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Methamphetamine rapidly decreases mouse vesicular dopamine uptake: role of hyperthermia and dopamine D2 receptors

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Abstract

Multiple high-dose administrations of the dopamine-releasing agent, methamphetamine, rapidly and persistently decrease vesicular dopamine uptake in purified vesicles prepared from striata of treated rats. Because important differences in the neurotoxic effects of stimulants have been documented in rats and mice, the purpose of this study was to determine if methamphetamine-induced effects in rats occur in mice and to elucidate mechanisms underlying these effects. Results reveal methamphetamine treatment rapidly decreased mouse striatal vesicular dopamine uptake; a phenomenon associated with a subcellular redistribution of vesicular monoamine transporter-2 (VMAT-2) immunoreactivity. Both methamphetamine-induced hyperthermia and dopamine D2 receptor activation contributed to the stimulant-induced deficits in vesicular dopamine uptake. Unlike methamphetamine, the dopamine reuptake inhibitors, methylphenidate and cocaine, rapidly increased vesicular dopamine uptake. The implications of these phenomena are discussed.

Keywords: Dopamine; Methamphetamine; VMAT-2; (Mouse)

1. Introduction

The VMAT-2 is largely responsible for regulating both intra- and extra-neuronal dopamine concentrations, as it sequesters dopamine within synaptic vesicles and thereby impacts both cytosolic dopamine levels and the quantity of dopamine available for exocytotic release. Amphetamine and its analogs disrupt this sequestration, presumably by redistributing dopamine to the cytosol thereby promoting reverse transport through the dopamine transporter and subsequent dopamine release (Sulzer et al., 1995).

Recent studies demonstrate an additional effect of amphetamine analogs on VMAT-2. Specifically, Hogan et al. (2000) reported that multiple high-dose administrations of methamphetamine decreased vesicular dopamine transport and binding of the VMAT-2 ligand, dihydrotetrabenazine, as assessed 24 h after treatment in purified vesicles prepared from the striata of treated mice. Importantly, these investigators also found that total dihydrotetrabenazine binding in whole mouse striatal homogenates was not affected at this 24-h time point. In addition, Brown et al. (2000) demonstrated that multiple methamphetamine administrations rapidly (within 1 h) decreased rat vesicular dopamine uptake in a purified vesicular preparation similar to that employed by Hogan and coworkers. This effect was largely associated with dopaminergic nerve terminals, since destruction of the striatal serotonergic projections prior to methamphetamine treatment did not alter the vesicular uptake deficit caused by the stimulant (Brown et al., 2000).

Subsequent studies elucidated mechanisms underlying the methamphetamine-induced decrease in vesicular dopamine uptake. Specifically, Brown et al. (2001) demonstrated that pretreatment with a dopamine D2 receptor antagonist attenuated the deficit in rat vesicular dopamine uptake caused by a single methamphetamine administration. Moreover, Riddle et al. (2002) demonstrated that the methamphetamine-induced deficit in rat vesicular dopamine uptake was associated with a loss of VMAT-2 immunoreactivity in the purified vesicular preparation.

It is unclear whether effects of methamphetamine are also observed in other animal models. This is of particular importance since previous studies have identified differential effects of amphetamine analogs between species. For instance, MDMA has been demonstrated to be neurotoxic to striatal serotonergic neurons in rats but not in mice (Battaglia et al., 1988; Stone et al., 1987). Likewise, species

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differences have also been documented for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Przedborski et al., 2001), *para*-chloroamphetamine (Fuller, 1978; Miller et al., 1971; Steranka and Sanders-Bush, 1979), and fenfluramine (Miller et al., 1971; Steranka and Sanders-Bush, 1979). Hence, the purpose of this study was to investigate whether methamphetamine alters striatal vesicular dopamine transport in mice. The mechanisms underlying this phenomenon and their implications with regard to the neurotoxic effects of methamphetamine are discussed.

