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The Role of Temperature, Stress, and Other Factors in the Neurotoxicity of the Substituted Amphetamines 3,4-Methylenedioxymethamphetamine and Fenfluramine

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Abstract

Amphetamines (AMPs) can cause long-term depletions in striatal dopamine (DA) and serotonin (5-HT), and these decrements are often accepted as *prima facie* evidence of AMP-induced damage to the dopaminergic and serotonergic projections to striatum. Rarely are indices linked to neural damage used to evaluate the neurotoxicity of the AMPs. Here, we determined the potential neurotoxic effects of two substituted AMPs, *d*-methylenedioxymethamphetamine (d-MDMA) and *d*-fenfluramine (d-FEN) in group-housed female C57BL6/J mice. Astrogliosis, assessed by quantification of glial fibrillary acidic protein (GFAP), was the main indicator of d-MDMA-induced neural damage. Assays of tyrosine hydroxylase (TH), DA, and 5-HT were used to determine effects on DA and 5-HT systems. Since AMPs are noted for both their stimulatory and hyperthermia-inducing properties, activity, as well as core temperature, was monitored in several experiments. To extend the generality of our findings, these same end points were examined in singly housed female C57BL6/J mice and in group-housed male C57BL6/J or female B6C3F1 mice after treatment with d-MDMA. Mice received either d-MDMA (20 mg/kg) (singly housed mice received dosages of 20, 30, or 40 mg/kg) or d-FEN (25 mg/kg) every 2 h for a total of four sc injections. d-MDMA caused hyperthermia, whereas d-FEN induced hypothermia. d-MDMA cause a large (300%) increase in striatal GFAP that resolved by 3 wk and a 50–75% decrease in TH and DA that was still apparent at 3 wk, d-FEN did not affect any parameters in striatum. d-MDMA is a striatal dopaminergic neurotoxicant in both male and female C57BL6/mice, as evidenced by astrogliosis and depletions of DA in this area in both sexes. The greater lethality to males suggests they may be more sensitive, at least to the general toxicity of d-MDMA, than females. d-MDMA (20 mg/kg) induced the same degree of damage whether mice were housed singly or in groups. Higher dosages in singly housed mice induced greater lethality, but not greater neurotoxicity. d-MDMA was also effective in inducing striatal damage in mice of the B6C3F1 strain. Significant increases in activity were induced by d-MDMA, and these increases were not blocked by pretreatment with MK-801, despite the profound lowering of body tempera-

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ture induced by this combination. A lowering of body temperature, whether by a 15°C ambient temperature (approx 2°C drop), pretreatment with MK-801 (1.0 mg/kg prior to the first and third d-MDMA injections; approx 5–6°C drop) or restraint (approx 5–6°C drop), was effective in blocking the neurotoxicity of d-MDMA in both C57BL6/J and B6C3F1. The stimulatory effects of d-MDMA appeared to have little impact on the neurotoxicity induced by d-MDMA or the protection conferred by MK-801. These data suggest that in the mouse, the neurotoxic effects of d-MDMA, and most likely other AMPs, are linked to an effect on body temperature.

Index Entries: AMPs; d-MDMA; d-FEN; GFAP; stress; MK-801; dopamine; neurotoxicity; striatum; temperature.

Introduction

Certain of the substituted amphetamines (AMPs), including amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, and fenfluramine, can cause substantial depletions of dopamine (DA), serotonin (5-HT), and their respective metabolites in the striatum and cortex (Fuller, 1978; Wagner et al., 1980; Schmidt, 1987; Sonsalla et al., 1989, 1991). These decrements are accepted by many as evidence of damage to the dopaminergic and serotonergic projections to these areas. Such changes may indeed reflect damage to axonal projections or terminals, but alternatively, may reflect pharmacological regulation of dopaminergic and serotonergic function (Lee et al., 1983; Battaglia et al., 1987; Lesch et al., 1993). Moreover, most studies of AMP-induced neurotoxicity, regardless of the end points examined, have utilized a rat model; relatively little attention has been focused on the effects of these agents in the mouse. Thus, one purpose of this work was to examine the neurotoxicity of two of the AMPS, *d*-3,4-methylenedioxymethamphetamine (d-MDMA) and *d*-fenfluramine (FEN), in the C57BL6/J mouse using a general marker of neuronal injury, reactive gliosis. The putative neurotoxicity of both d-MDMA and d-FEN has been the focus of controversy (Ricaurte et al., 1985; Kleven and Seiden, 1989). The use of this general marker of damage provides a means for determining the significance of AMP-induced reductions in biochemical markers of dopaminergic and serotonergic innervation. Reactive gliosis has proven to be a sensitive indicator of chemical-induced injury of the CNS, especially where targets of injury are not known, and when loss of neural perikarya does not occur or is not obvious (as is the case with the AMPs) (O'Callaghan, 1993; O'Callaghan and Miller, 1993).

The effects of the AMPs, regardless of whether they are indicative of neuronal damage, can be markedly species-, strain-, and sex-dependent. For example, MDMA appears to affect predominantly the cortical and striatal serotonergic systems in the rat (e.g., *see* Stone et al., 1987; Logan et al., 1988) and the striatal dopaminergic system in the mouse (Logan et al., 1988). Further, different strains of mice demonstrated marked differences in sensitivity to the DA depleting actions of MDMA (Zheng and Laverty, 1993). To obtain a more complete assessment of the neurotoxicity of MDMA, we also examined the striatal dopaminergic damaging properties of this AMP in both male and female C57BL6/J mice as well as an additional strain, the B6C3F1.

Striking differences are observed in the core temperatures of rats exposed to d-FEN and d-MDMA at room temperature (~21–22°C) with d-MDMA often causing a lethal hyperthermia and in d-FEN-treated subjects a marked hypothermia (Gordon et al., 1991; Miller et al., 1991; Miller and O'Callaghan, 1994). Subsequent work has identified a similar pattern in the C57BL6/J mouse (O'Callaghan and Miller, submitted for publication). We reasoned the hyperthermia associated with d-MDMA exposure may be a key element in its neurotoxicity and examined the role of body temperature by two principal means: (1) altering ambient temperature and (2) pretreatment with agents or conditions that lower body temperature. Stimulation, as evidenced by increased activ-

ity, is another prominent action of the AMPS, and certain experimental settings or conditions, including group housing or various stressors, can exacerbate both (Chance, 1946; Hohn and Lasagna, 1960; Askew, 1962; Herman et al., 1984; Gold et al., 1988; Gordon et al., 1991; Badiani et al., 1992). There has, however, been little work concerning the interactions of these factors with the neurotoxicity of the AMPs. Thus, we examined alterations in ambient temperature, pretreatments affecting core temperature, and aggregation for their impact on the dopaminergic damage induced by MDMA. The increased stimulation caused by AMP exposure is often considered to be a factor in the hyperthermia that also accompanies exposure (*see* Bushnell, 1986). To address the contributions of stimulation to the neurotoxic effects of d-MDMA, we monitored motor activity as well as core temperature in several of our experiments. Our data indicate core temperature plays a key role in the striatal damage induced by MDMA, but its stimulatory actions appear not to play a role in its neurotoxicity.

