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The role of hyperthermia and metabolism as mechanisms of tolerance to methamphetamine neurotoxicity

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Abstract

Pretreatment with multiple methamphetamine injections prior to a high-dose methamphetamine challenge administration can attenuate long-term deficits in striatal and hippocampal serotonin content caused by the stimulant. The present data extend previous findings by demonstrating that rats pretreated with escalating doses methamphetamine did not exhibit dopamine deficits in the striatum, nor serotonin deficits in striatal, frontal cortical, or hippocampal tissues, 7 days after a challenge methamphetamine administration. This protection was not due to attenuation of methamphetamine-induced hyperthermia or altered brain methamphetamine concentrations. These data differ from previous findings thereby highlighting that different mechanisms contribute to the tolerance of the neurotoxic effects.

Keywords: Pharmacokinetics; Neurotoxicity; Dopamine; 5-HT (5-hydroxytrytamine, serotonin); Hyperthermia

1. Introduction

Multiple high-dose administrations of methamphetamine cause long-lasting depletions of central monoamine neurotransmitters in the brain (for review, see Gibb et al., 1994). Pretreatment with methamphetamine prior to a high-dose methamphetamine challenge administration can lead to the development of tolerance to the acute neurotoxic effects on dopamine and serotonin systems (Schmidt et al., 1985; Gygi et al., 1996), as assessed 18 h after the last challenge injection, and 4 days after the challenge administration (Stephans and Yamamoto, 1996). Hyperthermia plays an important role in mediating the long-term neurotoxic effects of methamphetamine on striatal dopamine neurons (Bowyer et al., 1994; Albers and Sonsalla, 1995). For instance, Bowyer et al. (1992) and Ali et al. (1996) reported that lowering the environmental temperature to 4 °C while administering a neurotoxic regimen of methamphetamine, as well as administering pharmacological drugs that produce hypothermia in combination with a neurotoxic regimen of methamphetamine, attenuates the methamphetamine-induced depletion of striatal dopamine. Collectively, these experiments demonstrate a link between hyperthermia and methamphetamine-induced neurotoxicity, suggesting that the attenuation of methamphetamine-induced hyperthermia could play a role in tolerance to the neurotoxic effects of the stimulant. Therefore, the purpose of this experiment was to determine whether development of tolerance to the neurotoxic effects of methamphetamine after an escalating dose-paradigm is due the attenuation of methamphetamine-induced hyperthermia during the challenge administration.

2. Materials and methods

2.1. Materials

(±)-Methamphetamine hydrochloride was provided by the National Institutes on Drug Abuse (Rockville, MD).

2.2. Animals

Male Sprague–Dawley rats (Simonsen Laboratory, Gilroy, CA, 300–325 g) were housed four per cage in hanging wire cages in a temperature-controlled room on a 14:10-h light/dark cycle. Rats had free access to food and water. All experiments were approved by the University of Utah

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Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The rats that received multiple high-dose administration of methamphetamine, as described in the text below, had mortality which varied from 0% to 25% after the regimen.

2.3. Pharmacological procedures

Upon receipt, animals were housed four per cage in wire cages in an IACUC-approved housing facility for 1 week. The evening before the experiment, rats were re-housed, eight rats per cage, in plastic tubs (33-cm length × 28-cm width × 17-cm height) in a separate procedure room (23.5–24.0 °C), in order to facilitate comparison with the previous studies in the laboratory. The tolerance-dosing regimen established by Gygi et al. (1996) consisted of saline or methamphetamine pretreatments on days 1, 3, and 5. On day 1, a total of four injections of either saline or 2.5 mg/kg of methamphetamine were administered (s.c.) with a 2-h interval between each dose. The doses for methamphetamine were increased to 5.0 mg/kg on day 3 and 7.5 mg/kg on day 5. Rats were allowed a 40-h drug-free period between each pretreatment dosing regimen. Sixty-six hours after the last pretreatment regimen on day 5, rats were either challenged with four injections of saline or 10 mg/kg methamphetamine, s.c., with a 2-h interval between each injection. Where indicated, core body (rectal) temperatures were assessed every hour, for a total of 8 h, during the pretreatment and challenge dosing regimens. Rectal temperatures were measured using a BAT-12 model thermometer and rectal probe (model RET-2; Physiotemp Instruments, Clifton, NJ, USA). Rats were decapitated 7 days after the last challenge administration.

