for saline; however, the temperature of MK-801-treated mice remained elevated at the 24-hr time point. Pretreatment with MK-801 either prevented the AMP-induced elevation in core temperature (d-MDA, fig. 2, middle panel) or significantly lowered the temperature below

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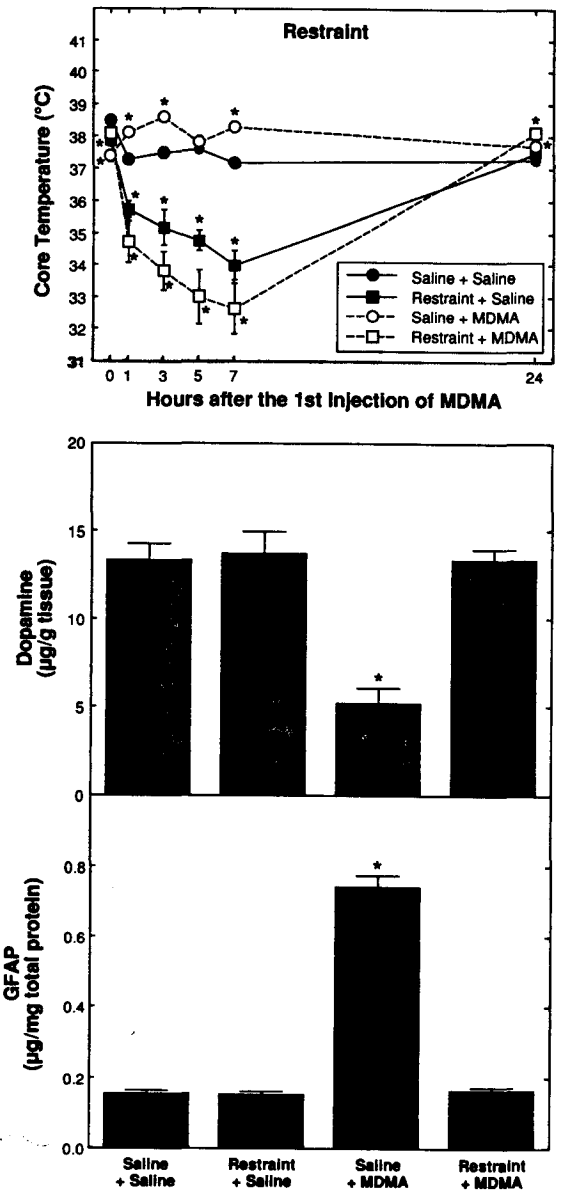

Fig. 7. The effects of restraint on the alterations in core temperature, striatal DA and striatal GFAP induced by d-MDMA (20 mg/kg × 4). Mice were restrained from 0.5 hr before the first injection of d-MDMA until 0.5 hr after the fourth injection of d-MDMA. Core temperature measurements were made 0.5 hr after assignment to a new cage or after restraint (base line or 0) and 1, 3, 5, 7 and 24 hr after the first d-MDMA injection. For the measurements at 1, 3, 5 and 7 hr, this reading also represents a reading 1 hr after d-MDMA injections 1, 2, 3, and 4, respectively. Striatal samples were obtained 72 hr after treatment with d-MDMA. Each value represents the mean ± S.E.M. of six mice; the S.E.M. is not shown if it is smaller than the radius of the point. * Significantly different from saline + saline.

that recorded for mice receiving saline or MK-801 alone (d-METH, fig. 2, top panel; d-MDMA, bottom panel). MK-801 did not prevent the AMP-induced increase in temperature observed at the 24-hr time point.