Materials and Methods

Materials

d-MK-801 were purchased from Sigma Chemical Co. (St. Louis, MO) and Research Biochemicals, Inc. (Natick, MA). d-MDMA was generously supplied by the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). d-FEN was the generous gift of Les Laboratoire Servier (Fleury-les-Aubrais, France). The materials used in the GFAP assay have been described in detail in O'Callaghan (1991). The materials used in the purification of TH from rat clonal pheochromocytoma cells and in the TH immunoassay have been described previously (O'Callaghan et al., 1990). Rabbit polyclonal antisera to TH (#9022) was a gift from John F. Reinhard, Jr. (The Wellcome Research Laboratories, Research Triangle Park, NC). BCA protein assay reagent and bovine serum albumin were obtained from Pierce Chemical Co. (Rockford, IL). Reagents used for high-performance liquid chromatography (HPLC) were of HPLC grade and were purchased from Burdick and Jackson (Muskegon, MI); HPLC standards were purchased from Sigma. All other reagents were of at least analytical grade and were obtained from a variety of commercial sources.

Animals

Female and male C57BL/6J mice were obtained from Jackson Labs (Bar Harbor, ME) or female B6C3F1 from Charles River Laboratories (Portage, MA) and ranged from 2–6 mo when used in these experiments (*see* individual figure legends for exact age). On receipt, the mice were housed in groups of 10–12 in a temperature- (21 ± 1°C) and humidity- (50 ± 10%) controlled colony room maintained under filtered positive-pressure ventilation on a 12-h light 12-h dark cycle beginning at 6:00 AM EDT. The dimensions of the plastic tub cages were 46 cm in length × 25 in. in width × 15 cm in height; cage bedding consisted of heat-treated pine shavings spread at a depth of approx 4 cm. Food (Purina rat/mouse chow) and water were available ad libitum.

Temperature Measurement

Rectal temperature was recorded with a Bat-10 thermometer coupled to a RET-3 mouse rectal probe (time constant of 0.5 s; Physitemp, Inc., Clinton, NJ) lubricated with mineral oil. To measure their rectal temperatures, mice were placed under a "Quonset hut"-shaped piece of foam approximately the length of the mouse 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 fast-rise time of the rectal probe, permitted reliable measurements of rectal temperature to be obtained in <30–40 s/mouse. Temperature sampling times varied from experiment to experiment. In the experiments where ambient temperature was manipulated, core temperature was monitored at 1 h after the

last d-MDMA injection. In other experiments, core temperature was monitored throughout the dosing period. *See* individual figure legends for exact details.

Dosing

Preliminary observations indicated that administration of d-MDMA, at the dosage and regiment selected for this study, caused significant lethality if mice were housed 10–12/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, were moved to a new cage of the same size. After assignment to a new cage, mice were weighed and approx 0.5 h later, a baseline temperature was taken. At 0.5 h following the baseline temperature, mice received their first of four sc injections of d-MDMA or d-FEN; each injection was given 2 h apart. Mice received either saline, d-MDMA (20, 30, or 40 mg/kg) or d-FEN (25 mg/kg); dosages were as the base. The first injection was always given between 10:00 and 11:00 AM to minimize circadian influences on toxicity. In the experiments evaluating the effects of lowered ambient temperature or altered housing, mice were individually housed without bedding in small plastic cages (22 cm long × 14 cm wide × 15 cm high) or with bedding in large plastic cages (46 cm long × 25 cm wide × 15 cm high), respectively. Mice were placed in the 15°C ambient temperature approx 0.5 h prior to obtaining a baseline temperature. In the experiments that evaluated the effects of MK-801 (1.0 mg/kg as the base) pretreatment, it was given sc, 0.5 h prior to the first and third injections of d-MDMA. This dosage of MK-801 was selected based on published work (Sonsalla et al., 1989).

Restraint

When subjected to restraint, mice were placed in the restrainers approx 0.5 h prior to the first AMP injection. Plastic centrifuge tubes (50 mL) were adapted for use as restrainers that snugly restrained the mice and prevented their turning from front to back, although they could rotate from a supine to 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, and were placed in an incubator maintained at 20.5°C. Baseline temperature measurement was taken immediately prior to placement the restrainer. At the time an injection was to be given, mice were removed from the restrainer, a temperature measurement was obtained, and the injection was then given. Mice remained in the restrainer for 0.5 h following the final injection. After removal from the restrainer, temperature was measured, and mice were housed 6/cage until collection of tissue for assay; other mice in the cage had received the same treatment.

Activity Measurement

The activity of grouped mice ($n = 6$) placed in large (46 cm long × 25 cm wide × 15 cm high with bedding 1 cm deep) plastic cages was monitored with a cage monitoring system (San Diego Instruments, San Diego, CA). This monitoring system consisted of a frame equipped with three infrared photocell pairs that fit around the perimeter of the cage. Three separate pairs of cells each project an infrared beam that bisected the width of the cage at 12.5, 17.0, and 32 cm. Frame heights were adjusted to a height (approx 2 cm) that would allow interruption of the beams by passage of a mouse in front of the beam. Any interruption of a beam is logged as an activity count. Because mice were tested in groups of six no statistical comparisons were made between different treatment groups.

Brain Dissection and Tissue Preparation

Immediately after decapitation, whole brains were removed from the skull. The left and right

striatum were dissected free-hand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI), weighed, and stored at 70°C until biochemical assay. Striatum from the left side of the brain was weighed, frozen on dry ice, and stored at –70°C for subsequent analysis of DA or 5-HT by HPLC. Striatum from the right side was weighed, homogenized with a sonic probe (model XL-2005, Heat Systems, Farmingdale, NY) in 10 vol of hot (90–95°C) 1% SDS, and stored frozen at –70°C before immunoassay of GFAP or TH.

GFAP Immunoassay, Protein Assay, DA, and 5-HT Analysis

GFAP was assayed according to modifications of a previously described sandwich ELISA (O'Callaghan, 1991). The method of Smith et al. (1985) was used to determine total protein concentrations. HPLC with electrochemical detection was used to determine the levels of DA and 5-HT (*see* O'Callaghan and Miller [1994] for details).

Statistics

The Statistical Analysis System (SAS Institute Inc., 1986) was used to analyze the data. Individual variables were subjected to an analysis of variance followed by Duncan's Multiple Range Test for comparisons of means. An α-level of 0.05 was used to determine significance. Statistical comparisons are confined to comparisons within a given experiment, since the reported studies were not conducted during the same period.

Results

Time–Response Analysis

Injection of four dosages of d-MDMA, but not d-FEN, resulted in large increases in the concentration of striatal GFAP (Fig. 1). The onset was rapid and was at least 150% of control by 24 h after the fourth injection with a maximum of approx 300% by 72 h. Over the following 21 d, GFAP showed a gradual decline toward baseline. The d-MDMA-induced increase in striatal GFAP was accompanied by a decrement in striatal DA and TH (Fig. 1) with an approx 75% decrement in both that showed no recovery by 21 d postdosing. As was the case for GFAP, d-FEN did not affect DA or TH. Striatal 5-HT was reduced by both d-MDMA and d-FEN, but a return to control levels by 21 d was observed for both compounds (Fig. 1). It should be noted these 5-HT depletions are much smaller than the DA decrement observed following d-MDMA.