2.4. Monoamine tissue content

Dopamine and serotonin content were measured by high-performance liquid chromatography (HPLC) coupled to electrochemical detector according to a modification of the method described by Chapin et al. (1986). Tissues were sonicated in 1 ml of tissue buffer (0.05 M sodium phosphate/0.03 M citric acid with 15% methanol (vol/vol), pH 2.5), then centrifuged at 18,800 × *g* for 15 min at 4 °C to separate the supernatant from the protein. The supernatant was centrifuged at 18,800 × *g* for 10 min at 4 °C then 20 μl of the supernatant was injected onto the HPLC (Dynamax AI-200 Autosampler and SD-200 pump; Varian, Walnut, CA) coupled to an electrochemical detector (E_{ox} = + 0.70 V; Varian Star 9080, Walnut, CA). A Whatman PartiSphere C-18 column (250 × 4.6 mm, 5 μm) was used to separate the monoamines. The mobile phase consisted of methanol (23% vol/vol), sodium octyl sulfate (0.03% wt/vol), ethylenediaminetetraacetic acid (EDTA) (0.1 mM), sodium phosphate dibasic (0.05 M), and citric acid (0.03 M). The pH of the mobile phase was 2.87, and the flow rate was 1 ml/min. Protein content was determined as described by Lowry et al. (1951).

2.5. Methamphetmine concentrations

Methamphetamine concentrations were determined according to a modified procedure described by Kokoshka et al. (2000). Brain samples were weighed and sonicated in 1 ml of doubled deionized water using a Branson Sonifier 250. Deuterated internal standards were then added and a liquid–liquid extraction procedure performed. Methamphetamine concentrations in brain extracts were determined with a ThermoFinnigan TSQ7000 tandem mass spectrometer

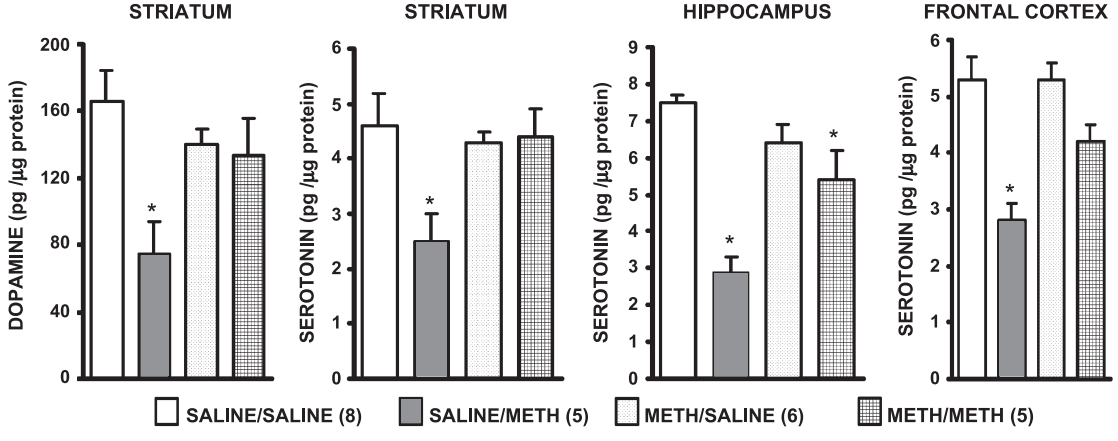

Fig. 1. Quantitative analysis of dopamine and serotonin concentration in striatum, hippocampus, and frontal cortex 7 days following the tolerance-dosing regimen with methamphetamine (METH). Sprague–Dawley rats from the Simonsen laboratory were pretreated with four injections (s.c.) of escalating doses of methamphetamine (ranging from 2.5 to 7.5 mg/kg/injection; 2-h intervals between each injection) or saline vehicle (1 ml/kg/injection), with 40-h drug-free periods between each dosing regimen, and then challenged with a multiple high-dose methamphetamine regimen (four injections, 10 mg/kg/injection, s.c.; 2-h intervals) or saline vehicle, 66 h after the last pretreatment regimen. Data are expressed as mean values ± S.E.M. *Value significantly different from saline-treated controls ($P \leq 0.05$). Values in parentheses represent the number of rats per group.

operating in atmospheric chemical ionization mode. Quality control specimens (300 ng/ml brain homogenate) were co-extracted with each batch and were within 20% of theoretical target concentrations. The limit of quantitation for this assay was 1 ng/ml.