Effect of elevated ambient temperature on the MK-801 block of d-MDMA-induced neurotoxicity. Because MK-801-induced hypothermia may underlie its neuroprotective action in AMP-treated mice, we examined the neuroprotective action of MK-801 when mice were housed at an elevated ambient temperature (fig. 3). When saline or MK-801 alone was given at an ambient temperature of 27°C, core temperature 1 hr after the last injection was significantly elevated above that obtained at 20.5°C (fig. 3, top panel). When d-MDMA alone was given at an ambient temperature of 20.5°C or 23.5°C, core temperature was significantly elevated above that obtained for saline-treated animals at 20.5°C; in these mice, an ambient temperature of 23.5°C caused a greater elevation in core temperature than did an ambient temperature of 20.5°C. D-MDMA alone could not be given at 27°C because of the likelihood of extreme hyperthermia and lethality. At an ambient temperature of 20.5°C, pretreatment with MK-801 not only blocked the hyperthermia associated with d-MDMA but, consistent with the data in figure 2, caused a greater than 50% reduction in core temperature. This hypothermia could, however, be overcome. At an ambient temperature of 27°C, pretreatment with MK-801 resulted in a core temperature equivalent to that observed in mice given SAL or MK-801 at 27°C or in mice given d-MDMA alone at 20.5°C.

The ambient temperature at dosing also affected the neurotoxicity of d-MDMA (fig. 3, middle and bottom panels). The d-MDMA-induced decrease in DA and increase in GFAP in striatum were greater at an ambient temperature of 23.5°C than at 20.5°C. Neither saline nor MK-801 altered striatal DA (middle panel) or GFAP (bottom panel) at either ambient temperature. In agreement with our previous findings (O'Callaghan and Miller, 1994), MK-801 completely blocked the GFAP elevation and DA decrease induced by d-MDMA at an ambient temperature of 20.5°C. At an ambient temperature of 27°C, however, MK-801 only attenuated the MDMA-induced decrease in DA and increase in GFAP.

Effect of MK-801 on core temperature and MPTP-induced neurotoxicity. In contrast to the data obtained for AMPs, MK-801 does not protect against MPTP-induced neurotoxicity (Sonsalla *et al.*, 1989; O'Callaghan and Miller, 1993), possibly because it failed to lower core temperature. Therefore, we determined whether MK-801 affected core temperature in MPTP-treated mice (fig. 4). MPTP alone (a single 12.5-mg/kg dosage) resulted in a significant decrease in core temperature at 1 and 3 hr after dosing relative to mice given either saline or MK-801 alone (fig. 4, top panel). MK-801 administered 0.5 hr before and 3 hr after MPTP caused a significant hypothermia that endured for at least 7 hr. By 24 hr, mice receiving both MK-801 and MPTP had core temperatures significantly above those of mice given saline or MK-801 alone. Although MK-801 exacerbated the hypothermia induced by MPTP, it had no effect on either the decrease in DA (fig. 4, middle panel) or the increase in GFAP (fig. 4, bottom panel) caused by MPTP.

Effect of elevated ambient temperature on core temperature, striatal DA and GFAP in d-FEN-treated mice. Data in the accompanying paper (O'Callaghan and Miller, 1994) established that d-FEN, unlike d-METH, d-MDA and d-MDMA, lowers body temperature and does not alter striatal DA or GFAP. Because the data obtained in the present study link the neurotoxic effects of d-METH, d-MDA and d-MDMA to hyperthermia, they suggest that d-FEN might be neurotoxic if its ability to lower core temperature could be overcome. In agreement with the data in figure 3, an ambient temperature of 27°C causes an elevation in core temperature in saline-treated mice (fig. 5, top panel). d-FEN was able to overcome the significant hyperthermia due to an elevation in ambient temperature; indeed, mice given d-FEN were significantly hy-

pothermic relative to controls whether ambient temperature was 20.5 or 27°C. d-FEN did not alter striatal DA (fig. 5, middle panel) or GFAP (fig. 5, bottom panel) when given at an ambient temperature of 20.5 or 27°C.

