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Tolerance to the neurotoxic effects of methamphetamine in young rats

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Abstract

The present study examined whether exposure to methamphetamine during adolescence (as determined in post-natal day 40 rats) might alter its effects when used in young adulthood (as assessed in post-natal day 90 rats). Results confirm that high-dose methamphetamine administration (4 × 10 mg/kg/injection, s.c., 2-h intervals) decreases striatal dopamine uptake and transporter ligand binding in post-natal day 90 rats; effects that were blocked if animals received six biweekly methamphetamine pretreatments (15 mg/kg; s.c.) beginning at post-natal day 40. This neuroprotection was not likely due to pharmacokinetic tolerance, since brain methamphetamine concentrations did not differ 1 h after the high-dose methamphetamine regimen among treated rats regardless of pretreatment. The methamphetamine biweekly pretreatment attenuated the hyperthermia caused by the neurotoxic methamphetamine regimen; a phenomenon that may have contributed to the neuroprotection. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Methamphetamine; Aging; Tolerance; Hyperthermia; Striatum; Dopamine transporter

1. Introduction

Over the past decade, there has been an increase in the illicit synthesis and abuse of methamphetamine. Much of this abuse occurs in secondary student (adolescent) populations between 14 and 18 years of age. For instance, in 2000, 7.9% of 12th graders, 6.9% of 10th graders, and 4.2% of 8th graders reported using methamphetamine at least once in their lifetime (Johnston et al., 2000). This usage is especially problematic since exposure to this drug during teenage years may result in long-term neuronal damage. This concern is based, in part, on findings that high-dose methamphetamine administration causes persistent dopamine deficits in rodents (Hotchkiss and Gibb, 1980; Hotchkiss et al., 1979; Wagner et al., 1980; Ricaurte et al., 1982), non-human primates (Woolverton et al., 1989) and possibly humans (Wilson et al., 1996). Because these neurochemical deficits can persist months, these changes likely reflect long-term damage to dopamine nerve terminals. Hence, an understanding of the age-related effects of methamphetamine exposure is of importance.

Several recent studies demonstrated age-dependent differences in the response of dopamine systems to high-dose methamphetamine treatment. For instance, Lucot et al. (1982), Wagner et al. (1981), Pu and Vorhees (1993) and Cappon et al. (1997) have observed that multiple methamphetamine administrations cause long-term deficits in the dopamine system of older rats (age greater than post-natal day 60), but have little persistent impact on dopamine systems when given to juvenile or young adolescent animals (aged post-natal day 14–40). More recently, Kokoshka et al. (2000) demonstrated that at least some of these age-dependent differences may be caused by pharmacokinetic factors. Although these data demonstrate that younger animals (at least rodents) are less affected than older animals by methamphetamine treatment, these studies do not address whether methamphetamine administration to younger animals alters the pattern of response when exposed in adulthood. Hence, the purpose of this study was to investigate whether treatment of adolescent rats (post-natal day 40; Ojeda et al., 1980) alters the response to high-dose methamphetamine administration once these animals reach young adulthood (post-natal day 90; Ojeda et al., 1980). Results reveal that biweekly pretreatment with the stimulant early in adolescence may make adult rats resistant to the long-term effects of the stimulant. In contrast to previous reports of tolerance, the tolerance observed in this study is not likely due to pharmacokinetics, but may relate to a reduction in methamphetamine-induced hyperthermia. Possible reasons for this difference may be

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associated with a difference in age of the animals used and/or the difference in the pretreatment dosing paradigms.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Simonsen Laboratories, Gilroy, CA) were group-housed (eight animals/cage) with an alternating light/dark cycle (lights on 14 h/day) and treated in an ambient temperature of 24 °C. Food and water were provided ad libitum. Where indicated, core body (rectal) temperatures were measured using a digital thermometer (Physiotemp Instruments, Clifton, NJ) every hour prior to each drug or vehicle administration and continuing until 4 h after the final drug administration. Rats were sacrificed by decapitation. All animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and chemicals

[7,8-^{3}H]Dopamine (46 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL). [^{3}H]WIN35428 ([*N*-methyl-^{3}H]-WIN35428 ((−)-2-β-carbomethoxy-3-β-(-4-fluorophenyl)tropane 1,5-napthalenedisulfonate; 84.5 Ci/mmol) was purchased from New England Nuclear (Boston , MA). Methamphetamine hydrochloride was furnished by the National Institute on Drug Abuse (Bethesda, MD). Drugs were administered as indicated in figure legends and doses were calculated as the respective free bases.

