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Persistence of tolerance to methamphetamine-induced monoamine deficits

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Abstract

Methamphetamine is a highly addictive and potent stimulant, the use of which has increased significantly in recent years. In addition to the severe behavioral and societal consequences associated with methamphetamine abuse, methamphetamine can cause persistent damage to monoaminergic nerve terminals in rats, as measured by either monoamine concentrations or activity of the rate limiting synthetic enzymes, tyrosine hydroxylase and tryptophan hydroxylase. Repeated, sub-neurotoxic doses of methamphetamine, however, can cause rats to become resistant to the neurotoxic effects of multiple high-dose administrations of methamphetamine; a phenomenon known as tolerance. This study investigates the persistence of tolerance evoked by pretreatment with escalating-dose administrations of methamphetamine. Rats were pretreated over several days with low, escalating doses of methamphetamine, followed by high-dose methamphetamine challenge after variable recovery periods. Results revealed that tolerance to monoaminergic deficits persisted for at least one week, but was completely eliminated by 31 days. There were no differences in the distribution of methamphetamine or its major metabolite, amphetamine, between methamphetamine-pretreated animals and saline-pretreated animals 2 h after the final methamphetamine challenge injection, and there were no regional differences in methamphetamine concentrations between the frontal cortex, hippocampus or striatum. We also observed that while methamphetamine pretreatment attenuated the hyperthermia caused by the high-dose methamphetamine challenge, significant reductions in methamphetamine-induced hyperthermia were not required for the development of tolerance with this regimen.

Keywords: Methamphetamine; Tolerance; Persistence; Neurotoxicity; Dopamine; Hyperthermia

1. Introduction

Methamphetamine is a potent and highly addictive stimulant, the use of which increased substantially in the late 1990s (NIDA, 2002). In addition to the negative societal consequences that result from methamphetamine abuse, there are significant toxicological repercussions that are seen in both rodent models and primates (including humans). Multiple high-dose administrations of methamphetamine cause pronounced monoaminergic deficits in the central nervous system (for reviews, see Brown and Yamamoto, 2003; Cadet et al., 2003; Gibb et al., 1994; Kita et al., 2003). Pretreatment with repeated or escalating sub-toxic doses of methamphetamine has been shown to confer protection against (or tolerance to) deficits in the activity of the monoamine generating enzymes tyrosine hydroxylase and tryptophan hydroxylase, as well as brain concentrations of dopamine and serotonin (5-HT) (Abekawa et al., 1997; Gygi et al., 1996; Johnson-Davis et al., 2003, 2004; Schmidt et al., 1985b; Stephans and Yamamoto, 1996; Thomas and Kuhn, 2005). Understanding the mechanisms of tolerance could give insights into how to mitigate some of the deficits associated with high-dose methamphetamine.

The mechanisms contributing to methamphetamine toxicity have been extensively investigated by a number of researchers (for reviews, see Cadet et al., 2003; Davidson et al., 2001;

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Fleckenstein et al., 2000; Kita et al., 2003). In addition, drug addiction and tolerance have been studied in terms of conditioning processes, in which conditioned environmental stimuli can elicit drug-seeking behavior (Poulos et al., 1981) and opponent-process theory, which proposes that tolerance to many stimuli, including drugs, can be explained by a change in baseline affect (Solomon, 1980; Solomon and Corbit, 1974). However, the factors influencing the development of tolerance to methamphetamine-induced monoaminergic deficits have not been as thoroughly studied. One mechanism that has been proposed is a change in the pharmacokinetic distribution of methamphetamine in tolerant animals. Some researchers have shown that brain concentrations of methamphetamine following a multiple high-dose challenge are significantly lower in methamphetamine-pretreated animals (Alburges et al., 1990; Gygi et al., 1996; Schmidt et al., 1985a). In contrast, other, more recent studies have not demonstrated differences in methamphetamine concentrations in the brains of tolerant animals compared to saline-treated controls (Johnson-Davis et al., 2003; Riddle et al., 2002). Another proposed mechanism underlying tolerance is the attenuation of hyperthermia seen in methamphetamine-pretreated animals (Riddle et al., 2002), although recent data suggest that this may not be a prerequisite (Johnson-Davis et al., 2003). One explanation for these varying results is that all of these studies used different tolerance-producing regimens, suggesting the possibility of multiple mechanisms underlying this phenomenon.

One important aspect of tolerance to methamphetamine that has yet to be examined is the persistence of this phenomenon. Multiple, high-dose administrations of methamphetamine have been administered 1–3 days after final methamphetamine pretreatment with escalating-dose paradigms (Alburges et al., 1990; Gygi et al., 1996; Johnson-Davis et al., 2003, 2004; Schmidt et al., 1985b) and one week after a chronic, daily pretreatment regimen (Stephans and Yamamoto, 1996). All of these studies resulted in similar, profound tolerance to methamphetamine-induced neurotoxicity. However, each of these investigations used different pretreatment regimens. A systematic investigation of the persistence of tolerance using a single pretreatment and challenge paradigm has not been conducted. This study evaluates the persistence of tolerance to monoaminergic deficits conferred by escalating-dose methamphetamine treatment. The results reveal that tolerance induced by escalating doses of methamphetamine persists for one to two weeks but is eliminated by 31 days.