Effect of ETOH, PB, DDC, d-FEN or COC on core temperature and MDMA-induced neurotoxicity. A variety of pharmacological agents reported to cause hypothermia were tested for their ability to lower core temperature and protect against the neurotoxic effects of d-MDMA (fig. 6). In general, the pharmacological agents that caused the greatest degree of hypothermia when given in combination with d-MDMA were the most neuroprotective. Thus, like MK-801 (fig. 3), pretreatment with ETOH and PB effected a complete (ETOH) or nearly complete (PB) block of the d-MDMA-induced decrease in striatal DA and increase in striatal GFAP. Note that the core temperature profiles obtained in the ETOH- and the PB-pretreated mice correspond closely to that obtained in MK-801-pretreated mice (fig. 2, lower panel). DDC and d-FEN pretreatments were not as effective as ETOH and PB pretreatments in causing hypothermia and they afforded only partial protection against the effects of d-MDMA on DA and GFAP (note that DDC was a very effective hypothermia-inducing agent when given alone, but not when given with d-MDMA). Pretreatment with COC was not effective in causing hypothermia, and it did not protect against the effects of d-MDMA on DA and GFAP.

Effect of restraint on core temperature and MDMA-induced neurotoxicity. Restraint alone or in combination with d-MDMA was a very effective procedure for producing hypothermia (fig. 7, top). Mice restrained and given d-MDMA are more hypothermic than those restrained and given saline. Restraint-induced hypothermia completely blocked the effects of d-MDMA on DA and GFAP.

Discussion

We have demonstrated that the neurotoxic effects d-METH, d-MDA and d-MDMA in the C57BL/6J mouse are linked, in some way, to their action(s) on body temperature. Conditions that elevate core temperature enhance AMP-induced neurotoxicity, whereas conditions that lower core temperature afford partial or complete neuroprotection. These findings add to the rich and varied history documenting the prevention or blunting of brain damage by lowering body temperature (Busto *et al.*, 1987, 1989; Fay, 1959; Green *et al.*, 1992; Ikonomidou *et al.*, 1989; Little, 1959; Lougheed and Kahn, 1955; Widmann *et al.*, 1993).

In the rat, striatal GFAP elevations and long-term decreases in DA are seen only with METH regimens that cause near lethal hyperthermia (J. P. O'Callaghan and D. B. Miller, personal observations; O'Callaghan and Miller, 1993). Indeed, an ice-bath "rescue" often is required to prevent METH-induced lethality under these dosing conditions (Bowyer *et al.*, 1994). When mice—especially grouped mice—are given sufficiently high doses of AMPs, they also may display severe and lethal hyperthermia (Askew, 1962). Our data, however, make it clear that substantial damage to mouse striatum can occur at dosages of d-METH and other AMPs that produce only minimal to moderate hyperthermia (*e.g.*, see figs. 1 and 2). The dosages of d-METH capable of effecting striatal damage in the rat and mouse are quite close, but the small body size of the mouse may allow for an efficient dissipation of heat, effectively preventing the production of lethal hyperthermia at neurotoxic dosages (for a discussion of body size and thermoregulation, see Gordon, 1993).

The three approaches we used to alter AMP neurotoxicity by affecting temperature, *i.e.*, lowered ambient temperature, pharmacological intervention and restraint, were all effective in preventing or attenuating AMP-induced neurotoxicity. In general, the latter two approaches were neuroprotective by virtue of the fact that they lowered core temperature, not because they prevented the small degree of AMP-induced hyperthermia. For example, conditions that resulted in large decrements in core temperature, such as restraint, ETOH and MK-801, resulted in complete protection against AMP-induced neurotoxicity, whereas conditions that resulted in a more modest degree of hypothermia, such as DDC, resulted in only partial neuroprotection. In contrast, cocaine, which completely blocked d-MDMA-induced hyperthermia but did not cause hypothermia, did not offer any protection against d-MDMA-induced neurotoxicity. These observations suggest that induction of hypothermia in AMP-treated mice is central to attenuation or blockade of AMP-induced neurotoxicity. Although the protective effects of lowered ambient temperature did not appear to require a reduction in core temperature (fig. 1), in these experiments it is possible that core temperature was decreased during the period of dosing but had recovered to control level by the time a measurement was taken (1 hr after the last AMP injection). PB-induced hypothermia followed just such a time course, and this pretreatment was nearly as effective as lowered ambient temperature in preventing d-MDMA-induced neurotoxicity. The effects of elevated ambient temperature also suggest that core temperature is an important determinant of AMP-induced neurotoxicity. For example, even a 3°C change in ambient temperature (20.5–23.5°C) further elevates the d-MDMA-induced increase in core temperature and elevates the neurotoxic effects of this AMP.