Effects on Core Temperature

d-MDMA and d-FEN had dramatically different effects on core temperature (Fig. 2). d-MDMA cause a small, about 1°C, elevation during and after the dosing period. d-FEN, in contrast, caused a marked (>3°C) decline in core temperature that persisted up to at least 1 h after the last injection. One day after dosing, both the d-FEN- and d-MDMA-treated mice showed a core temperature above saline controls.

Effect of Lowered Ambient Temperature on Core Temperature and d-MDMA-Induced Neurotoxicity

Mice given d-MDMA at an ambient temperature of 22°C displayed significant elevations in core temperature at 1 h after the last injection compared to controls injected with saline (Fig. 3, top panel). In contrast, mice maintained at 15°C (a total of 9 h) from 0.5 h before the first injection until 0.5 h following the fourth injection, regardless of treatment, did not have core temperatures different from mice housed at 22°C. d-MDMA administered at an ambient temperature of 22°C caused large elevations (>300%) in striatal GFAP and a large (>75%) decrease in DA (Fig. 3, middle and bottom panels), changes indicative of striatal damage. However, if d-MDMA was administered

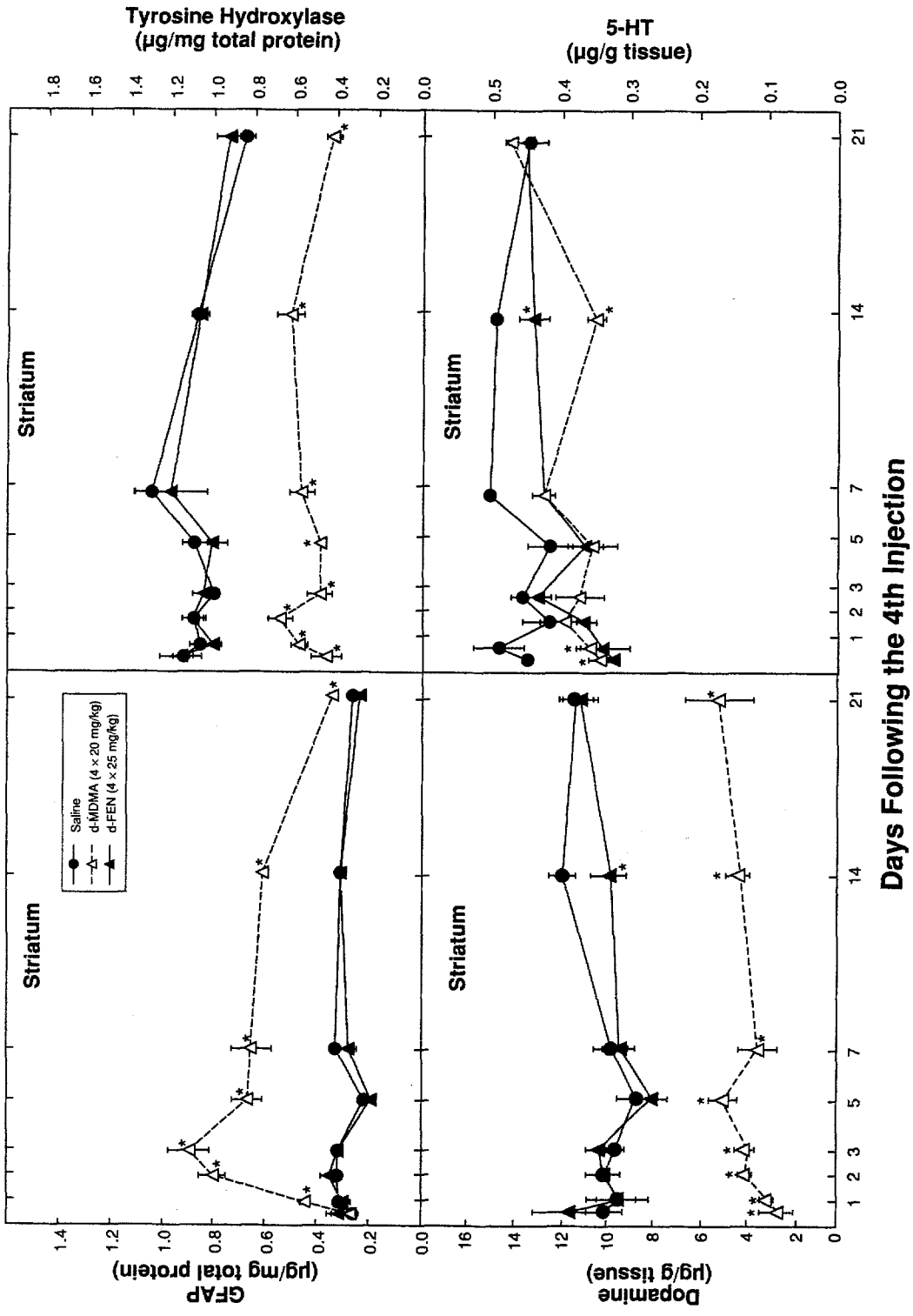

Fig. 1. Time-course of the effects of d-MDMA (20 mg/kg × 4) and d-FEN (25 mg/kg × 4) on the concentration of striatal GFAP, TH, DA, and 5-HT in female C57BL6/J mice. Mice were approx 3 mo of age at the time of dosing. Each value represents the mean ± SEM for six mice. Figure is adapted from O'Callaghan and Miller (1994). *Significantly different from corresponding SAL.

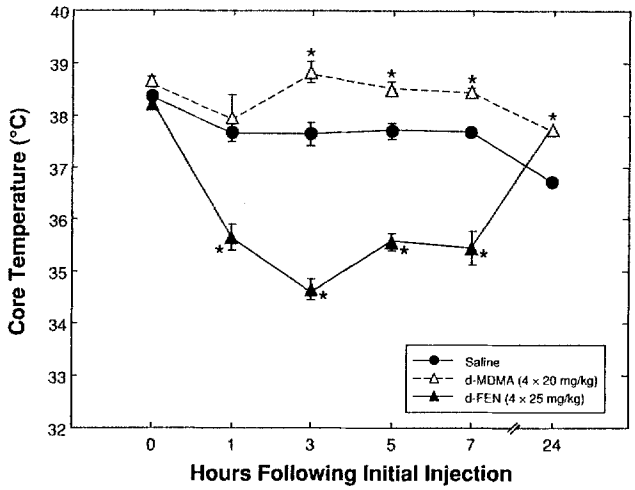

Fig. 2. Time-course of the effects of d-MDMA (20 mg/kg × 4) and d-FEN (25 mg/kg × 4) on core temperature in female C57BL6/J mice. Mice were approx 3 mo of age at the time of dosing. Each value represents the mean ± SEM for six mice. Figure is adapted from O'Callaghan and Miller (1994). *Significantly different from corresponding SAL.

at an ambient temperature of 15°C, no change in either striatal GFAP or DA was observed.