2. Materials and methods

2.1. Drugs

±Methamphetamine–HCl for drug administration was supplied from RTI International (Research Triangle Park, NC) in cooperation with the National Institute of Drug Abuse (NIDA) Research Drug Supply Program. Analytical standards of methamphetamine, amphetamine, *p*-hydroxymethamphetamine, *p*-hydroxyamphetamine, methamphetamine-d8, and amphetamine-d5 were obtained from Cerilliant® (Round Rock, TX). Dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid, serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and *N*-methyl serotonin were all purchased from Sigma (St. Louis, MO).

2.2. Animals

Male Sprague-Dawley rats (200–225 g) were obtained from Charles River Laboratories. Rats were allowed to acclimate for 1 week to the temperature-controlled animal facility and to a 14:10 h light/dark cycle before experiments. Animals were housed 3 per cage in clear plastic rat cages. Food and water were available *ad libitum*. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Pharmacological procedures

The tolerance-inducing regimen was based on the work of Schmidt et al. with some modifications (Schmidt et al., 1985a; Schmidt et al., 1985b). For escalating methamphetamine pretreatment, rats received 5 subcutaneous (s.c.) injections of either phosphate buffered saline (0.9%, pH 7.4) or 2.5, 5.0, and 7.5 mg/kg methamphetamine on days 1, 3, and 5, respectively. Injections were administered at 6 h intervals beginning at 7:00 AM on day one. For example, a methamphetamine-pretreated group would receive 2.5 mg/kg doses on Tuesday at 7:00 AM, 1:00 PM, 7:00 PM, 11:00 PM and the following morning (Wednesday) at 7:00 AM, resulting in the 5 administrations of 2.5 mg/kg methamphetamine. This dosing schedule would be repeated with 5.0 mg/kg doses beginning on Thursday morning and with 7.5 mg/kg doses beginning on Saturday morning. Following the pretreatment, half of the saline-pretreated animals and half of the methamphetamine-pretreated animals were challenged with 5 injections of 12.5 mg/kg methamphetamine at 4-h intervals either 24 h, 7 days 14 days, or 31 days after the final pretreatment injection. The remaining animals received only saline injections during this challenge phase. Thus, 4 different groups were evaluated: 1) saline-pretreatment–saline challenge (SS), 2) methamphetamine-pretreatment–saline challenge (MS), 3) saline-pretreatment–methamphetamine challenge (SM), and 4) methamphetamine-pretreatment–methamphetamine challenge (MM). Rats were sacrificed by decapitation either 2 or 22 h after the final challenge injection of either methamphetamine or saline. Core body temperatures were recorded every 2 h during the challenge period and 1/2 h prior to and 1 h after each dose during the pretreatment period. Temperatures were recorded using a THERMES temperature data acquisition system and rat rectal probes (model RET-2; Physitemp, Clifton, N.J.).

2.4. Tissue collection

Immediately after decapitation, blood was collected in a heparin treated Vacutainer™ tube (Becton, Dickinson and Company, Franklin Lakes, NJ), inverted several times, and

placed on ice. The samples were centrifuged at 1250 ×*g* for 10 min to separate the plasma from the cellular fraction. The plasma was then placed in separate microcentrifuge tubes and frozen at −70 °C. Brains were removed from the skull, and the frontal cortex, hippocampus and striatum were dissected, frozen on dry ice and stored at −70 °C.

2.5. Monoamine analysis

Dopamine, DOPAC, homovanillic acid, 5-HT and 5-HIAA content were measured by high-performance liquid chromatography (HPLC) coupled to electrochemical detection according to a modification of the method described by Chapin et al. (1986). Brain tissues were sonicated using a Sonics Vibra Cell VC505 ultrasonic processor equipped with a 1/8″ tapered microtip (Sonics and Materials, Inc., Newton, CT). Tissues were sonicated in 1 ml tissue buffer (0.05 M Na phosphate/0.03 M citric acid with 15% methanol (vol/vol), pH 2.5) including 500 µl *N*-methyl serotonin as an internal standard, then centrifuged at 16,000 ×*g* for 30 min at 4 °C to separate the supernatant from the protein containing fraction. The pellet was saved for analysis of total protein content. The supernatant was transferred to a new tube, centrifuged again for 30 min. and again transferred to a new tube. 50 µl of the final supernatant was injected onto the HPLC. The HPLC system consisted of a Waters 717 plus autosampler, 600 series pump and controller, and a 464 electrochemical detector (Waters Corp. Milford, MA). A glassy carbon electrode was used with an oxidation potential of 0.8 V. Analyte quantitation was based on area ratios as compared to the internal standard using linear curve fits with $1/x$ weighting. The limit of quantitation for each analyte was 2.5 ng/ml of homogenate.