2.6. Statistical analysis

Monoamine content and rectal temperatures were analyzed using analysis of variance (ANOVA) with Tukey–Kramer post-hoc tests. Methamphetamine concentrations were analyzed with factorial ANOVA (treatment and brain region as independent variable; SPSS version 11.5). The assumption of equality of variance was confirmed with Levene's test ($F_{(5,26)}=0.383$, $P>0.05$). Statistical significance was set at $P \leq 0.05$. Data represent means ± standard error of the mean (S.E.M.).

3. Results

Results presented in Fig. 1 demonstrate that a multiple high-dose challenge administration of methamphetamine (4×10 mg/kg/injection, s.c.) decreases striatal dopamine tissue content ($F_{(3,20)}=0.015$, $P<0.05$) and serotonin tissue content in striatum ($F_{(3,20)}=0.05$, $P<0.05$), hippocampus ($F_{(3,20)}=0.0001$, $P<0.05$), and frontal cortex ($F_{(3,20)}=0.0003$, $P<0.05$), 7 days after treatment. However, pretreatment with multiple injections of escalating doses of methamphetamine prior to the high-dose methamphetamine challenge attenuated the methamphetamine-induced depletion of striatal dopamine and serotonin. Tolerance to the serotonergic neurotoxic effects of methamphetamine also occurred in the hippocampus and frontal cortex (Fig. 1). The pretreatment regimen per se did not significantly alter dopamine and serotonin concentrations.

On each day of the methamphetamine pretreatment regimen, the Methamphetamine/Methamphetamine group and the Methamphetamine/Saline group had similar elevated core body temperatures that were statistically different from the saline-treated rats (data not shown). On the day of the challenge, the high-dose methamphetamine challenge administrations elevated core body temperatures in both the Saline/Methamphetamine and Methamphetamine/Methamphetamine groups (Fig. 2). There was a trend for the Methamphetamine/Methamphetamine group to have slightly lower body temperatures than the Saline/Methamphetamine group throughout the 30–150-min time-points but it was only statistically different at the 90-min time-point ($F_{(3,28)}=0.018$, $P \leq 0.05$). Throughout the rest of the experiment, rectal temperatures between the Methamphetamine/Methamphetamine and Saline/Methamphetamine were similar.

Noteworthy are findings that methamphetamine concentrations in the three brain regions of the pretreated and saline-pretreated rats were not statistically different