2.3. Synaptosomal [^{3}H]DA uptake, [^{3}H]WIN35428 binding and METH concentrations

Synaptosomal [^{3}H]dopamine (0.5 nM final concentration) uptake and [^{3}H]WIN35428 (0.5 nM final concentration) binding were determined in striatal synaptosomal preparations as described previously (Kokoshka et al., 1998; Metzger et al., 2000). Methamphetamine concentrations were determined by gas chromotography coupled to mass spectroscopy as described previously (Haughey et al., 2000).

2.4. Statistics

Statistical analyses among groups were conducted using analysis of variance, followed by a Fisher Least Significance test. Differences among groups were considered significant if the probability of error was less than 5%. The data represent means ± 1 standard error of the mean (S.E.M.).

3. Results

Results presented in Fig. 1 confirm that high-dose treatment with methamphetamine, administered in a pattern

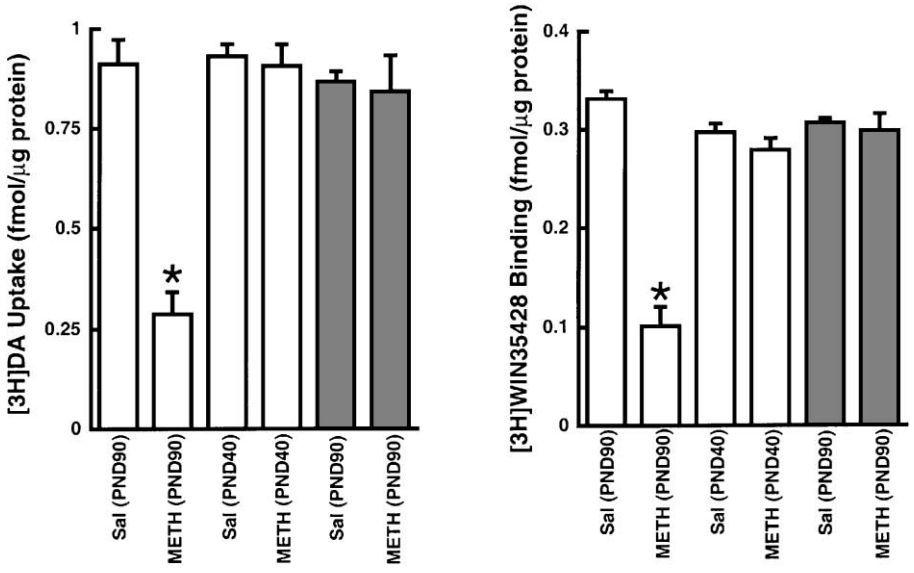

Fig. 1. Rats designated "PND 90" (post-natal day 90) received a single injection of methamphetamine (METH; solid columns; 15 mg/kg; s.c.) or saline vehicle (open columns; 1 ml/kg, s.c.) on two consecutive days weekly for six consecutive weeks. One week after the final treatment (i.e., at a time at which rats had attained the age post-natal day 90), rats received four injections of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (1 ml/kg, s.c.). For comparison, rats designated "PND 40" (post-natal day 40) were not pretreated, but received four injections of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (1 ml/kg, s.c.) at the post-natal day 40 age. Rats were decapitated 1 week later. Columns represent the mean [^{3}H]dopamine (DA) uptake or [^{3}H]WIN35428 binding and vertical lines 1 S.E.M. of determinations in striatal tissue of 7–8 rats. * Value for methamphetamine-treated rats that is significantly different from vehicle-treated controls ($P < 0.05$).

designed to mimic a "binge" treatment (4 × 10 mg/kg/injection, s.c., 2-h intervals), causes long-term dopamine deficits in post-natal day 90, but not post-natal day 40 rats, as determined 7 days after treatment by assessing striatal [^{3}H]dopamine uptake and binding of the dopamine transporter ligand, [^{3}H]WIN35428. Results presented in Fig. 1 also demonstrate that the methamphetamine-induced dopamine deficits observed 7 days after binge treatment of post-natal day 90 rats were prevented if animals first received multiple biweekly methamphetamine pretreatments beginning at post-natal day 40. Specifically, rats received a single injection of methamphetamine at a dose of 15 mg/kg (s.c.) on two consecutive days, and this biweekly treatment was repeated for six consecutive weeks. One week after the final treatment (i.e., at a time at which rats had attained the age post-natal day 90), rats received the high-dose binge methamphetamine treatment (4 × 10 mg/kg/injection, s.c., 2-h intervals). Rats were then decapitated 1 week later, and striatal [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding were assessed. As demonstrated in Fig. 1, the binge treatment decreased both striatal [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding by approximately 70% in animals pretreated with saline. This effect was completely prevented in rats that received the biweekly methamphetamine pretreatment regimen. In addition, results presented in Fig. 2 demonstrate that the biweekly pretreatment regimen with methamphetamine attenuated the acute hyperthermia caused by the subsequent binge methamphetamine administration.