2.6. Protein analysis

Protein analysis was determined by bicinchoninic acid (BCA) assay (Bell, 1973; Lowry et al., 1951; Smith et al., 1985). Pellets from tissues were dissolved in 0.5 ml of 1.0 N NaOH. The resulting suspension was diluted 1:10 in 0.9% NaCl containing 0.05% sodium azide (NaN_3). This diluted material was assayed using a BCA total protein assay kit from Sigma (St. Louis, MO).

2.7. Amphetamine analysis

Analysis of amphetamine, methamphetamine, OH-amphetamine, and OH-methamphetamine was performed according to the following procedure. For plasma samples, 0.5 ml of plasma was extracted. For brain samples, tissues (5–20 mg of frontal cortex, 30–60 mg of hippocampus, or 18–30 mg of striatum) were homogenized in 0.5 ml of water prior to extraction. Internal standards (amphetamine-d5 and methamphetamine-d8) were added to all samples. Method blanks (amphetamine and methamphetamine free plasma or blank brain tissue), unknowns, calibration curve samples, and QC samples were extracted with 5 ml of 4:1 *n*-butyl chloride:acetonitrile (Burdick and Jackson, Muskegon, MI) after pH adjustment with 100 µL of concentrated NH_4OH (29% w/w; Fisher Chemical, Tustin, CA). After rocking for 30 min, samples were centrifuged at 1250 ×*g* for 10 min. The organic layer was transferred to a new glass tube, evaporated to dryness under a stream of air at 20 °C, and reconstituted in 100 µl of 95:5 acetonitrile:0.1% formic acid (Fisher Chemical, Tustin, CA). 20 µl of the final reconstituted sample was injected onto a liquid chromatograph/tandem mass spectrometer.

2.8. LC-MS-MS analysis

Chromatographic separation was achieved on a MetaChem MetaSil Basic column (3.0 × 100 mm; 3 µM pore size; Varian, Lake Forest, CA). The solvents used were 0.1% formic acid and 100% acetonitrile. The solvent gradient was 95:5 formic acid: acetonitrile until 1 min. From 1 min to 13 min, the proportion of acetonitrile was increased from 5% to 42%. The proportion of acetonitrile was then reduced to 5% by 13.5 min and remained there for the duration of the analytical run (18 min). The column temperature was 30 °C. The mass spectrometric system consisted of a Surveyor® HPLC autosampler and pump (ThermoFinnigan; San Jose, CA) coupled to a TSQ® Quantum (ThermoFinnigan; San Jose, CA). Mass spectrometric analysis consisted of electrospray ionization, followed by selective reaction monitoring. Mass to charge transitions were as follows: amphetamine: 136.1 → 91; methamphetamine: 150.1 → 91; amphetamine-d5: 141.1 → 93; methamphetamine-d8: 158.1 → 92.

2.9. Statistical analysis

Statistical analyses were conducted using the StatPro™ add-in function of Microsoft Excel®. For monoamine concentrations, one way analyses of variance (ANOVA), assuming equal variances, with post hoc testing were performed on the results from SS, MS, SM, and MM tissues. Statistical analysis of methamphetamine and amphetamine concentrations was performed as follows: For each analyte (amphetamine or methamphetamine) a one way ANOVA was employed comparing the concentrations of the SM treated animals and MM treated animals in frontal cortex, hippocampus and striatum. Plasma concentrations of methamphetamine and amphetamine in SM and MM treated animals were analyzed using Student's *t*-test. Temperature data were analyzed by ANOVA and post hoc testing at each individual time point. Differences for all analyses were considered significant at $P<0.05$.

3. Results

Fig. 1A and B present the striatal concentrations of dopamine and 5-HT in rats challenged 24 h, 7 days, 14 days or 31 days after the final pretreatment injection with 5 injections of 12.5 mg/kg of methamphetamine and sacrificed 24 h after the last challenge dose. We chose this sacrifice time based upon earlier tolerance work (Gygi et al., 1996; Schmidt et al., 1985b), recognizing that this may not be toxicity in the absolute sense, but is likely predictive of toxicity. Preliminary studies using