2. Materials and methods

2.1. Experimental animals

Male CF-1 mice (25–36 g; Charles River, Portage, MI) were housed in groups of 4 in plastic cages, maintained under conditions of controlled temperature of 24 °C on a 14/10 h light/dark cycle, unless otherwise specified in figure legends. Food and water were provided ad libitum. On the day of the experiment, mice were housed in groups of 8 in plastic cages. Core (rectal) body temperatures were determined using a digital rectal thermometer (Physiotemp Instruments, Clifton, NJ). Mice were sacrificed by decapitation. All procedures were conducted in accordance with guidelines approved by the Animal Care Committee of the University of Utah and the National Institutes of Health.

2.2. Drugs and chemicals

Methamphetamine hydrochloride and methylphenidate hydrochloride were supplied generously by the National Institute on Drug and Abuse. [7,8-^{3}H]Dopamine (47–50 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL) and [2-^{3}H]dihydrotetrabenazine (20 Ci/mmol) was purchased from American Radioligand Chemicals, Inc. (St. Louis, MO). Tetrabenazine was kindly donated by Drs. Jeffrey Erickson and Helene Varoqui (Louisiana State University Health Sciences, New Orleans, LA). (*R*)-(+)-7chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390) and eticlopride were purchased from Sigma (St. Louis, MO). Drugs were administrated as indicated in the legends of appropriate figures, and doses were calculated as the respective free bases. Drugs were dissolved in 0.9% saline.

2.3. Preparation of mouse striatal synaptic vesicles

Synaptic vesicles were obtained from synaptosomes prepared from mouse striatum as described previously (Brown et al., 2002; Sandoval et al., 2002). Briefly, fresh tissue was homogenized in ice-cold 0.32 M sucrose. The homogenate was centrifuged (800 × *g* for 12 min; 4 °C), and the supernatant (S1) was carefully removed and centrifuged (22,000 × *g* for 15 min; 4 °C) to obtain the synaptosomal-containing pellet (P2). The resulting P2 were resuspended and homogenized in ice-cold distilled deionized water. Osmolarity was restored by addition 25 mM HEPES and 100 mM potassium tartrate (pH 7.5, 4 °C). Samples were centrifuged for 20 min at 20,000 × *g* (4 °C). The resultant S3 removed and $MgSO_4$ added (final concentration of 1 mM, pH 7.5, 4 °C) and centrifuged at 100,000 × *g* for 45 min. The final P4 were resuspended at 50 mg/ml (original tissue wet weight).

2.4. Vesicular [^{3}H]dopamine uptake and [^{3}H]dihydrotetrabenazine binding

Vesicular [^{3}H]dopamine uptake was performed by incubating 100 μl (~ 2.5 μg protein) of synaptic vesicle samples at 30 °C for 3 min in assay buffer (final concentration in mM: 25 HEPES, 100 potassium tartrate, 1.7 ascorbic acid, 0.05 EGTA, 0.1 EDTA, 2 ATP-Mg^{2+}, pH 7.5) in the presence of [^{3}H]dopamine (30 nM final concentration). The reaction was terminated by addition of 1 ml cold wash buffer (assay buffer containing 2 mM $MgSO_4$ substituted for the ATP-Mg^{2+}, pH 7.5) and rapid filtration through Whatman GF/F filters soaked previously in 0.5% polyethylenimine. Filters were washed three times with cold wash buffer using a Brandel filtering manifold. Radioactivity trapped in filters was counted using a liquid scintillation counter. Nonspecific values were determined by measuring vesicular [^{3}H]dopamine uptake at 4 °C in wash buffer.