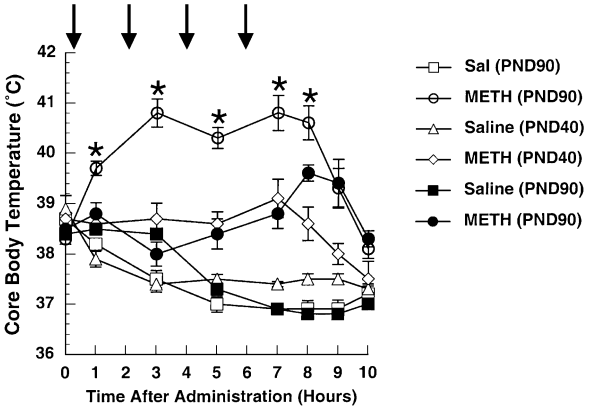

Fig. 2. Rats designated "PND 90" (post-natal day 90) received a single injection of methamphetamine (METH; solid symbols; 15 mg/kg; s.c.) or saline vehicle (open symbols; 1 ml/kg, s.c.) on two consecutive days weekly for six consecutive weeks. One week after the final treatment (i.e., at a time at which rats had attained the age post-natal day 90), rats received four injections (as indicated by the inverted arrows) of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (1 ml/kg, s.c.). For comparison, rats designated "PND 40" (post-natal day 90) were not pretreated, but received four injections of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (1 ml/kg, s.c.) at the post-natal day 40 age. Body temperatures were monitored as indicated in Materials and methods. Symbols represent the means and vertical lines 1 S.E.M. of determinations in 7–8 rats. * Values for methamphetamine-treated rats that are significantly different from vehicle-treated controls ($P<0.05$).

Furthermore, post-natal day 40 rats that received no pretreatment were not hyperthermic.

Noteworthy is the fact that, in contrast to the neuroprotective effects of biweekly pretreatment, pretreatment once weekly at a dose of 15 mg/kg (s.c.) for 6 weeks beginning at post-natal day 40 did not prevent the persistent methamphetamine-induced dopamine deficits when animals received the neurotoxic binge treatment on post-natal day 90. Moreover, a single pretreatment of rats at post-natal day 40 with the neurotoxic binge methamphetamine regimen did not prevent the persistent dopamine deficits when animals received a second binge treatment on post-natal day 90 (data not shown).

To investigate the possibility that pharmacokinetics contributed to the neuroprotective effects of the biweekly methamphetamine pretreatment regimen, striatal and whole brain methamphetamine concentrations were assessed. Results revealed that these values did not change as a result of the pretreatment regimen. Specifically, the respective mean whole brain and striatal concentrations of methamphetamine 1 h after binge treatment were (in ng/mg tissue): saline pretreatment post-natal day 90 rats, 9.1 ± 0.9 and 10.9 ± 1.1; methamphetamine pretreatment post-natal day 90 rats, 9.3 ± 1.3 and 10.6 ± 1.0. Post-natal day 40 rats that received the binge treatment (without any prior exposure to methamphetamine) had whole brain and striatal methamphetamine concentrations that are significantly less than saline-treated controls ($P<0.05$; 6.6 ± 0.9 and 4.9 ± 1.2 ng/mg tissue, respectively).

4. Discussion

Despite the use of amphetamine analogs such as methamphetamine by adolescents in high school and even junior high school, little research is being conducted to look at this stimulant in this age group. This lack of study is due, in part, to findings that methamphetamine causes little or no long-term dopamine deficits in younger animals (particularly rodents; Lucot et al., 1982; Wagner et al., 1981; Pu and Vorhees, 1993; Cappon et al., 1997). Recently, Kokoshka et al. (2000) confirmed these findings and observed that after a methamphetamine treatment identical to that employed in the present study, brain methamphetamine concentrations in younger animals were approximately 50% of those observed in adult animals suggesting age-dependent pharmacokinetic factors contribute to these developmental differences. Although these studies demonstrate differences in the response of younger and older rats to methamphetamine treatment, they do not address whether methamphetamine administration to younger animals alters the pattern of response when exposed in adulthood.

