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Environmental Research 92 (2003) 48–53

Environmental Research

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Elevated environmental temperature and methamphetamine neurotoxicity

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Received 4 February 2002; received in revised form 28 August 2002; accepted 30 September 2002

Abstract

Amphetamines have been of considerable research interest for the last several decades. More recent work has renewed interest in the role of ambient temperature in both the toxicity and neurotoxicity of these drugs. We have determined that the striatal dopaminergic neurotoxicity observed in the mouse is linked in some fashion to both body and environmental temperature. Most studies of d-methamphetamine (d-METH) neurotoxicity are conducted at standard laboratory ambient temperatures (e.g., ~21–22°C) and utilizing a repeated dosage regimen (e.g., three to four injections spaced 2 h apart). A lowering of the ambient temperature provides neuroprotection, while an elevation increases neurotoxicity. d-METH causes long-term depletions of striatal dopamine (DA) that are accompanied by other changes that are indicative of nerve terminal degeneration. These include argyrophilia, as detected by silver degeneration stains, and an elevation in glial fibrillary acidic protein (GFAP), a marker of reactive gliosis in response to injury, as well as a long-term decrease in tyrosine hydroxylase (TH) protein levels. Here we show that increasing the ambient temperature during and for some time following dosing increases the neurotoxicity of d-METH. Mice (female C57BL6/J) given a single dosage of d-METH (20 mg/kg s.c.) and maintained at the usual laboratory ambient temperature show minimal striatal damage (an ~15% depletion of DA and an ~ 86% increase in GFAP). Substantial striatal damage (e.g., an ~70% depletion of DA and an ~200% elevation in GFAP) was induced by this regimen if mice were maintained at 27°C for 24 or 72 h following dosing. An increase in neurotoxicity was also apparent in mice kept at an elevated temperature for only 5 or 9 h, but keeping animals at 27°C for 24 or 72 h was the most effective in increasing the neurotoxicity of d-METH. Our data show how a relatively minor change in ambient temperature can have a major impact on the degree of neurotoxicity induced by d-METH. Single-dose regimens may aid in uncovering the as yet unknown mechanism(s) of substituted amphetamine neurotoxicity because they reduce the inherent complexity present in repeated dosage regimens.
Published by Elsevier Science (USA).

1. Introduction

Many factors impact toxicity. The toxic agent, the exposure situation, and the organism itself are primary among these factors (Casarett and Doull, 1973). The exposure situation is by far the least studied, although it is known that variables related to exposure such as environmental temperature can have a significant impact on toxicity. In general, toxicologists evaluate agents for toxic qualities under ideal conditions that involve little or no stress to the organism. Most laboratories are maintained at temperature, humidity, and lighting conditions that are considered optimal for the health of the animal. Although it is acknowledged that exposure to toxic agents in the real world rarely occurs under ideal conditions, it is uncommon for less than ideal conditions to be utilized in a laboratory setting (see Gordon (2003) for a further discussion of these issues).

The substituted amphetamines, such as amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA), are a good example of a series of related compounds which have toxic and neurotoxic properties which are affected by environmental temperature. Early work described the hyperthermia associated with exposure to these agents and the possible role that this increased body temperature might play in aggregate toxicity (Askew, 1962;

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0013-9351/03/$ - see front matter Published by Elsevier Science (USA).
doi:10.1016/S0013-9351(02)00051-8

Chance, 1946; Swinyard et al., 1961). However, the impact of environmental temperature on the toxic, and in particular neurotoxic, properties of these agents had been left unexamined until recently (Bowyer and Holson, 1995; Bowyer et al., 2001b; Miller and O'Callaghan, 1994). Recent interest in environmental temperature and substituted amphetamine toxicity has, in part, been generated by the reports of deaths due to 3,4-methylenedioxymethampetamine or "Ecstasy." It is speculated that the hyperthermic actions of the compound experienced in conjunction with the high ambient temperatures found in dance clubs or at "raves" are responsible for the exacerbation of the toxicity (Chadwick et al., 1991; Randall, 1992).