Effect of Single Housing on d-MDMA-Induced Neurotoxicity

Since the mice subjected to an ambient temperature of 15°C were housed singly to prevent conservation of heat by huddling, it was important to determine that d-MDMA was neurotoxic to mice under these housing conditions. For most of the dosing period, mice receiving 20, 30, or 40 mg/kg of d-MDMA had core temperatures above that of saline-injected controls (Fig. 4, top panel). By the end of the dosing period (7.5 h after the first injection), the mice receiving 20 mg/kg were still significantly hyperthermic. The temperatures of mice receiving 30 mg/kg of d-MDMA were no different than saline controls, whereas those mice receiving 40 mg/kg of d-MDMA were hypothermic relative to controls. However, there was a great deal of variability in the core temperatures of the mice in the 30 and 40 mg/kg groups as evidenced by the large standard errors.

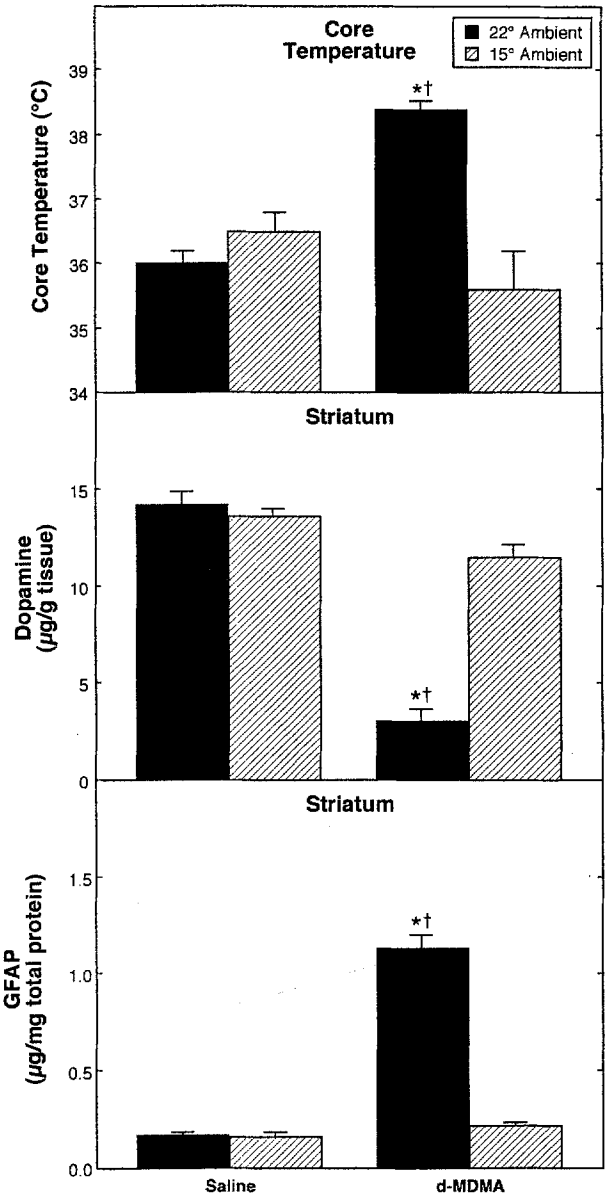

Fig. 3. The influence of lowered ambient temperature on the alterations in core temperature, striatal DA, and striatal GFAP induced by d-MDMA in the C57BL6/J female mouse. Mice were approx 3 mo of age at the time of dosing, and striatal samples were obtained 72 h after treatment. Each value represents the mean ± SEM. Figure is adapted from Miller and O'Callaghan (1994). *Significantly different from SAL at same ambient temperature. †Significantly different from same treatment group kept at 15°C.

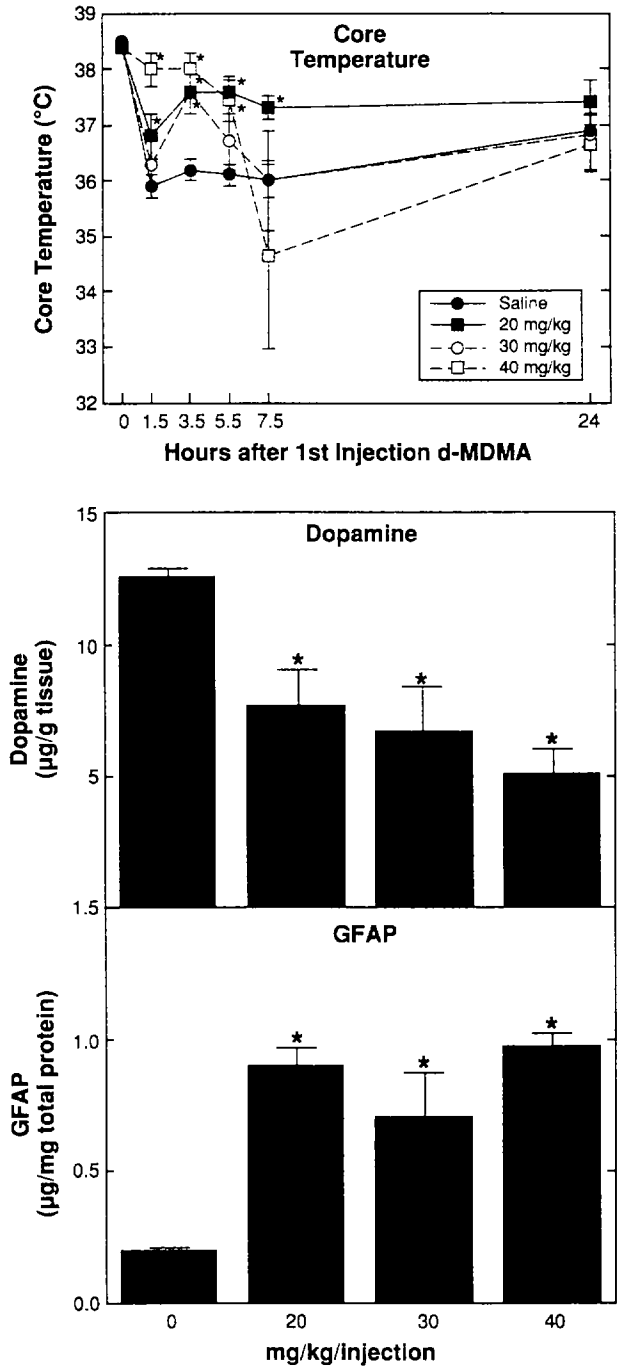

Fig. 4. The effects of 0 (n = 6), 20 (n = 5), 30 (n = 4), or 40 (n = 4) mg/kg of d-MDMA on core temperature, striatal DA, and GFAP in C57BL6/J female mice singly housed. Mice were approx 4 mo of age at the time dosing, and striatal samples were obtained 72 h after treatment. Overall ANOVAs revealed no effect of dose of d-MDMA on dopamine or GFAP. Each value represents the mean ± SEM. *Significantly different from SAL.

However, equivalent neurotoxicity was induced by all dosages as indicated by equivalent DA depletions (Fig. 4, middle panel) and GFAP elevations (Fig. 4, bottom panel).

d-MDMA-Induced Neurotoxicity, Core Temperature, Activity, and MK-801 Protection in Female C57BL6/J and B6C3F1 Mice

Female C57BL6/J female mice receiving d-MDMA (Fig. 5, upper left) displayed core temperatures that were elevated relative to saline controls most of the dosing period and elevated relative to the MK-801 group following the second injection of d-MDMA. As has been reported (Pechnick et al., 1989), mice receiving MK-801 had elevated temperatures relative to the saline controls. In contrast, a combination of MK-801 and d-MDMA caused a profound, protracted hypothermia that was still evident at the end of the dosing period. MK-801 did not prevent the d-MDMA-induced increase in temperature observed at 24 h.