Binding of [^{3}H]dihydrotetrabenazine was performed as described previously (Brown et al., 2000, 2001, 2002). Briefly, 200 μl (~ 6 μg protein) of the synaptic vesicle preparation was incubated in wash buffer in the presence of [^{3}H]dihydrotetrabenazine (2 nM final concentration) for 10 min at 25 °C. The reaction was terminated by addition of 1 ml cold wash buffer and rapid filtration through Whatman GF/F filters soaked in 0.5% polyethylenimine. Filters were washed three times with ice-cold wash buffer. Radioactivity trapped in filters was counted using a liquid scintillation counter. Nonspecific binding was determined by co-incubation with 20 μM tetrabenazine. All protein concentrations were determined by a BioRad protein assay (BioRad Laboratories, Hercules, CA).

2.5. Preparation of striatal subcellular fractions

Fresh striatal tissue was homogenized in ice-cold 0.32 M sucrose and centrifuged (800 × *g* for 12 min: 4 °C). The resulting supernatant (S1) was then centrifuged (22,000 × *g* for 15 min: 4 °C), and the pellets (P2); whole synaptosomal fraction (plasmalemmal membrane plus vesicular subcellular fraction) were resuspended in cold distilled deionized water at a concentration of 50 mg/ml (original wet weight of tissue). Resuspended tissue was aliquoted into two test tubes. One aliquot was centrifuged (22,000 × *g* for 20 min; 4 °C) to separate plasmalemmal membranes from the synaptic vesicle-enriched fraction. The resulting supernatant

(S3) contained the vesicular subcellular fraction of interest, and the pellets (P3; plasmalemmal membrane fraction) were resuspended in cold distilled deionized water.

2.6. Western blot analysis

VMAT-2 antibody was purchased from Chemicon International, Inc. (Temecula, CA). Binding of VMAT-2 antibody was performed using 60 μl of whole synaptosomal, plasmalemmal membrane or vesicle subcellular fractions. Samples were added to 20 μl of loading buffer (final concentration: 2.25% sodium dodecyl sulfate, 18% glycerol, 180 mM Tris Base (pH 6.8), 10% β-mercaptoethanol and bromophenol blue). Approximately 60 μg P2, 40 μg protein P3, and 20 μg protein S3 were boiled for 10 min and loaded in a 10% sodium dodecyl sulfate-polyacrylamide gel. Following electrophoresis, samples were transferred to polyvinylidene difluoride hybridization transfer membrane (New England Nuclear, Boston, MA). All subsequent incubation steps were performed at room temperature while shaking. Each membrane was first blocked for 2 h in 100 ml of Tris buffer saline with Tween (TBST; 250 mM NaCl, 50 mM Tris pH 7.4 and 0.05% Tween 20) containing 5% nonfat dry milk. Each membrane was then incubated with anti-VMAT-2 antibody (1:4000 dilution) in 12 ml of TBST with 5% milk for 1 h and then washed five times (2 × 1 min wash: 3 × 5 min wash) in 70 ml TBST with 5% milk. The membranes then were incubated for 1 h with the goat $F(ab')_2$ anti-rabbit immunoglobulin antibody (Biosource International, Camarillo, CA) at a 1:2000 dilution in TBST with 5% milk. This secondary antibody had been affinity-isolated, preabsorbed with human immunoglobulin, and conjugated with horseradish peroxidase. The membranes were then washed five times (2 × 1 min wash: 3 × 5 min) with 70 ml TBST, and then developed with Renaissance Western Blot Chemiluminescence Reagent Plus (New England Nuclear, Boston, MA), according to manufacturer specification. Multiple exposures of blots were obtained to ensure development within the linear range of the film (Kodak Biomax MR). Bands on blots were quantified by densitometry measuring net intensity (the sum of the background-subtracted pixel values in the band area) using Kodak 1D image-analysis software.