Over the last several years we and others have been examining the impact of environmental temperature on the neurotoxicity of the substituted amphetamines (Bowyer and Holson, 1995; Bowyer et al., 2001a,b; Johnson and Miller, 2001; Miller and O'Callaghan, 1994; O'Callaghan and Miller, 2001). When these agents are given to the mouse under certain dosing conditions (e.g., repeated dosing) they cause long-term depletions of striatal dopamine (DA) that are accompanied by other changes that are indicative of nerve terminal degeneration. These include argyrophilia, as detected by silver degeneration stains, and an elevation in glial fibrillary acidic protein (GFAP), a marker of reactive gliosis in response to injury, as well as a long-term decrease in tyrosine hydroxylase (TH) protein levels (O'Callaghan and Miller, 1994). Environmental temperature can modulate these neurotoxic effects (Miller and O'Callaghan, 1994). For example, slight modulations in room temperature can alter neurotoxicity such that a change in temperature from 20.5°C to 23.5°C significantly exacerbates neurotoxicity. Further, maintaining mice at 15°C during repeated dosing is totally neuroprotective (Johnson et al., 2000). Here we examined whether increasing the ambient temperature to 27°C following a single injection of a dosage of d-methamphetamine (d-METH) capable of causing only minimal striatal damage (i.e., limited gliosis and DA depletion) would produce a greater degree of neurotoxicity. In effect, our data show that increasing the environmental temperature following d-METH exposure is equivalent to increasing the dosage.

2. Materials and methods

2.1. Materials

The following 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, USA), and d-MDMA (Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD, USA), BCA protein assay reagent and bovine serum albumin (Pierce Chemical Co., Rockford, IL, USA); reagents used for HPLC were of HPLC grade (ESA, Chelmsford, MA, USA). The materials used in the GFAP assay have been described in detail in O'Callaghan (1991).

2.2. Animals

Female C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) were used to be consistent with our previous work (e.g., Miller and O'Callaghan, 1994). Mice were obtained at ~8 weeks of age and maintained in a colony certified by the American Association for Accreditation of Laboratory Animal Care. All procedures were conducted according to protocols approved by the Institutional Animal Care and Use Committee and in accordance with the NRC "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996). Upon receipt, mice were housed in groups of 6–8 in plastic tub cages (30.5 cm × 30.5 cm × 15 cm) with heat-treated pine shavings. Cages were kept 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 06:00 Eastern Daylight Time. Water and food (ProLab ISOPRO RMH 3000, irradiated containing 22%, 5%, 5%, 6%, and 2.5% crude protein, crude fat, crude fiber, ash, and added minerals, respectively) were available ad libitum.

2.3. Dosing

On the day of dosing mice were removed from their original cages and regrouped six per cage in new cages. Mice were then weighed and their tails given a unique identifying number utilizing a laboratory marker (Sharpie Nontoxic Permanent Marker). Rectal temperatures were taken immediately and the cages were placed in a temperature- (±0.3°C) and humidity- (±2%) controlled environmental chamber (Caron Model 6030, Marietta, OH, USA) maintained at 27°C and a relative humidity of 55%. Following a 2-h acclimation period, mice were removed from the chamber, their rectal temperatures were recorded, and they were injected s.c. with d-METH (20 mg/kg as the base) or with 0.9% saline (SAL). Following the injection the mice were maintained in a standard animal room at ~21–22°C for 72 h (SAL and d-METH groups) or in an environmental chamber at 27°C for 72 h (SAL group) or for 5, 9, 24, or 72 h (d-METH group) to be consistent with the time the tissue was obtained in our previous work regarding d-METH neurotoxicity (O'Callaghan and Miller, 1994). Rectal temperatures were recorded at 1, 3, 5, 7, and 9 h following the injection of saline or d-METH. When mice were maintained at an elevated

ambient temperature they were briefly removed from the environmental chamber, one cage at a time to record rectal temperatures. Mice maintained at standard animal room temperature (~21–22°C) were kept in the room where the environmental chamber was located. The temperatures of the animal room and the environmental chamber were automatically recorded and no deviations from the ranges reported above were observed during the experimental period.

2.4. Temperature measurement

A Bat-10 thermometer coupled to a RET-3 mouse rectal probe (Physitemp, Inc., Clinton, NJ, USA) lubricated with mineral oil and inserted to a premarked depth of 2.8 cm was used to record rectal temperature. It is possible to obtain a reliable measurement of rectal temperature in the mouse in less than 30 s utilizing previously developed procedures (Miller and O'Callaghan, 1994) in conjunction with a fast-rise time probe.