Considering the known stimulatory effects of the AMPs, it is not surprising that mice receiving d-MDMA were much more active (Fig. 5, lower left) than saline controls with the increase being most prominent at the beginning of the dosing period. These mice were still more active than saline controls at the end of dosing, but stereotypic behaviors, not as likely to be counted, rather than forward locomotion, may have predominated. Prominent increases in activity were also displayed throughout the dosing period by mice receiving MK-801 Surprisingly, considering the degree of hypothermia displayed, mice receiving MK-801 and d-MDMA were more active than saline controls during the dosing period. Interestingly, these same mice displayed an even more prominent elevation in activity during the lights-out period.

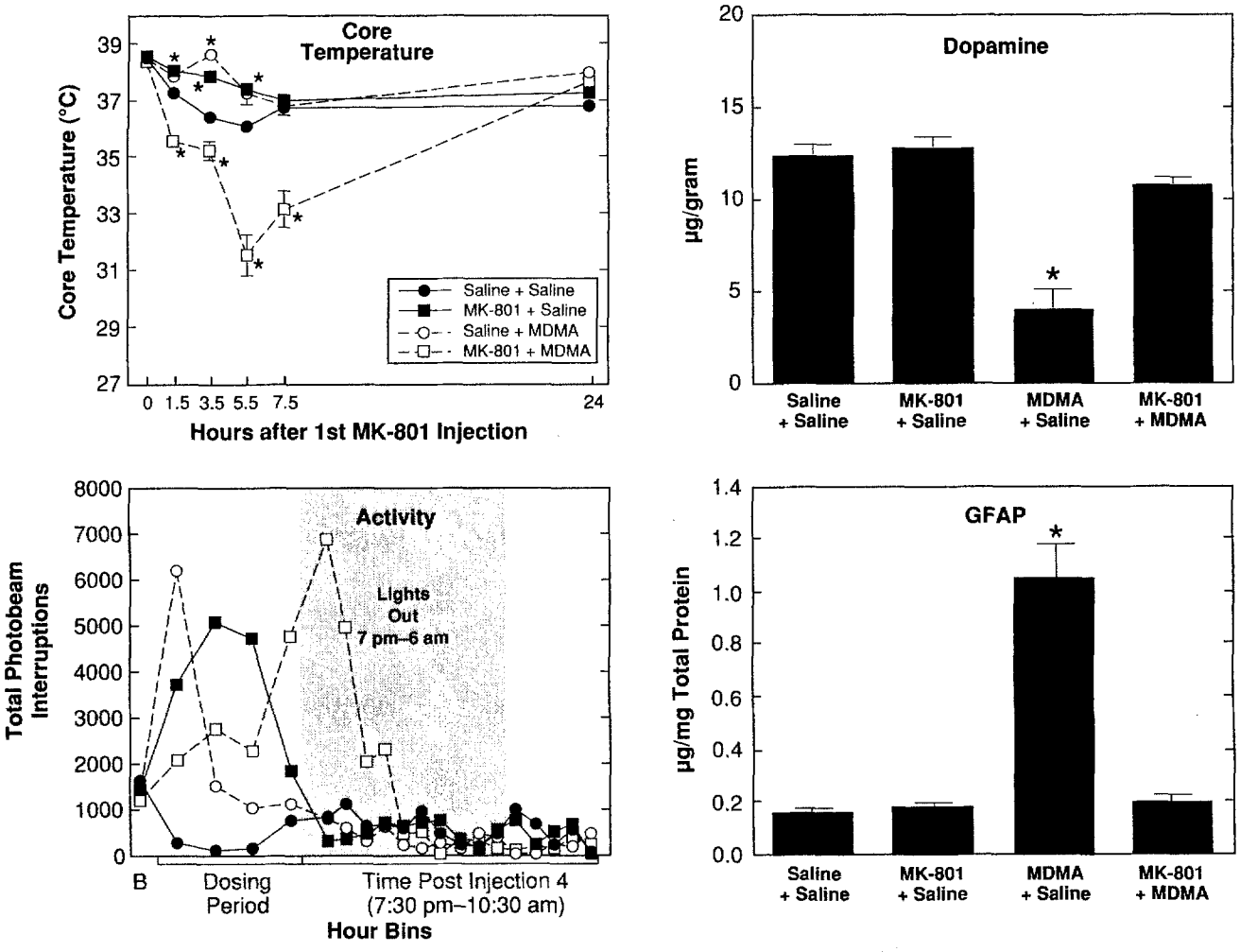

Fig. 5. The effects of d-MDMA (20 mg/kg) on the core temperature, activity, striatal DA, and striatal GFAP in female C57BL6/J mice with and without MK-801 (1.0 mg/kg) pretreatment. Mice were approx 4 mo of age at the time of dosing. Each value represents the mean ± SEM for six mice. *Significantly different from SAL-SAL.

As we have reported previously (Miller and O'Callaghan, 1993, 1994; O'Callaghan and Miller, 1994) four injections of d-MDMA significantly elevated (>400%) levels of GFAP in striatum (Fig. 5, lower right) accompanied by a significant depletion (~70%) of DA in this same area (Fig. 5, upper right). MK-801 pretreatment completely blocked the neurotoxicity of d-MDMA, while having no effect when given alone on either striatal GFAP or DA.

B6C3F1 female mice receiving d-MDMA plus saline had core temperatures significantly elevated above both saline control and MK-801 plus saline-treated mice for most of the duration of the dosing period (Fig. 6, upper left). An elevation of approx 1°C was apparent by an hour after the first injection and was still evident after the fourth injection. As we have previously observed (Miller and O'Callaghan, 1994), mice receiving MK-801 and saline had similar core temperatures that declined across the period of testing. Pretreatment with MK-801 plus saline not only prevented the elevation in temperature caused by d-MDMA, but pro-