2.7. Striatal dopamine content

On the day of the assay, frozen striatal samples were sonicated 3–5 s in cold tissue buffer (0.05 M sodium phosphate/0.03 M citric acid buffer with 15% methanol (v/v); pH 2.5), and centrifuged for 15 min at 22,000 × *g*. Resulting tissue pellets were retained and protein was determined according to the method of Lowry et al. (1951). The resulting supernatant was centrifuged a second time for 10 min at 20,000 × *g*. A total of 20 μl of supernatant was injected onto a high-performance liquid chromatography system coupled to an electrochemical detector (+0.73 V) for separation and quantification of dopamine levels using the method of Chapin et al. (1986).

2.8. Data analysis

Statistical analyses were conducted using one- or two-way analysis of variance (ANOVA) followed by Fisher's least significant difference post-hoc comparison where appropriate. For data presented in Fig. 4, eticlopride/methamphetamine data were pooled for two-way ANOVA, and subsequently divided into two groups according to ambient temperature for post-hoc comparison between these groups, and with the saline-pretreated, methamphetamine-treated group. Differences among groups were considered significant if the probability of error was less than 5%.

3. Results

Results presented in Fig. 1 demonstrate that multiple high-dose injections of methamphetamine (four injections, 10 mg/kg/injection, s.c., 2-h intervals) rapidly decreased both vesicular [^{3}H]dopamine uptake and [^{3}H]dihydrotetrabenazine binding, as assessed 1 and 24 h after treatment in purified synaptic vesicles. The deficit observed 1 h after treatment reflected a redistribution of VMAT-2 immunoreactivity among subcellular fractions (Fig. 2). Specifically, methamphetamine had little effect on total VMAT-2 immunoreactivity in synaptosomes prepared from treated mice (P2). However, upon osmotic lysis and subsequent fractionating of synaptosomes into a vesicular-enriched fraction and a remaining membrane-associated fraction, a redistribution was observed such that methamphetamine treatment decreased VMAT-2 immunoreactivity in the vesicle preparation (S3), while increasing it (albeit not significantly) in a corresponding membrane-associated fraction (P3).

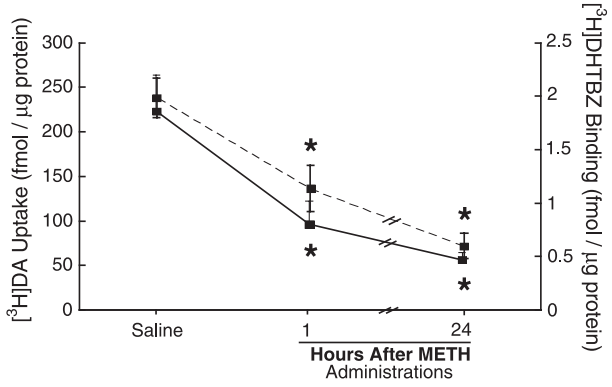

Fig. 1. Treated mice received four injections of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) and were sacrificed 1 or 24 h later. Control mice received four injections of saline vehicle (5 ml/kg/injection, s.c.) and were sacrificed 1 h later (zero-time controls). Circles and squares represent mean vesicular dopamine uptake and dihydrotetrabenazine binding, respectively, and vertical lines 1 S.E.M. of determinations in six mice. *Values significantly different from zero-time controls ($P \leq 0.05$).

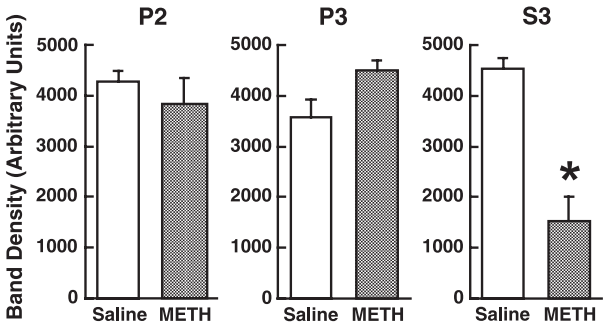

Fig. 2. Mice received four injections of methamphetamine (METH; 10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (5 ml/kg/injection, s.c.) and were sacrificed 1 h later. Columns represent means and vertical lines 1 S.E.M. of determinations in four mice. *Values significantly different from saline-treated controls ($P \leq 0.05$).