It has been proposed that EAAs play a critical role in nigrostriatal dopaminergic neurotoxicity resulting from exposure of mice to METH (Sonsalla *et al.*, 1989; 1991). Our data suggest that the neuroprotective actions of the EAA receptor antagonist MK-801 against AMP-induced neurotoxicity can be attributed in large measure to the propensity of this compound to lower core temperature. As noted above, the greatest neuroprotection against AMP-induced neurotoxicity was achieved by agents that cause large decreases in core temperature, a category of compounds that includes MK-801. Reversal of this hypothermic action of MK-801 in d-MDMA-treated mice by elevation of ambient temperature nearly abolished the neuroprotective actions of MK-801. Thus the neuroprotective action of MK-801 was at least partially dependent on its ability to lower core temperature in AMP-treated mice. Previous findings also have implicated temperature as a prominent mechanism of the neuroprotective action of MK-801. For example, MK-801 provides protection against the neurodegenerative effects of ischemia. Although this effect of MK-801 has been attributed to antagonism of the neurodegenerative effects of EAAs (Albers *et al.*, 1992), others question this interpretation because the protection against ischemic damage is associated with profound hypothermia (Buchan, 1992; Buchan and Pulsinelli, 1990; Corbett *et al.*, 1990). Although our findings and those of others do not rule out a role for EAAs in AMP-induced neurotoxicity, the alterations in core temperature caused by MK-801 when given in combination with AMPs in mice and in rats (Bowyer *et al.*,

1992, 1993, 1994) must be taken into consideration in studies of the neuroprotective effects of NMDA receptor antagonists.

In agreement with previous findings (O'Callaghan and Miller, 1993; Sonsalla *et al.*, 1989), MK-801 did not block the MPTP-induced decrease in striatal DA and the increase in striatal GFAP. As was the case for AMPs, however, MK-801 caused a profound hypothermia when given in combination with MPTP. We draw two general conclusions from these findings. First, lowered core temperature cannot be a generic means of protection against dopaminergic neurotoxicity in the C57BL/6J mouse, because MPTP-induced gliosis and DA depletion equivalent to that caused by AMPs (fig. 4 and O'Callaghan *et al.*, 1990) was not blocked by an equivalent degree of hypothermia induced by MK-801. Second, altered metabolism secondary to reduced core temperature may not play a role in the neuroprotective effects of hypothermia in AMP-treated mice. This second observation is based on the fact that metabolic activation as well as active transport by the catecholamine uptake carrier are essential for induction of neurotoxicity by MPTP (Mayer *et al.*, 1986; O'Callaghan *et al.*, 1990). These processes might be assumed to be temperature-dependent, yet a nearly 5°C reduction in core temperature by MK-801 failed to alter the neurotoxic effects of MPTP. A final argument against a metabolic interpretation for hypothermic neuroprotection comes from the work that demonstrates an exacerbation of d-amphetamine neurotoxicity by pretreatment with iprindole, an intervention known to slow the metabolism of this AMP and to increase the DA depletion associated with exposure (Fuller and Hemrick-Luecke, 1982). If anything, these latter data suggest that hypothermia might make AMPs more potent neurotoxicants. None of these arguments detract from the need to examine the role of altered metabolism and toxicokinetics in the temperature-dependence of AMP-induced neurotoxicity (Campbell, 1994).