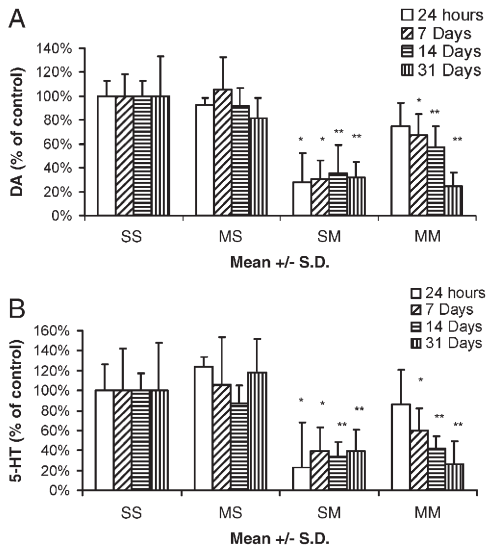

Fig. 1. A. Normalized dopamine concentrations of striatal tissues from rats pretreated and subsequently challenged with either: 1) saline followed by saline (SS), 2) escalating-dose methamphetamine followed by saline (MS), 3) saline followed by high-dose methamphetamine (SM), or 4) escalating-dose methamphetamine and high-dose methamphetamine (MM). The values for each time point were normalized to their respective SS concentration. Exact treatment schedules and doses are described in Materials and Methods. Values are expressed in ng/mg protein and are normalized to the concentration in the SS group. The time in the upper right hand corner refers to the timing of the challenge following the final pretreatment injection. *$P<0.05$ vs. all other groups; **$P<0.05$ vs. SS and MS. B. Normalized 5-HT concentrations of striatal tissues from rats treated as described in Fig. 1. The time in the upper right hand corner refers to the timing of the challenge following the final pretreatment injection. *$P<0.05$ vs. all other groups; **$P<0.05$ vs. SS and MS. All data are expressed as mean±S.D.

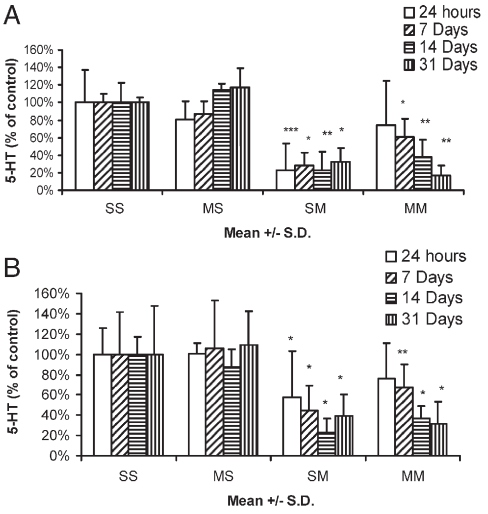

Fig. 2. A. Normalized 5-HT concentrations of frontal cortex tissues from rats treated as described in Fig. 1. The time in the upper right hand corner refers to the timing of the high-dose challenge following the final pretreatment injection. *$P<0.05$ vs. all other groups; **$P<0.05$ vs. SS and MS; ***$P<0.05$ vs. SS group only. B. Normalized 5-HT concentrations of hippocampal tissues from rats treated as described in Fig. 1. The time in the upper right hand corner refers to the timing of the challenge following the final pretreatment injection. *$P<0.05$ vs. SS and MS groups; **$P<0.05$ vs. MS group only.

different doses and injection schedules indicated that, for the particular rats used in these experiments, 12.5 mg/kg methamphetamine every 4 h resulted in maximum monoamine deficits. The data in Fig. 1 demonstrate that this pretreatment paradigm protects rats from monoamine deficits induced by the high-dose methamphetamine regimen without causing significant dopamine or 5-HT depletions in the MS group (pretreated with methamphetamine, challenged with saline). ANOVA revealed that, for animals challenged with high-dose methamphetamine at 24 h, the reductions in the dopamine and 5-HT concentrations for the SM animals were statistically significantly reduced compared to all other groups ($F=16.7$ and 8.9 for dopamine and 5-HT, respectively; degrees of freedom (df)=25 for both analytes; $P<0.05$).

Similar to the striatum, the frontal cortex also exhibited tolerance to high-dose methamphetamine after escalating-dose pretreatment. Fig. 2A shows that there is a significant reduction in the 5-HT concentrations of the SM group relative to the SS group which was not present in the MM groups ($F=3.1$; $df=25$; $P<0.05$). 5-HT concentrations in the hippocampus of the SM and MM groups were not significantly different from each other (Fig. 2B). ($F=4.8$; $df=25$).

Fig. 3 shows the core body temperatures of all rats during the high-dose challenge phase of the experiment. These results reveal that the temperatures of the SM rats were significantly elevated relative to the MM rats at all time points except at 13 and 15 h ($P<0.05$). For all time points, excluding the first and last, core temperatures for the SM and MM groups were significantly greater than both the SS and MS groups ($P<0.05$).

Previous reports of tolerance to methamphetamine have indicated a distributional shift of methamphetamine from the brain to the plasma of methamphetamine-pretreated (tolerant) rats as compared to saline-pretreated controls (Alburges et al., 1990; Gygi et al., 1996; Schmidt et al., 1985a). In contrast to these previous studies, no differences were observed in the distribution of methamphetamine or amphetamine in the striatum, hippocampus, or frontal cortex of methamphetamine pretreated vs. control