Results presented in Fig. 3 demonstrate that pretreatment with the dopamine D1 receptor antagonist, SCH23390 (2 mg/kg, i.p.), did not prevent the decrease in vesicular dopamine uptake caused by methamphetamine treatment. In contrast, pretreatment with the dopamine D2 receptor antagonist, eticlopride (2 mg/kg, i.p.) attenuated this decrease (Fig. 4A). In addition, eticlopride pretreatment attenuated the increase in core body temperature caused by methamphetamine treatment (Fig. 4B). Maintenance of hyperthermia in mice treated with eticlopride and methamphetamine (i.e., by placing mice in a 28.5 °C environment instead of the ambient environment of 23 °C) diminished the ability of eticlopride to prevent the methamphetamine-induced decrease in vesicular dopamine uptake (Fig. 4A and B).

To investigate further the role of hyperthermia in the methamphetamine-induced decrease in vesicular dopamine uptake, mice were treated with the stimulant while maintained in an ambient environment of 6 or 28.5 °C so as to prevent or promote, respectively, drug-induced increases in

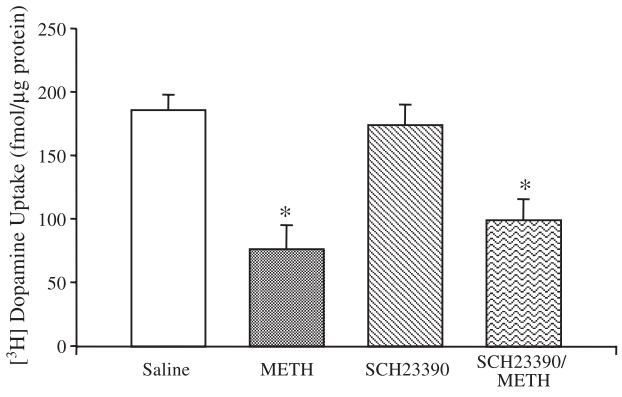

Fig. 3. Mice received four injections of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (5 ml/kg/injection, s.c.) and were sacrificed 1 h later. In addition, mice received SCH23390 (2 mg/kg, i.p.) or saline vehicle (5 ml/kg, i.p.) 15 min prior to each injection of methamphetamine or saline vehicle. Columns represent means and vertical lines 1 S.E.M. of determinations in six mice. *Values significantly different from saline-treated controls ($P \leq 0.05$).