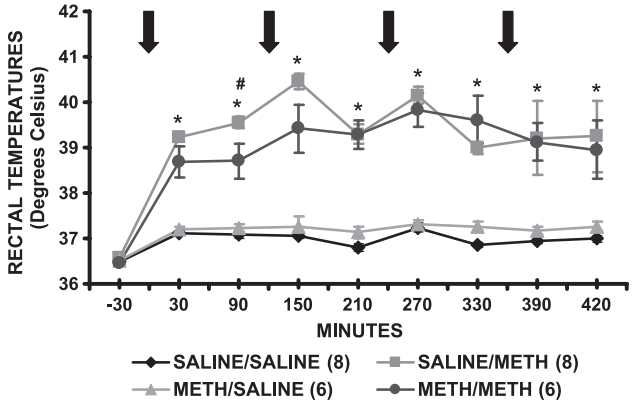

Fig. 2. The effect of the tolerance-dosing regimen with methamphetamine (METH) on body temperature. Core body temperatures were recorded every 1 h after each methamphetamine or saline injection. Sprague–Dawley rats from the Simonsen laboratory were pretreated with four injections (s.c.) of escalating doses of methamphetamine (ranging from 2.5 to 7.5 mg/kg/injection; 2-h intervals between each injection) or saline vehicle (1 ml/kg/injection), with 40-h drug-free periods between each dosing regimen, and then challenged with a multiple high-dose methamphetamine regimen (four injections, 10 mg/kg/injection, s.c.; 2-h intervals) or saline vehicle, 66 h after the last pretreatment regimen. Arrows indicate the time of methamphetamine injection. Data are expressed as mean values ± S.E.M. *Saline/Methamphetamine and Methamphetamine/Methamphetamine groups have values that were significantly different from saline-treated controls ($P \leq 0.05$). #Methamphetamine/Methamphetamine group is significantly different from the Saline/Methamphetamine group. Values in parenthesis represent the number of rats per group.

from each other. A 3 (brain region) by 2 (treatment) factorial ANOVA was performed to compare methamphetamine concentrations. The main effect for brain region was not significant ($F_{(2,26)}=0.065$, $P>0.05$); and the main effect for treatment was also not significant ($F_{(1,26)}=0.343$, $P>0.05$). No interaction effect was observed ($F_{(2,26)}=0.827$, $P>0.05$). Thus, it appears that neither brain region or methamphetamine pretreatment had any effect on methamphetamine concentrations. Methamphetamine concentrations in the striatum, hippocampus, and frontal cortex of methamphetamine-pretreated rats were 5.4 ± 0.8, 6.5 ± 1.1, and 5.8 ± 0.6 ng/mg of tissue, respectively. Methamphetamine concentrations in the striatum, hippocampus, and frontal cortex of saline-pretreated rats were 5.7 ± 0.8, 4.6 ± 1.1, and 6.0 ± 1.1 ng/mg of tissue, respectively.

4. Discussion

The purpose of the present study was to investigate the importance of hyperthermia in tolerance afforded by pretreatment with escalating doses of methamphetamine. Studies have shown that pretreatment with multiple injections of escalating doses of methamphetamine, with drug-free periods between each dosing regimen, attenuates the acute striatal monoaminergic deficits observed 18 h after the

neurotoxic challenge administration (Schmidt et al., 1985; Gygi et al., 1996). Our data confirm and extend these findings by demonstrating that this tolerance to the neurotoxic effects of methamphetamine can persist at least 7 days after the challenge administration. Specifically, rats pretreated similarly with methamphetamine did not exhibit dopamine depletions in striatum, nor serotonin deficits in striatal, frontal cortical, or hippocampal tissues.

Several laboratories have shown that hyperthermia plays an important role in methamphetamine-induced neurotoxicity (Bowyer et al., 1994; Albers and Sonsalla, 1995; Ali et al., 1996). Moreover, employing a bi-weekly methamphetamine pretreatment regimen demonstrated that attenuation of methamphetamine-induced hyperthermia contributes to the tolerance to the neurotoxic effects of methamphetamine (Riddle et al., 2002). Thus, it was important to determine whether the development of tolerance to the neurotoxic effects of methamphetamine was due to the attenuation of methamphetamine-induced hyperthermia during the challenge administration. Contrary to findings by Riddle et al., Fig. 2 demonstrates that with the exception of a single time-point at 90 min, the pretreatment regimen did not prevent the increase in core body temperatures. Although there was a slight lowering of body temperatures in the Methamphetamine/Methamphetamine group at the 30–150-min time-points, rectal temperatures were similar to the Saline/Methamphetamine group throughout the rest of the experiment. Although the possibility that the small decrease at 30–150 min contributed to the tolerance, findings that body temperature were equivalent at all remaining time-points suggest that the difference in temperature did not contribute to the tolerance, 7 days after treatment.