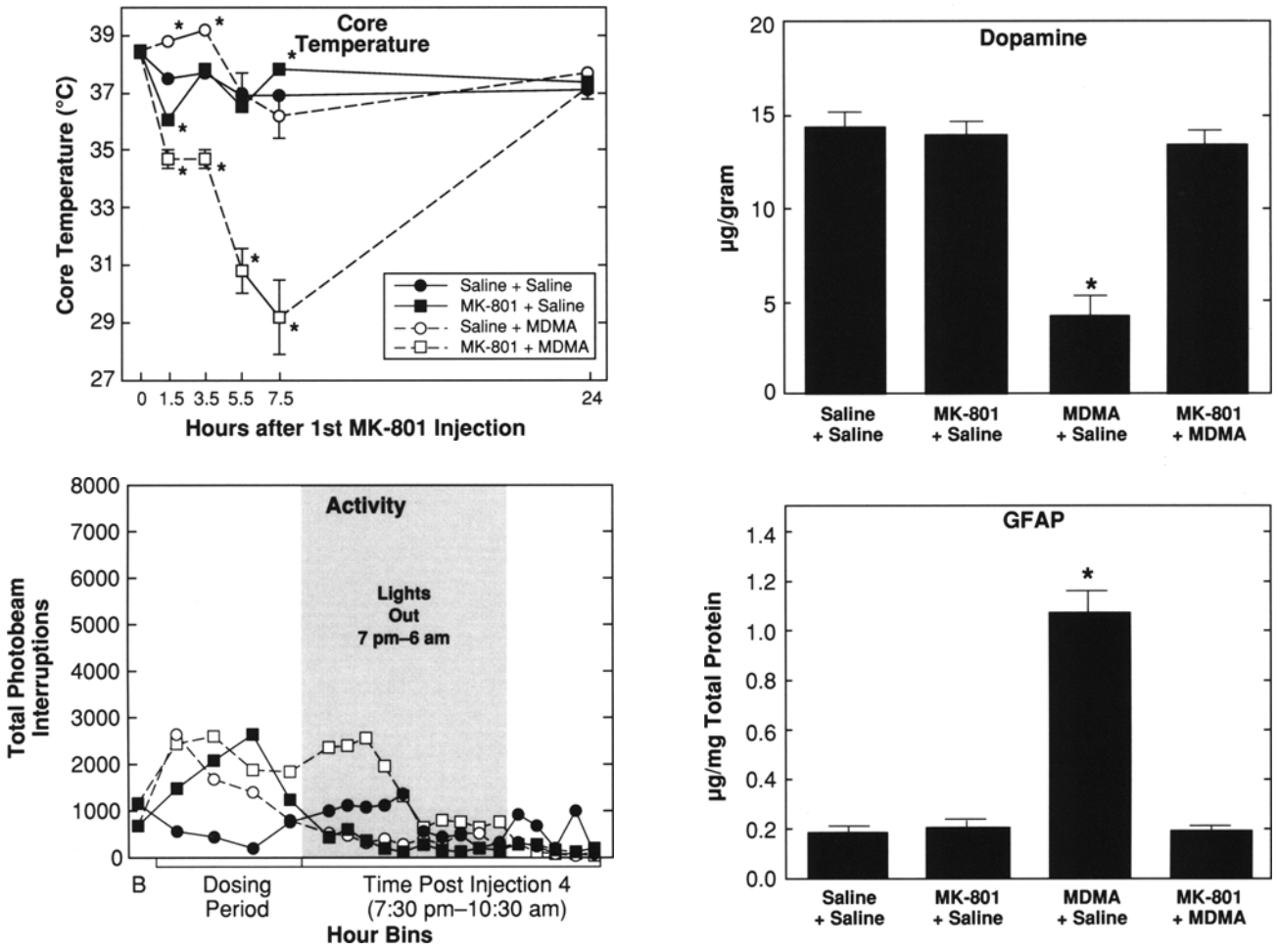

Fig. 6. The effects of d-MDMA (20 mg/kg) on the core temperature, activity, striatal DA, and striatal GFAP in female B6C3F1 mice with and without pretreatment with MK-801 (1.0 mg/kg). Mice were approx 6 mo of age at the time of dosing. Each value represents the mean ± SEM for six mice. *Significantly different from SAL.

duced in this strain, as in the C57BL6/J, a protracted hypothermia. B6C3F1 mice displayed a greater hypothermia than C57BL6/J with the lowest temperature around 29°C in contrast to 32°C. Unlike the C57BL6/J, no differences among groups were noted by 24 h.

Like C57BL6/J mice (Fig. 6, lower left), B6C3F1 mice receiving d-MDMA were more active than those given saline (Fig. 6, lower left) with the increases most prominent in the early portion of the dosing period. MK-801 also elevated activity above control levels with a return to saline levels by the lights-out period. Mice given both d-MDMA and MK-801 displayed a prominent increase in activity beginning during the dosing period and lasting throughout most of the dark period. Although d-MDMA and MK-801 are clearly stimulatory in both C57BL6/J and B6C3F1, the degree of stimulation observed in the B6C3F1 was minor compared to that displayed by the C57BL6/J (Fig. 6, lower left). Conversely, since activity counts require movement directly in front of the photocell, the lower activity counts of the B6C3F1 may represent increased stereotypy rather than less stimulation in this strain. A determination of

the exact effects of AMPs on activity and stereotypy will require a direct comparison of these two strains utilizing direct observation techniques or an automated device able to separate motor stimulation from stereotypy.

The effect of d-MDMA on striatal DA and GFAP in B6C3F1 mice (Fig. 6, right side) was almost identical to that found in C57BL6/J mice (Fig. 5, right side). Treatment with four injections of d-MDMA was neurotoxic to this strain as demonstrated by a GFAP elevation in striatum >400% that was accompanied by a significant depletion of DA (~70%)

Effect of MK-801 on Core Temperature and d-MDMA-Induced Neurotoxicity in C57BL6/J Male Mice

Male mice of this strain receiving d-MDMA displayed a hyperthermia (Fig. 7) that was prominent by 1.5 h and reached a peak of ~40°C by 3.5 h after the first injection. Core temperature was still significantly above saline control by 5.5 h, but was no different than saline by 7.5 h after the first injection. By 24 h their core temperature was several degrees lower than the other groups and may reflect general toxicity; four of the six original mice died after the fourth injection (only temperatures of the survivors are presented). A slight but significant hyperthermia was also caused by MK-801; these elevations of 0.5–1.0°C were apparent during most of the dosing period, but had resolved by 7.5 h. MK-801 pretreatment prevented the severe hyperthermia (and lethality) associated with d-MDMA but did not produce the profound hypothermia seen in female mice of this strain (Fig. 5, upper left).

d-MDMA dearly causes damage to the striatum of male C57BL6/J mice (Fig. 7) as it does to female mice (Fig. 5) of the same strain. A significant elevation in striatal GFAP levels was accompanied by large decrements in DA. Pretreatment with MK-801 totally blocked the neurotoxic effects of d-MDMA-induced neurotoxicity, as it does in females of the C57BL6/J strain.