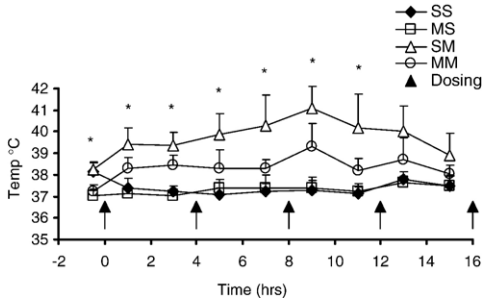

Fig. 3. Temperature data from animals challenged with high-dose methamphetamine 24 h after the final pretreatment dose. Sprague-Dawley rats (Charles River, Canada) were pretreated with 5 injections (s.c.) of escalating doses of methamphetamine (2.5, 5.0, and 7.5 mg/kg/injections; 6 h intervals between each injection) or saline vehicle (1 ml/kg), with 24 h drug-free intervals between each dosing regimen. 24 h after the final pretreatment dose, animals were challenged with either multiple dose methamphetamine (5 injections; 12.5 mg/kg/injection; 4-h intervals) or saline vehicle (1 ml/kg/injection). Data are expressed as mean±S.D. $N=6$ for SS and MS groups. $N=4$ for SM group. $N=10$ for MM group. *SM value significantly larger than MM value ($P<0.05$).

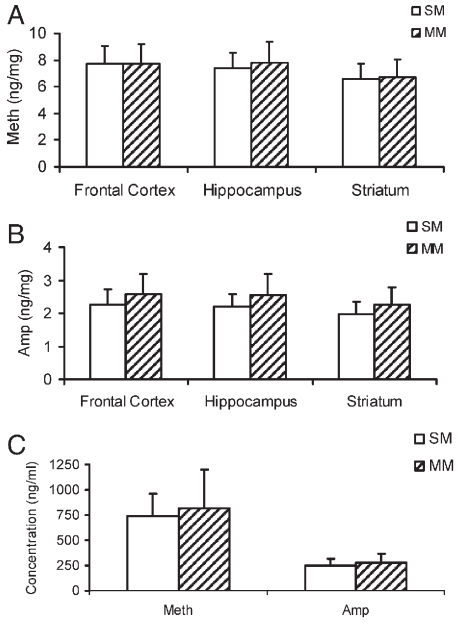

Fig. 4. Methamphetamine and amphetamine concentrations in the frontal cortex, hippocampus, striatum and plasma of rats treated as described in Fig. 1 and sacrificed 2 h after the final methamphetamine injection. $N=5$ for SM group. $N=11$ for MM group. Values represent mean±S.D.

animals (Fig. 4A and B; $F=1.36$; $df=47$). Likewise, plasma concentrations of methamphetamine and amphetamine were nearly identical in the SM and MM groups (Fig. 4C). Fig. 4A and B also demonstrate that there are no regional differences (frontal cortex, hippocampus, and striatum) in the concentration of methamphetamine and amphetamine in the rat brain 2 h after the final challenge dose of methamphetamine.

3.1. Persistence of tolerance: 7 days

Previous studies from our lab, using a different pretreatment protocol with shorter dosing intervals, demonstrated that animals challenged 66 h after the final pretreatment dose of methamphetamine also exhibited tolerance to a high-dose methamphetamine challenge (Johnson-Davis et al., 2003). To determine the persistence of escalating-dose-induced tolerance to methamphetamine-induced monoaminergic deficits, we repeated the pretreatment paradigm described in the Materials and Methods section, but administered the high-dose challenge regimens of methamphetamine 24 h, 1 week, 2 weeks, and 31 days after the final pretreatment injection.

Fig. 1A and B show the striatal monoamine concentrations from rats challenged with five doses of 12.5 mg/kg methamphetamine 7 days after the final pretreatment injection. Although slight decreases in dopamine and 5-HT concentrations were observed in the MM group as compared to SS and MS treated rats, there is clear protection from methamphetamine-induced deficits as compared to the saline-pretreated rats (SM) ($F=39.9$ and 30.4 for dopamine and 5-HT, respectively; $df=29$ for both analytes). Concentrations of dopamine and 5-HT in the methamphetamine-pretreated group (MM) were nearly twice those of the SM group. No pretreatment toxicity was seen in the MS group.

Monoamine concentrations for the frontal cortex and hippocampus of rats treated with high-dose methamphetamine challenge 7 days after pretreatment are shown in Fig. 2A and B, respectively. The frontal cortex data are similar to the striatal monoamine data, showing a slight reduction in the protection afforded by the methamphetamine pretreatment when compared to animals challenged 24 h after pretreatment (Fig. 2A). Tolerance is still evident, with a statistically significant difference between 5-HT concentrations in the SM and MM groups ($F=26.7$; $df=27$; $P<0.05$). In the hippocampus, the difference between 5-HT concentrations in the SM and MM groups is not statistically significant (F-stat=8.8; $df=29$).

Fig. 5 shows the core body temperatures during high-dose methamphetamine challenge regimen for the animals challenged 7 days after pretreatment. At only one point is the temperature for the SM group significantly higher than that of the MM group (5 h after the first dose), although a pattern of slightly elevated temperatures in the SM group compared to the MM group is again evident. Both methamphetamine challenged groups (SM and MM) were significantly warmer than the saline challenged groups (SS and MS) for most of the experiment (Fig. 5).