2.5. Brain dissection and tissue preparation

Tissue was obtained at 72 h following dosing, a time point at which GFAP elevations and dopamine depletions are maximal for d-METH (see O'Callaghan and Miller, 1994, for a complete time course). Mice were decapitated and whole brains were excised with the aid of a blunt forceps. Left and right striatum were dissected freehand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI, USA) using a pair of fine-curved forceps, weighed, frozen on dry ice, and stored at −70°C for subsequent analysis of DA by HPLC and GFAP by ELISA. The right striatum was homogenized with an ultrasonic probe (Model XL-2005, Heat Systems, Farmingdale, NY, USA) in 10 vol of hot (90–95°C) 1% SDS immediately prior to GFAP analysis. Left striatum were homogenized in 400 μL perchloric acid and the supernatant was analyzed by HPLC.

2.6. GFAP immunoassay, protein assay, and DA analysis

GFAP was assayed according to previously detailed methods (see O'Callaghan and Miller, 1994). Total protein concentration was determined by the method of Smith et al. (1985). DA was assayed by HPLC with electrochemical detection (for complete details see Johnson et al., 2000).

2.7. Statistics

All data analysis was conducted with JMP R 3.2 (1997) (SAS Institute Inc., Cary, NC, USA). Individual variables were evaluated by ANOVA followed by Duncan's test for mean comparisons. The α level used to determine significance was 0.05 in all cases. The temperature measures were converted to a composite measure (TAUC). The TAUC was calculated for each mouse by the application of Simpson's Rule to temperatures measured at times 0, 1, 3, 5, and 7 h following drug administration. This composite measure represents the area under the curve of a plot of temperature (°C) versus time (h), and has units of °C h.

3. Results

3.1. Effects of elevated ambient temperature on animal status and the hyperthermia caused by d-METH

Although mice receiving a single dose of d-METH (20 mg/kg) were hyperactive and hyperthermic, there were no unusual behaviors (e.g., self-mutilation, etc.) or lethality associated with this dosing regimen. Informal observation revealed no overt differences between mice treated with saline or d-METH by 24 h after injection.

All mice given a single dosage of 20 mg/kg of d-METH showed a significant elevation in body temperature whether maintained at normal (~21–22°C) or an elevated (27°C) ambient temperature. Approximately equal increases in TAUC (Fig. 1) were found for all of

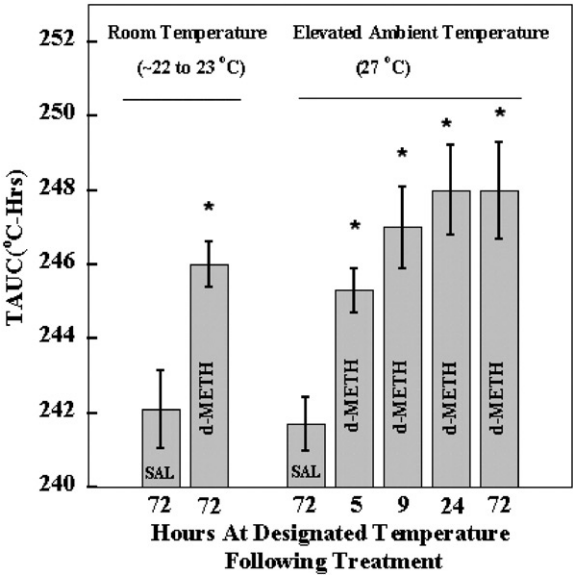

Fig. 1. Effects of elevated ambient temperature (27°C) on the rectal temperature of mice given a single injection of SAL or d-METH (20 mg/kg s.c.). The temperature measures were converted to a composite measure (TAUC). The TAUC was calculated for each mouse by the application of Simpson's Rule to temperatures measured at times 0, 1, 3, 5, and 7 h following drug administration. This composite measure represents the area under the curve of a plot of temperature (°C) versus time (h), and has units of °C h. Each value represents the mean ± SEM of six mice. No differences were noted between groups given SAL and maintained at 22–23°C or 27°C. *, Significantly different from SAL.

the groups given d-METH. This hyperthermia had apparently resolved by 24 h, as there were no significant differences between the groups at this time point (data not presented).