Noteworthy are findings that the methamphetamine-pretreatment did not alter brain methamphetamine concentrations attained after methamphetamine challenge in the striatum, hippocampus, and frontal cortex 2 h after the challenge administration. This differs from the findings of Schmidt et al. (1985) and Gygi et al. (1996) that methamphetamine tolerance was due to alterations in methamphetamine redistribution between the brain and plasma of the tolerant rats. Specifically, methamphetamine-pretreated rats had half the amount of methamphetamine in the brain and twice the amount of methamphetamine in plasma, when compared to non-pretreated rats. A lack of pharmacokinetic differences is consistent, however, with findings by Riddle et al. (2002) using the bi-weekly challenge paradigm. These seemingly contradictory findings highlight the fact that there are likely multiple mechanisms underlying tolerance to the neurotoxic effects of methamphetamine depending on the dosing protocols employed. For instance, studies by Schmidt et al. (1985) and Gygi et al. (1996) used a larger methamphetamine dose for the challenge administration. Another important factor may be age of the rat. Studies by Schmidt et al. (1985) and Gygi et al. (1996) employed studies using younger rats approximately 200 g. Still another important factor is vendor. Notably, rats in this study were obtained from Simonsen laboratories (Gilroy, CA) and that tolerance using the same dosing paradigm could not be attained in rats of the same strain obtained from Charles River Laboratories (Raleigh, North Carolina; data not shown).

In conclusion, this present study demonstrates that tolerance to the neurotoxic effects of methamphetamine using the specific dosing paradigm described above were not due to an attenuation of hyperthermia or alterations in methamphetamine pharmacokinetics. These studies, compared with others, emphasize that many mechanisms likely contribute to tolerance and that great care must be taken when comparing among studies.

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References

Albers, D.S., Sonsalla, P.K., 1995. Methamphetamine-induced hyperthermia and dopaminergic neurotoxicity in mice: pharmacological profile of protective and nonprotective agents. J. Pharmacol. Exp. Ther. 275, 1104–1114.

Ali, S.F., Newport, G.D., Slikker Jr., W., 1996. Methamphetamine-induced dopaminergic toxicity in mice. Role of environmental temperature and pharmacological agents. Ann. N.Y. Acad. Sci. 801, 187–198.

Bowyer, J.F., Tank, A.W., Newport, G.D., Slikker Jr., W., Holson, R.R., 1992. The influence of environmental temperature on the transient effects of methamphetamine on dopamine levels and dopamine release in rat striatum. J. Pharmacol. Exp. Ther. 260, 817–824.

Bowyer, J.F., Davies, D.L., Schmued, L., Broening, H.W., Newport, G.D., Slikker Jr., W., Holson, R.R., 1994. Further studies of the role of hyperthermia in methamphetamine neurotoxicity. J. Pharmacol. Exp. Ther. 268, 1571–1580.

Chapin, D.S., Lookingland, K.J., Moore, K.E., 1986. Effects of LC mobile phase composition retention times for biogenic amines and their precursors and metabolite. Curr. Sep. 7, 68–70.

Gibb, J.W., Hanson, G.R., Johnson, M., 1994. Neurochemical mechanisms of toxicity. In: Cho, A.K., Segal, D.S. (Eds.), Amphetamine and its Analogs. Academic Press, San Diego, CA, pp. 269–295.

Gygi, M.P., Gygi, S.P., Johnson, M., Wilkins, D.G., Gibb, J.W., Hanson, G.R., 1996. Mechanisms for tolerance to methamphetamine effects. Neuropharmacology 35, 751–757.

Kokoshka, J.M., Fleckenstein, A.E., Wilkins, D.G., Hanson, G.R., 2000. Age-dependent differential responses of monoaminergic systems to high doses of methamphetamine. J. Neurochem. 75, 2095–2102.

Lowry, O.H., Rosebrough, N., Farr, A., Randall, R., 1951. Protein measurements with folin phenol reagent. J. Biol. Chem. 193, 265–275.

Riddle, E.L., Kokoshka, J.M., Wilkins, D.G., Hanson, G.R., Fleckenstein, A.E., 2002. Tolerance to the neurotoxic effects of methamphetamine in young rats. Eur. J. Pharmacol. 435, 181–185.

Schmidt, C.J., Sonsalla, P.K., Hanson, G.R., Peat, M.A., Gibb, J.W., 1985. Methamphetamine-induced depression of monoamine synthesis in the rats: development of tolerance. J. Neurochem. 44, 852–855.

Stephans, S., Yamamoto, B., 1996. Methamphetamine pretreatment and the vulnerability of the striatum to methamphetamine neurotoxicity. Neuroscience 72, 593–600.