To determine whether treatment during "adolescence" (i.e., post-natal day 40) alters the response in "young adulthood" (i.e., post-natal day 90), rats received several distinct pretreatment regimens followed by a subsequent

binge methamphetamine regimen. The effect of these treatments on dopamine neurons was evaluated. Neither a single binge treatment nor a weekly single injection altered the pattern of response to the neurotoxic methamphetamine regimen. In contrast, biweekly methamphetamine pretreatment prevented the persistent dopamine deficits caused by the neurotoxic methamphetamine regimen. Biweekly pretreatment also attenuated the hyperthermia caused by the subsequent neurotoxic methamphetamine administration. It is established that prevention of methamphetamine-induced hyperthermia can prevent the long-term dopaminergic deficits caused by methamphetamine treatment (Bowyer et al., 1994; Farfel and Seiden, 1995). Specifically, it has been suggested that alterations in intraneuronal dopamine disposition promote the formation of dopamine-associated reactive oxygen species (LaVoie and Hastings, 1999; Fleckenstein et al., 2000; Fumagalli et al., 1999), and that the prevention of hyperthermia attenuates this phenomenon (LaVoie and Hastings, 1999). Thus, it is likely that tolerance to the hyperthermia effects of methamphetamine contributes to the mechanism whereby pretreatment during rat adolescence makes adult rats resistant to methamphetamine-induced dopamine deficits. Central mechanism(s) underlying methamphetamine-induced hyperthermia have not been elucidated fully, although dopamine likely contributes as it has been demonstrated that administration of dopamine antagonists or dopamine depletion following α-methyltyrosine administration attenuates methamphetamine-induced increases in body temperature (Metzger et al., 2000). In addition, it has been suggested that methamphetamine stimulates norepinephrine release from sympathetic nerve terminals, which then enhance thermogenesis in skeletal muscle under the permissive action of glucocorticoids (Makisumi et al., 1998). Further studies are required to elucidate whether central and/or peripheral mechanisms underlie the tolerance to hyperthermia resulting from the biweekly pretreatment.

Several investigators have reported that pretreatment of rats with various dosing regimens of methamphetamine can effect tolerance to its long-term monoaminergic deficits such as that described in the present report. For instance, Schmidt et al. (1985) demonstrated that pretreatment with incrementally increasing doses of methamphetamine over a period of several days causes tolerance to the deficits in dopamine neuronal function induced by subsequent administration of multiple high-dose injections of methamphetamine. Likewise, Gygi et al. (1996) reported that pretreatment with an incrementally increasing dose regimen of methamphetamine over a period of several days causes tolerance to the deficits in serotonergic neuronal function caused by subsequent high-dose administration of methamphetamine. Additionally, Abekawa et al. (1997) demonstrated that rats behaviorally sensitized to methamphetamine or amphetamine develop tolerance to the striatal dopamine and serotonin deficits caused by methamphetamine treatment. One interesting finding in the studies by Gygi et al. (1996) was that brain concentrations of methamphetamine were reduced in tolerant rats while plasma concentrations were elevated. Hence, a redistribution of methamphetamine between brain and plasma (and not an increase in peripheral metabolism) appeared to effect the tolerance. In contrast to these previous findings, the present data demonstrate that brain methamphetamine concentrations did not differ among control rats and those made tolerant to the methamphetamine treatment. Although these data are limited in that concentrations of methamphetamine were only assessed at a single time point and altered pharmacokinetics cannot be absolutely excluded as a possible factor, these results suggest strongly that alterations in brain methamphetamine concentrations did not contribute to the neuroprotective effects induced by pretreatment in young animals.

In conclusion, the present study confirms previous reports of an age-related difference in the response of dopamine neurons to methamphetamine treatment. Moreover, this study confirms that some, but not all, types of methamphetamine pretreatment can protect against the long-term dopamine deficits caused by the administration of the stimulant. In contrast to previously described tolerances, the present phenomenon is likely not due to differences in pharmacokinetics caused by the methamphetamine pretreatment, but may be linked to a reduced hyperthermia response to methamphetamine caused by previous exposure to the stimulant.

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