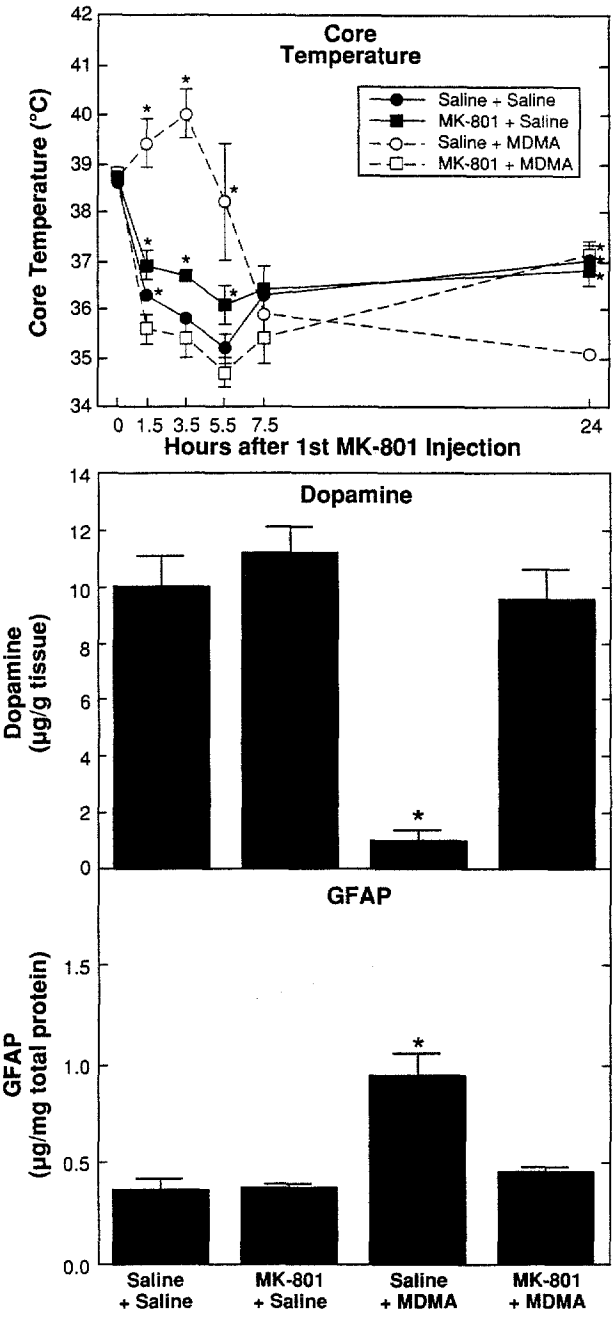

Fig. 7. The effects of d-MDMA (20 mg/kg) on the core temperature, striatal DA, and striatal GFAP in male C57BL6/J mice with and without pretreatment with MK-801 (1.0 mg/kg). Mice were approx 4 mo of age at the time of dosing. Each value represents the mean ± SEM for six mice except the group receiving d-MDMA ($N = 2$ because of deaths). Mice were approx 4 mo of age at dosing. *Significantly different from SAL.

Effect of Restraint on Core Temperature and d-MDMA-Induced Neurotoxicity

In female C57BL6/J mice, restraint alone or in combination with d-MDMA was a very effective procedure for producing hypothermia (left side of Fig. 8). Mice restrained and given d-MDMA are more hypothermic than those restrained and given saline. Further, restraint-induced hypothermia in this strain completely blocked the effects of d-MDMA on DA and GFAP. Surprisingly, considering C57BL is one of the parent strains of the B6C3F1 strain, restraint alone had little effect, producing only a very weak hypothermia in this strain (right side of Fig. 8). B6C3F1 mice given d-MDMA and restrained were severely hypothermic. Despite this degree of hypothermia, an elevation in GFAP was still evident, although it was about 50% lower than that seen with d-MDMA alone. The depletion in DA induced by d-MDMA, however, was completely blocked by restraint.

Discussion

Astrocytic reactivity results in an enhanced expression of GFAP that can be used to localize and quantify chemically induced damage to the CNS (O'Callaghan, 1993). Here, assays of GFAP indicated d-MDMA damages the striatum of C57BL6/J mice and B6C3F1 mice. Protracted decrements in DA and TH suggest that dopaminergic projections to striatum were the stimulus for this reactive gliosis. In contrast, d-FEN caused no changes in striatal GFAP, DA, or TH. The reduced levels of striatal 5-HT found with both compounds are likely not indicative of damage to serotonergic axons; decrements were small and not persistent. Our data suggest d-MDMA, but not d-FEN, is a dopaminergic neurotoxicant in the mouse. Other AMPs (e.g., methamphetamine) have been identified as dopaminergic neurotoxicants in both the rat and mouse, but d-MDMA is identified primarily as a serotonergic neurotoxicant (Ricuarte et al., 1985; Schmidt, 1987; Commins et al., 1987). However, our data indicate the rat and mouse respond differently to d-MDMA and are in agreement with the conclusion of others, namely that MDMA primarily affects the dopaminergic system in mice and the serotonergic system in rats (Logan et al., 1988).

d-MDMA clearly damages the striatum in mice and is able to induce this neurotoxicity at dosages that cause a mild to moderate hyperthermia. In contrast, striatal GFAP elevations and DA depletions are seen in the rat after AMP exposure only with dosing regimens that produce a lethal or near-lethal hyperthermia. In fact, these neurotoxic regimens often require an ice-bath "rescue" (Bowyer et al., 1993). Temperature plays a role in the damage induced by MDMA, but it is also obvious that a moderate hyperthermia alone will not cause striatal damage. Control mice, as well as mice given MK-801 (e.g., often displayed temperatures as high as mice given MDMA). Our data also indicate conditions capable of causing a lowered core temperature are neuroprotective, which is commensurate with other work documenting the prevention or blunting of brain damage by lowering body temperature (Fay, 1959; Busto et al., 1989; Widmann et al., 1993).

The three approaches we used to alter d-MDMA neurotoxicity by affecting temperature, i.e., lowered ambient temperature, pharmacological intervention through pretreatment with MK-801, and restraint, were all effective in preventing or attenuating AMP-induced neurotoxicity. In general, the latter two approaches were likely neuroprotective because they produced hypothermia. Although a lowered ambient temperature was neuroprotective, a core temperature drop was not evident (Fig. 3) under the conditions we employed. It is possible that core temperature was decreased during the period of dosing, but had recovered to control by the time of measurement (1 h after the last d-MDMA injection). Our data implicate temperature as a prominent mechanism in the neuroprotective action of MK-801 and call into question the notion that excitatory amino adds