3.2. Effects of elevated ambient temperature on the ability of a single dose of d-METH to induce striatal neurotoxicity

Striatal DA and GFAP levels did not differ between groups given SAL and maintained at 27°C for 0 or 72 h.

A single dosage of d-METH was effective at reducing striatal DA levels in mice kept at 27°C for 9, 24, or 72 h following dosing (Fig. 2). Mice kept at the usual laboratory temperature (~22–23°C) had a nonsignificant depletion of 24% relative to SAL, while those mice kept at an elevated temperature for 9, 24, or 72 h had depletions of 66%, 71%, and 72%, respectively. Even mice maintained at 27°C for only 5 h exhibited a greater depletion (37%), albeit a nonsignificant decrease, than mice maintained at ~21–22°C.

A similar pattern was evident for striatal GFAP levels (Fig. 3). All mice given d-METH and maintained at 27°C displayed significant elevations in striatal GFAP, indicating that a single exposure to d-METH can be made neurotoxic to striatum by elevating ambient temperature. Mice given d-METH and kept at 27°C for any length of time showed elevations ranging between ~170% at 5 and 9 h to ~200% at 24 and 72 h. A nonsignificant elevation (81%) in GFAP was observed in mice treated with d-METH and kept at ~21–22°C.

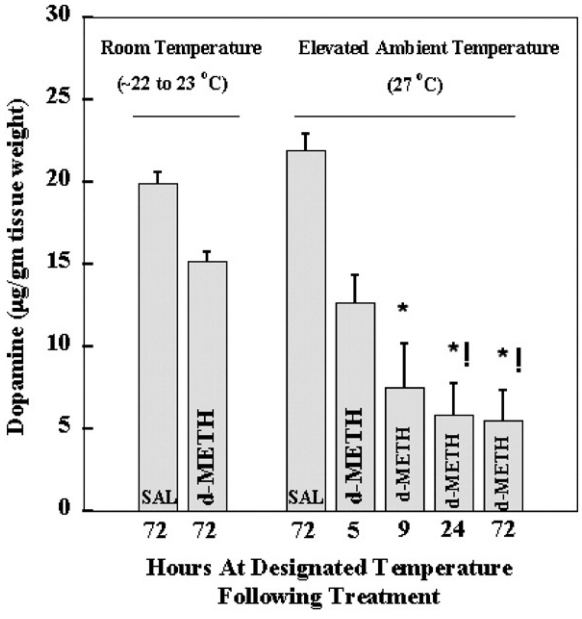

Fig. 2. Effects of varying periods of elevated ambient temperature (27°C) on striatal DA levels of mice given a single injection of SAL or d-METH (20 mg/kg s.c.). Each value represents the mean ± SEM of six mice. No differences were observed between groups given SAL and maintained at 22–23°C or 27°C. *, Significantly different from SAL; !, significantly different from 0 h.

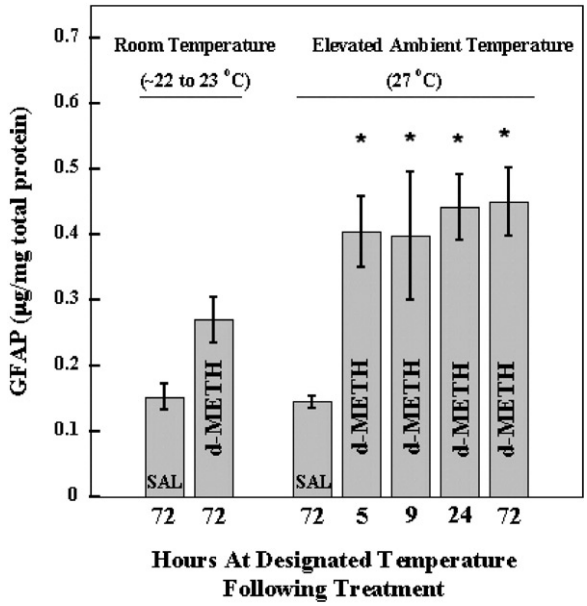

Fig. 3. Effects of varying periods of elevated ambient temperature (27°C) on striatal GFAP levels of mice given a single injection of SAL or d-METH (20 mg/kg s.c.). Each value represents the mean ± SEM of six mice. No differences were observed between groups given SAL and maintained at 22–23°C or 27°C. *, Significantly different from SAL.