3.2. Persistence of tolerance: 14 days

This study also included a group of rats that received a methamphetamine challenge regimen 14 days after the final pretreatment dose. Fig. 1A shows the striatal dopamine concentrations for animals sacrificed 22 h after a challenge with either saline or high-dose methamphetamine (12.5 mg/kg) 14 days after the final pretreatment injection. In contrast to animals challenged 24 h or 1 week after pretreatment, the protection from dopaminergic neurotoxicity was diminished for the methamphetamine-pretreated animals. Although the pattern of attenuated neurotoxicity is still evident, at this time point the difference between the SM and MM groups is not statistically significant ($F=19.2$; $df=29$). A similar pattern is seen in the striatal serotonin concentrations of the 14 day rats (Fig. 1B). The 14 day delay in the challenge has nearly eliminated the tolerance seen in the rats challenged at 24 h ($F=33$; $df=29$).

Fig. 2A reveals that tolerance had mostly disappeared in the frontal cortex of animals challenged 14 days after the final pretreatment injection. Reductions in 5-HT concentrations in the SM and MM groups compared to the SS and MS groups,

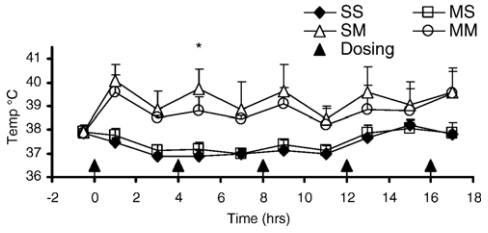

Fig. 5. Temperature data during high-dose methamphetamine challenge from the experiment in which animals were challenged with high-dose methamphetamine 7 days after the final pretreatment injection. Animals and pretreatment schedules are described in the legend of Fig. 1. Data are expressed as mean±S.D. $N=6$ for SS group and MS group. $N=8$ for SM group. $N=10$ for MM group. *SM value significantly larger than MM value ($P<0.05$).

respectively, were similar, and the two values were not statistically significant from each other ($F=36.9$; $df=28$). The same pattern was evident in the hippocampus, where 5-HT concentrations in the SM and MM groups were similarly decreased from the SS and MS groups, respectively (Fig. 2B) ($F=30.2$; $df=28$).

The core body temperatures for the 14 day challenge experiment are shown in Fig. 6. The pattern revealed is similar to the other experiments, in which a modest decrease in core body temperature is seen in the MM group as compared to the SM group. The SM group had significantly higher temperatures at 5, 7, and 9 h after the beginning of the challenge paradigm.

3.3. Persistence of tolerance: 31 days

In order to further elucidate the temporal features of methamphetamine-induced tolerance, we waited 31 days after pretreatment before challenging animals with either saline or multiple administrations of high-dose methamphetamine. Fig. 1A and B show the striatal monoamine concentrations from rats that were challenged 31 days after pretreatment with methamphetamine or saline. These data follow the same trend as the previous two time points and demonstrate that 31 days after escalating-dose pretreatment, protection from monoaminergic deficits caused by high-dose methamphetamine challenge is completely eliminated. For both dopamine and 5-HT there was no significant difference between the SM and MM groups, and both showed significant reductions compared to both the SS and MS groups ($F=23.4$ and 16.2, respectively for dopamine and 5-HT; $df=26$ for both analytes).

Fig. 2A and B shows the 5-HT concentrations of the frontal cortices and hippocampi of rats challenged with high-dose methamphetamine 31 days after escalating-dose pretreatment. As is the case with the striata of these animals, neither tissue is protected from the monoaminergic deficits resulting from high-dose challenge ($F=16.2$ and 18.6 for frontal cortex and hippocampal 5-HT, respectively; $df=24$).

Core body temperatures for rats challenged 31 days after pretreatment are shown in Fig. 7. At most time points, core temperatures of the SM and MM groups were significantly

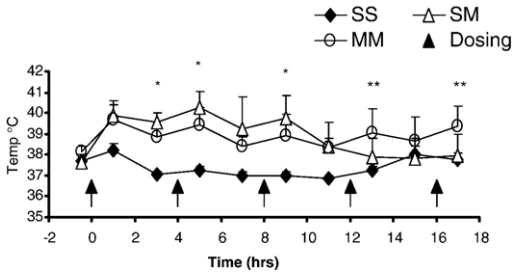

Fig. 6. Temperature data during high-dose methamphetamine challenge from animals challenged with high-dose methamphetamine 14 days after the final pretreatment injection. Animals and pretreatment schedules are described in the legend of Fig. 1. Data are expressed as mean±S.D. $N=6$ for SS group and MS group. $N=8$ for SM group. $N=10$ for MM group. *SM value significantly larger than MM value ($P<0.05$). **SM value significantly smaller than MM value ($P<0.05$).