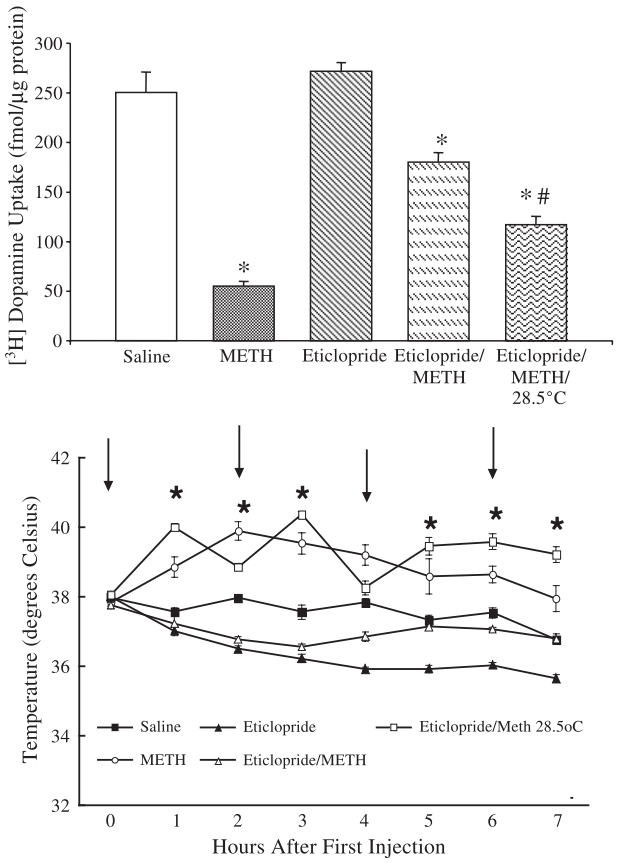

Fig. 4. Mice received four injections of methamphetamine (10 mg/kg/injection, s.c., 2-h intervals) or saline vehicle (5 ml/kg/injection, s.c.) and were sacrificed 1 h later. In addition, mice received eticlopride (2 mg/kg, i.p.) or saline vehicle (5 ml/kg, i.p.) 15 min prior to each injection of methamphetamine or saline vehicle. Columns represent means and vertical lines 1 S.E.M. of determinations in six mice (upper panel). *Values significantly different from saline-treated controls ($P \leq 0.05$). #Value significantly different from rats receiving methamphetamine per se or methamphetamine and eticlopride in a 28.5 °C environment. Corresponding body temperatures are presented in the lower panel, where vertical lines represent the time of each methamphetamine injection.

core body temperature. Maintenance in a 6 °C environment prevented methamphetamine-induced hyperthermia (i.e., temperatures approximated 36.5 °C throughout the pretreatment regimen) whereas methamphetamine-treated mice maintained in the warmer environment attained mean temperatures spanning 38.5–40.5 °C. As expected, methamphetamine treatment of mice maintained in a 28.5 °C environment decreased vesicular dopamine uptake by 80% (from 193 ± 15 to 39 ± 9 fmol/µg protein for saline- and methamphetamine-treated rats, respectively, $n = 5–6$). In contrast, mice maintained in the 4 °C environment displayed only a 35% decrease (from 200 ± 18 to 131 ± 15 fmol/µg protein for saline- and methamphetamine-treated rats, respectively, $n = 6$).

Noteworthy is the finding that not all psychostimulants decrease vesicular dopamine uptake in the purified vesicular fractions under study. Specifically, a single injection of methylphenidate (10 or 50 mg/kg, s.c.) or cocaine (30 mg/

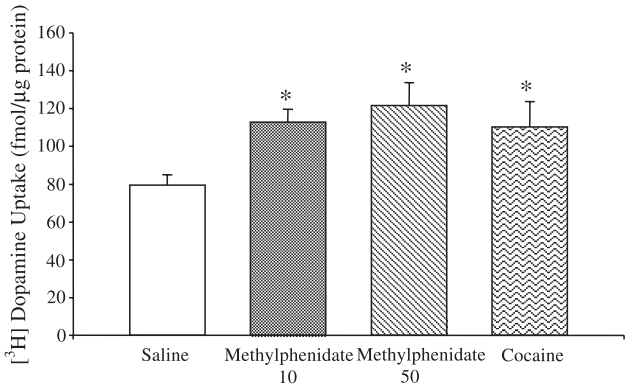

Fig. 5. Mice received a single injection of methylphenidate (10 or 50 mg/kg, s.c.), cocaine (30 mg/kg, i.p.) or saline vehicle (5 ml/kg s.c.) and were sacrificed 1 h later. Columns represent means and vertical lines 1 S.E.M. of determinations in six mice. *Values significantly different from saline-treated controls ($P \leq 0.05$).

kg, i.p.) increased vesicular dopamine uptake as assessed in vesicles prepared 1 h after treatment (Fig. 5).