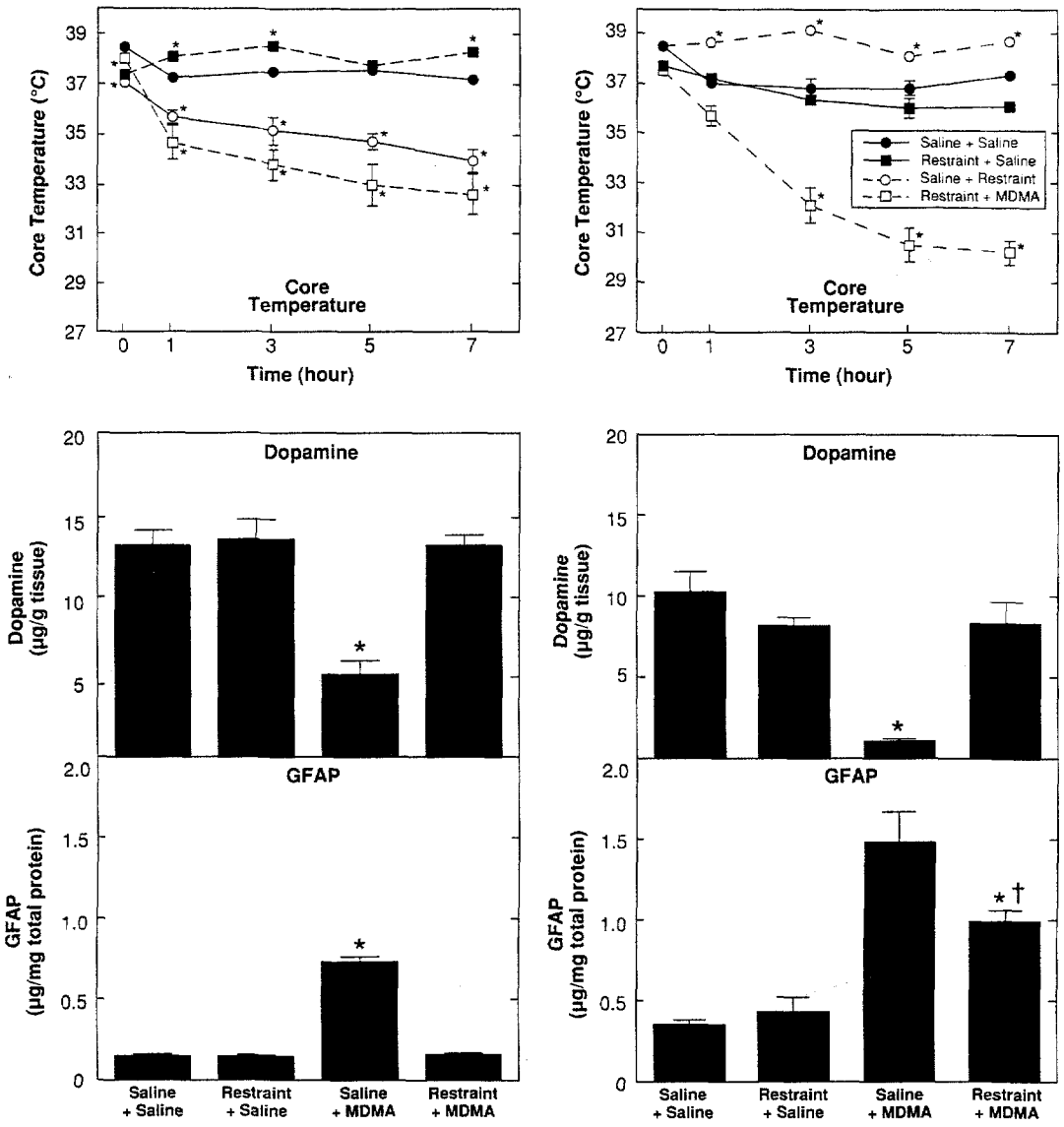

Fig. 8. The effects of restraint on the alterations in core temperature, striatal DA, and striatal GFAP induced by d-MDMA (20 mg/kg × 4) in female C57BL6/J **(left)** (3.5 mo of age) or B6C3F1 **(right)** (approx 5 mo of age) mice. Mice were restrained from 0.5 h before the first injection of d-MDMA until 0.5 h after the fourth injection of d-MDMA. Core temperature measurements were made at 0.5 h following assignment to a new cage or after restraint (baseline or 0) and at 1, 3, 5, and 7 h after the first d-MDMA injection. For the measurements at 1, 3, 5, and 7 h, this reading also represents a reading at 1 h after d-MDMA injection 1, 2, 3, and 4, respectively. Striatal samples were obtained at 72 h after treatment with d-MDMA. Each value represents the mean. ± SEM is not shown if it is smaller than the radius of the point. The portions of the figure concerning C57BL6/J mice is adapted from Miller and O'Callaghan (1994). *Significantly different from SAL.

play a prominent role in the neurotoxic actions of the AMPs (Sonsalla et al., 1989, 1991). Our findings suggest the alterations in core temperature must be taken into consideration in any studies concerning the mechanism of action of the AMPs or of compounds that protect against the neurotoxicity engendered by them.

d-MDMA was quite effective as a dopaminergic neurotoxicant in the mouse, and our limited data suggest its neurotoxicity did not depend on the strain and sex of the mouse or the housing conditions employed. In contrast, the neurotoxicity of another dopaminergic neurotoxicant, MPTP, is highly strain-dependent for unknown reasons (e.g., Heikkila et al., 1984; Sonsalla and Heikkila, 1988). The dopaminergic neurotoxicity of d-MDMA may be a general phenomenon in the mouse and not dependent on certain strain-dependent characteristics that could include anatomical and biochemical differences in the nigrostriatal system (e.g., Severson et al., 1981; Boehme and Ciaranello, 1982). Our data concerning the variable strain is, however, limited, and perhaps more important is the fact that the C57BL6/J mouse is one of the parental stocks for the hybrid strain B6C3F1. Because d-MDMA caused equivalent damage in female mice of either strain, it is likely the other parental stock of B6C3F1, the C3H strain, would also be sensitive. Of course, other dosing regimens may indicate differential sensitivity among various strains, and in fact, C57BL6/J mice have been shown to be markedly less sensitive than other strains to the DA depletions induced by d-MDMA when the interval between dosages is greatly increased (Zheng and Laverty, 1993). Metabolic and pharmacokinetic profiles of the AMPs in various mouse strains would aid in discovering common mechanisms underlying the neurotoxic effects of these agents in the mouse.

d-MDMA was effective in both male and female mice, although the higher lethality seen in male C57BL6/J mice may indicate a greater susceptibility to the general toxicity if not the neurotoxicity of this AMP. Limited survival of males given d-MDMA, however, makes any conclusions regarding this issue premature. It should be noted that a greater vulnerability of males in this situation is opposite to the sex-dependent effects associated with the other actions of AMPs (e.g., stimulation and sensitization) where the female is more affected (*see* Becker et al., 1982).

d-MDMA was effective at damaging striatum whether mice were aggregated or housed singly. The AMPs are synonymous with "aggregate toxicity," and we have found increased lethality when animals treated with d-MDMA at a dosage of 20 mg/kg are housed in groups of 10–12 (Miller and O'Callaghan, unpublished observations). However, it is apparent from our data that the neurotoxicity of d-MDMA at a dose of 20 mg/kg is approximately equal whether mice are housed in groups of six or housed alone. Singly housed mice were able to tolerate, although with some lethality, higher doses of d-MDMA (specifically 30 or 40 mg/kg/injection), but displayed elevations in GFAP and depletions in DA almost equivalent to mice receiving the dosage of 20 mg/kg. Our activity data also indicate there is a dissociation between body temperature and activity (i.e., elevated activity is not necessarily accompanied by elevated body temperature). Obviously, grouped mice given d-MDMA and MK-801 could be quite hyperactive, although hypothermic at the same time. Since temperature plays a role in MDMA-induced neurotoxicity and aggregation does elevate core temperature, future work should examine the role of aggregation at lower dosages of d-MDMA.

The inability of restraint to block completely the MDMA-induced striatal GFAP elevation in B6C3F1 mice (right side of Fig. 8) is in marked contrast to the protective effects of all other manipulations that lowered core temperature. Since the DA depletion was totally blocked by restraint, the remaining elevation in GFAP may be interpreted as evidence of damage to additional sites in the striatum of B6C3F1 mice. That MK-801 was able to protect against both the GFAP elevation and the DA depletion in this strain would indicate it may have neuroprotective properties unrelated to hypothermia. An examination of markers for other projections to or cell types in the striatum would be useful in interpreting the neurotoxic effects of d-MDMA in the B6C3F1 strain.

In summary, our data indicate the importance of body temperature in the neurotoxic

actions of MDMA in particular and the AMPs in general. Any treatment able to lower body temperature is likely to be at least partially neuroprotective against the neural damage induced by the AMPs. Despite the knowledge that temperature can affect the general toxicity of the AMPs, the importance of this variable for the neurotoxicity of these compounds is only now being appreciated (Bowyer et al., 1992, 1993).

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