In the previous paper (O'Callaghan and Miller, 1994) we showed that striatal levels of DA or GFAP were not affected in d-FEN-treated animals, nor was there evidence of fiber or terminal degeneration as assessed by silver staining (O'Callaghan and Miller, 1994). Because the striatal damage caused by d-METH, d-MDA and d-MDMA can be blocked by hypothermia, and because d-FEN caused profound hypothermia (O'Callaghan and Miller, 1994), we reasoned that d-FEN might be capable of causing dopaminergic neurotoxicity if its hypothermic effects could be blocked. However, striatal damage still was not evidenced in mice dosed with d-FEN and kept in a 27°C incubator. In fact, d-FEN was remarkably potent in preventing the elevation in core temperature displayed by control mice kept at this temperature; d-FEN-treated mice were still hypothermic even though kept at a warm temperature. It could be argued that d-FEN-treated mice must have core temperatures at least equivalent to those displayed after treatment with the other AMPs in order for us to state definitely that d-FEN is not capable of inducing dopaminergic neurotoxicity. However, our data showing that d-FEN protects against the damage induced by d-MDMA provides further evidence that d-FEN does not cause neural damage. Pretreatment with d-FEN prevented the hyperthermia associated with d-MDMA and was moderately effective in preventing d-MDMA-induced striatal damage. If d-FEN had neurotoxic properties in common with d-MDMA, as has been suggested on the basis of studies in the rat (Appel *et al.*, 1989; Commins *et al.*, 1985; Ricuarte *et al.*, 1985; Schuster *et al.*, 1986), then an exacerbation of MDMA neurotoxicity rather than a lessening might have been expected. Although we speculate that d-FEN protects the mouse striatum from d-MDMA-induced neurotoxicity because of its ability to produce hypothermia when given in combination with d-MDMA, other pharmacological properties of d-FEN may be responsible for its neuroprotective actions. For example, both d-FEN and d-MDMA are substrates for the serotonin uptake carrier in rats (Fuller *et al.*, 1988; McKenna and Peroutka, 1990). If d-FEN and d-MDMA share similar actions at sites on dopaminergic terminals in the mouse, then d-FEN may serve as a competitive inhibitor of d-MDMA at its yet unidentified target site(s). Despite these possibilities, our present and previous data, taken together, suggest that d-FEN does not share the neurotoxic properties associated with d-METH, d-MDA and d-MDMA.

Because various stressors exacerbate the general toxicity and lethality associated with the AMPs, restraint "stress" might be expected to increase the neurotoxicity of d-MDMA (Salama and Goldberg, 1969; Weiss *et al.*, 1961; Wilson, 1977). However, this manipulation afforded complete protection against the neurotoxicity of d-MDMA, probably because of the profound hypothermia associated with restraint. Other interpretations of the data are, however, possible. For example, steroids, including corticosterone, can protect against many types of CNS trauma (Norris and Hachinski, 1986; Tosaki *et al.*, 1985; Tuor *et al.*, 1993). Many stressors, including restraint, activate the HPA axis, resulting in elevated levels of circulating corticosterone, which could contribute to the neuroprotection afforded by restraint (Johnson *et al.*, 1992). However, the fact that AMPs themselves cause a release of corticosterone in both rat and mouse (Nash *et al.*, 1988; Pauly *et al.*, 1993) makes this interpretation of our restraint data less tenable.

In summary, we find the ability of AMPs to induce neurotoxicity to be highly dependent on their interactions with body temperature, and any treatment able to cause hypothermia when mice have been treated with AMPs has the potential to be neuroprotective. Although the effects of AMPs on body temperature and the role of body temperature in their lethality and toxicity have been continuously investigated since the 1940s (Askew, 1962; Chance, 1946; Swinyard *et al.*, 1961) the importance of body temperature in their neurotoxic actions is only now being recognized (Bowyer *et al.*, 1992, 1993, 1994; Schmidt *et al.*, 1990).

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