4. Discussion

Several of the substituted amphetamines, including METH, AMP, MDMA, and MDA can serve as striatal dopaminergic neurotoxicants in the mouse, especially when repeated dose regimens are used (Logan et al., 1988; Miller and O'Callaghan, 1994). These agents cause long-term depletions in DA that are accompanied by other changes indicative of nerve terminal degeneration. These include argyrophilia, as detected by silver degeneration stains, and an elevation in GFAP, a marker of reactive gliosis, as well as a long-term decrease in TH protein levels. Here, a single dosage of d-METH given at an ambient temperature of 21–22°C, the usual laboratory temperature, produced only a minimal neurotoxic response (i.e., a slight reduction in striatal DA accompanied by a minimal gliosis). However, administration of this dosage of d-METH while the animals were maintained at 27°C was quite effective in producing striatal dopaminergic neurotoxicity. Even maintaining animals at this temperature for as little as 5 h was effective although having them remain at an elevated temperature for longer periods (9–72 h) was more effective. These data confirm and extend the work from this and other laboratories showing that d-METH

can produce striatal neurotoxicity in the mouse without producing malignant hyperthermia (e.g., Miller and O'Callaghan, 1994). In fact, the higher ambient temperature effectively increased neurotoxicity without increasing the body temperature above that seen in the mice maintained at ~21–22°C and given d-METH. This can be contrasted to the rat in which METH neurotoxicity (e.g., GFAP elevations and long-term decreases in DA) generally occurs only with the use of regimens producing a lethal hyperthermia that often requires an "ice-bath rescue" (e.g., Bowyer et al., 1994; Bowyer et al., 2001a).

Links between temperature, both of the environment and the body, and the neurotoxicity of the substituted amphetamines are clearly established, but there is little agreement on the mechanistic role of temperature in the observed striatal neurodegeneration. We have found that many manipulations capable of lowering body temperature in the presence of the amphetamines (e.g., certain pharmacological treatments, restraint, removal of the adrenal, reduction in ambient temperature, etc.) will effectively reduce or block the neurodegeneration (e.g., Miller and O'Callaghan, 1994). However, there are compounds which lower temperature quite significantly during amphetamine exposure but are not fully protective (Miller and O'Callaghan, 1994 (Fig. 6), 1996). Others have found that reserpine is quite effective at lowering body temperature during METH exposure but provides no protection (Albers and Sonsalla, 1995). Here we show that an elevation in ambient temperature can increase METH striatal neurotoxicity without having an effect on core temperature. That is, neurotoxicity is increased but the increase in body temperature induced by the single dosage of METH is the same whether the mice are kept at the usual ambient temperature (~21–22°C) or an elevated temperature (27°C). In part, it is difficult to specify the exact link between temperature and substituted amphetamine neurotoxicity because it is not known exactly how the amphetamines cause terminal degeneration. A further complication is the lack of information concerning the respective roles of core and brain temperature. However, it is certain that a continued investigation of the role of temperature (environmental, brain, and body), especially in situations in which neurotoxicity occurs in the absence of hyperthermia, will be valuable in establishing the relationship between neurotoxic mechanism(s) and temperature. At the usual laboratory temperature d-METH is a robust neurotoxicant only when given repeatedly or when continuously administered. Such regimens are limiting in establishing exact dose- and time-effect relationships. Single dosage protocols may be more useful in investigating these types of relationships.

In summary, our results show that in the mouse a moderate increase in environmental temperature can increase the striatal neurotoxicity of d-METH without accompanying excessive or lethal hyperthermia. A continued exploration of the relationship between environmental temperature and the neurotoxicity of the substituted amphetamines may yield important clues to the neurotoxic mechanism of these agents. Single dosage regimens like the one utilized here may aid in better establishing dose- and time-effect relationships for the substituted amphetamines as they have for other striatal dopaminergic neurotoxicants (e.g., MPTP) (O'Callaghan et al., 1990a, b).

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