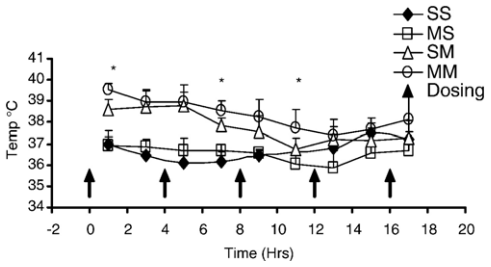

Fig. 7. Temperature data during high-dose methamphetamine challenge from animals challenged with high-dose methamphetamine 31 days after the final pretreatment injection. Animals and pretreatment schedules are described in the legend of Fig. 1. Data are expressed as mean±S.D. $N=6$ for SS group and MS group. $N=8$ for SM group. $N=7$ for MM group. *SM value significantly larger than MM value ($P<0.05$).

greater than those of the SS and MS groups. Once again, we see a pattern in which the differences between all of the groups are reduced towards the end of the challenge period (see the last 4 points in Fig. 7). One interesting observation is that in this experiment, the group with the highest temperatures was not the SM treated animals, as with the 24 h, 7 and 14 day challenge experiments, but the MM group. At 1, 7, and 11 h after the initial methamphetamine challenge injection, the mean temperature of the MM group was significantly greater than the mean temperature of the SM group, and there is a pattern throughout the time course of the challenge of slightly elevated temperatures in the MM group.

4. Discussion

Escalating non-toxic administrations of methamphetamine produce tolerance to the dopaminergic and serotonergic neurotoxicity induced by multiple high-dose administrations of methamphetamine. In this study, we determined the persistence of this tolerance as part of our goal of understanding its underlying mechanisms. Our results show that, in the striatum, the tolerance of both dopaminergic and serotonergic systems to multiple high-dose administration produced by sub-toxic escalating-dose treatment persists at least one week. However, it is not permanent, as after 2 weeks the protection is no longer statistically significant in pretreated rats, and after 31 days it is completely eliminated.

The observation that tolerance to methamphetamine is eliminated after one month is important when viewed in the context of drug addiction and abuse. Many drug abusers go through repeated cycles of active drug use and abstinence, often lasting several months (Galai et al., 2003; Self, 1997). This behavioral pattern has been explained as a conditioned response to drug cues (Poulos et al., 1981) or as an adaptation to an altered affective state caused by repeated drug use (Solomon, 1980; Solomon and Corbit, 1974). We have controlled for pretreatment toxicity by including a methamphetamine-pretreated group that is challenged with saline only (MS group). The lack of a pretreatment effect suggests that, in this particular case, tolerance to monoamine neurotoxicity does not simply result from raising the baseline of monoamine concentrations in pretreated animals. While tolerance to methamphetamine is

neuroprotective with respect to dopamine and 5-HT striatal deficits in the short term, the results presented in this study suggest that extended periods of abstinence, followed by binges during relapse, could place methamphetamine abusers at the same risk for neurotoxic damage as naïve individuals.

4.1. Monoamine toxicity

Although we used a unique combination of pretreatment and challenge protocols, the similarities to other investigations of methamphetamine tolerance are worth noting. This present paradigm was modeled upon work presented by Schmidt et al. (1985a,b). In that case, the inter-administration challenge interval was 6 h vs. 4 h and the rats were sacrificed 18 h after the final dose vs. 22 h in this experiment. However, the striatal results between these two experiments were similar. The same general pattern was also observed by Johnson-Davis et al. (2003) with another escalating-dose paradigm. Finally, our results are consistent with those of Stephans and Yamamoto (1996), in which single doses of methamphetamine were administered for 7 days followed by high-dose challenge seven days after pretreatment. They reported that the SM and MM groups had reductions in dopamine content of 61.9% and 40.7%, respectively, relative to the SS group, which are in close agreement with the data described in the present study. Collectively, the similar patterns of protection observed in these various studies suggest that a common mechanism, or group of mechanisms, is responsible for this phenomenon.

We have also demonstrated that tolerance to serotonergic toxicity in the striatum mimics that observed in the dopaminergic system. The pattern of striatal serotonin depletion and protection observed is comparable to that reported by Schmidt et al. (1985b) following a 24 challenge, by Stephans and Yamamoto (1996) in rats challenged 7 days after the final pretreatment administration, and by Johnson-Davis et al. (2003) in rats challenged with high-dose methamphetamine 66 h after the final pretreatment injection.

We also analyzed the frontal cortices and hippocampi for serotonin content. The frontal cortex showed the same pattern as the striatum, revealing a tolerance profile that was no longer significant at 14 days and had been completely eliminated by 31 days. The hippocampus, did not demonstrate as robust a tolerance profile as the other tissues. However, by 31 days, the pattern of attenuation was eliminated in this tissue as well.

4.2. Hyperthermia

Because methamphetamine-induced monoaminergic deficits have been repeatedly linked to hyperthermia (Albers and Sonsalla, 1995; Bowyer et al., 1994; Imam and Ali, 2001; Metzger et al., 2000), we monitored core body temperatures for all experiments. Two recent reports concluded, respectively, that tolerance was not solely due to a reduction in the hyperthermia associated with neurotoxic methamphetamine administration (Johnson-Davis et al., 2003), and that the protection against methamphetamine-induced reductions in VMAT-2 activity in tolerant animals was not due to the reductions in hyperthermia seen in methamphetamine-pretreated rats (Johnson-Davis et al., 2004). Similar results were seen with mice subjected to a tolerance regimen and subsequent high-dose methamphetamine challenge (Thomas and Kuhn, 2005).

We observe similar responses in the present experiments. In Fig. 3, the animals with higher core body temperatures during the challenge phase had greater monoamine reductions (see Figs. 1 and 2). Taken alone, this would imply that a major contributor for tolerance is the reduction in methamphetamine-induced hyperthermia. However, the data from the 7 day tolerance protocols (Fig. 5) reveal only a slight trend towards lower temperatures in the methamphetamine-pretreated groups, even though tolerance was established in these experiments (Figs. 1 and 2). Rats challenged 14 days after pretreatment (Fig. 6) showed some significant reductions in hyperthermia in the MM groups despite the observation that tolerance was no longer evident (Figs 1 and 2). These variable responses correspond with those of Johnson-Davis et al. (2003), indicating that reductions in hyperthermia may contribute to tolerance, but are not its only cause.

4.3. Distribution and metabolism of methamphetamine

One proposed mechanism for the development of tolerance is redistribution of methamphetamine and amphetamine from the brain to the blood, thus lowering the brain concentrations of methamphetamine and amphetamine. In contrast to the findings of some researchers, (Alburges et al., 1990; Gygi et al., 1996; Schmidt et al., 1985a) we observed no change in the distribution of methamphetamine or amphetamine 2 h after the final challenge dose, regardless of pretreatment (see Fig. 4). The ratios of methamphetamine and amphetamine in brain tissue vs. plasma were approximately 10:1 in both saline and methamphetamine-pretreated animals, and agree with more complete investigations of methamphetamine pharmacokinetics in which blood and tissue concentrations were monitored at multiple points following single intravenous or subcutaneous administrations (Cho and Melega, 2002; Melega et al., 1995; Riviere et al., 2000). The absolute concentrations of methamphetamine in brain tissue and plasma also correlate well with these studies, when normalized for the doses administered. In addition, the absolute brain concentrations of methamphetamine and amphetamine, and the lack of regional differences between the striatum, hippocampus, and frontal cortex reported here are consistent with the results of Johnson-Davis et al. (2003), who also used an escalating-dose tolerance paradigm, followed by a multiple-dose challenge administration. Finally, these results agree with those from a bi-weekly tolerance-induction paradigm (Riddle et al., 2002). The finding that the relative concentrations of methamphetamine and amphetamine in plasma and brain tissue differ from some previous studies could be influenced by a number of factors. The animals used for these experiments were received from a different supplier than other studies. There were also age differences among the animals used in the varying studies. Finally, we used a unique combination of pretreatment and challenge dosing. Nevertheless, our results indicate that pharmacokinetic differences following high-dose challenge do

not appear to be critical for the development of tolerance in this model.

In summary, the present experiments show that tolerance to the monoamine depleting effects of multiple, high-dose methamphetamine administrations produced by the administration of increasing doses of methamphetamine, persists for between seven days and two weeks, but is completely eliminated by 31 days. This protection is not a result of a change in methamphetamine metabolism, nor is it due to a redistribution of methamphetamine in either the brain or plasma of tolerant animals. We also have shown that, while methamphetamine pretreatment can attenuate the hyperthermia resulting from multiple high doses of methamphetamine, tolerance can be achieved in the absence of significant reductions in core body temperature. Johnson-Davis et al. (2003) have shown, using a slightly different protocol, that the reductions in vesicular dopamine uptake and vesicular monoamine transporter (VMAT-2) protein levels in the non-membrane associated fraction of isolated synaptosomes are significantly attenuated in pretreated animals. This could lead to lower intracellular dopamine concentrations in striatal dopamine nerve terminals. Limiting the accumulation of intraneuronal dopamine could reduce the amount of reactive oxygen species that can form as a result of dopamine oxidation (Fleckenstein et al., 1997; Giovanni et al., 1995), thus limiting toxicity. Future studies will investigate this possibility by exploring the role of oxygen and nitrogen radical formation in tolerance.

4.4. Conclusions

The present study demonstrates that tolerance to methamphetamine-induced monoamine depletions is a temporary phenomenon, which lasts 1–2 weeks but is completely eliminated by 31 days. We also have shown, in agreement with other recent reports (Johnson-Davis et al., 2003, 2004; Thomas and Kuhn, 2005) that neither a reduction in hyperthermia nor a change in methamphetamine pharmacokinetics appear to be critical contributors to tolerance. Finally, we believe that the discovery of the transient nature of tolerance to monoaminergic deficits could be important in light of the patterns of abstinence, relapse, and bingeing seen among methamphetamine abusers.

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