4. Discussion

Previous studies have demonstrated species differences in the effects of amphetamine analogs on monoaminergic neurons. For example, Stone et al. (1987) reported prolonged serotonergic deficits in rats administered a single injection of MDMA (10 mg/kg) that were not observed in mice treated with a low (15 mg/kg) or a high (60 mg/kg) single injection of MDMA. Long-term decreases were only observed in mice following exposure to multiple administrations of MDMA (6 × 15 mg/kg at 4-h intervals; Stone et al., 1987). Likewise, doses of MPTP necessary to induce central dopaminergic deficits differ between mice and rats (Przedborski et al., 2001). For instance, no significant decreases in dopamine content result in rats administered a dose comparable to that employed in the mouse model of dopaminergic neurodegeneration (Przedborski et al., 2001). Differences have also been reported for additional amphetamine analogs such as *para*-chloroamphetamine (Fuller, 1978; Miller et al., 1971; Steranka and Sanders-Bush, 1979) and fenfluramine (Miller et al., 1971; Steranka and Sanders-Bush, 1979). Such disparities demonstrate that not all stimulants cause neurotoxicity in the same manner across species and thereby warrant further investigations to attain better insight into how these agents induce their deleterious effects. Stimulant-induced alterations in vesicular dopamine uptake and redistribution of VMAT-2 protein have been implicated in the neurotoxic effects of these agents (Riddle et al., 2002; Sandoval et al., 2002). Thus, the goal of this study was to: (1) investigate the impact of methamphetamine on VMAT-2 in mice as compared to rats, and (2) ascertain mechanisms underlying effects observed in mice.

As noted in the Introduction, it has been demonstrated that multiple administrations of methamphetamine to rats causes a rapid decrease in vesicular dopamine uptake; an effect that is observed 1 and 24 h after drug treatment (Brown et al., 2000). A decrease in dihydrotetrabenazine binding has also been reported in mice 24 h after methamphetamine treatment (Hogan et al., 2000). Dopamine D2 receptor activation contributes to the rapid methamphetamine-induced decrease in purified vesicles from rat striata (Brown et al., 2001). Similarly, results presented in the current study demonstrate that multiple methamphetamine administrations rapidly decrease dopamine uptake in vesicles purified from mouse striata. As observed in rats, this deficit persists for 24 h, and dopamine D2 receptors contribute to this deficit.

The data from this study demonstrated that methamphetamine-induced hyperthermia contributed to the rapid decrease in vesicular dopamine uptake caused by multiple administrations of the stimulant to mice. The methamphetamine-induced reduction in mouse vesicular dopamine uptake was attenuated in methamphetamine-treated mice maintained in a cooler environment (i.e., 6 °C) providing further support for the role in hyperthermia. This is in contrast to the finding in rats by Brown et al. (2002) that hyperthermia did not contribute to the rapid decrease in striatal vesicular dopamine uptake observed after a single methamphetamine injection. In addition to a species difference, a possible explanation for the inconsistency is the difference in dosing paradigms (i.e., a single injection administered to rats vs. multiple administrations to mice in the present study). Further studies investigating factors contributing to the effects of multiple administration of methamphetamine in rats are required.

Noteworthy are findings presented in Fig. 2 suggesting that high-dose methamphetamine treatment (4 × 10 mg/kg, s.c., 2-h intervals) was without effect on total VMAT-2 protein immunoreactivity in whole synaptosomes (i.e., the P2 fraction) prepared from the striata of treated mice. However, when the synaptosomes were lysed and fractionated into non-membrane (S3) and membrane-associated (P3) fractions, decreases and slight (albeit insignificant) increases, respectively, in VMAT-2 immunoreactivity were observed. Since there is approximately twice the amount of VMAT-2 immunoreactivity in P3 vs. S3 (data not shown), a significant decrease in S3 may not be sufficient to significantly increase VMAT-2 in the P3 fraction. Taken together, these data suggest that methamphetamine may affect a redistribution of VMAT-2 within nerve terminals. Differences between the effects in purified vesicular and more inclusive fractions are not without precedent; specifically, Hogan et al. (2000) first reported a decrease in dihydrotetrabenazine binding 24 h after methamphetamine treatment in a mouse purified vesicular preparation similar to the one used in the present study, without a concurrent change in whole homogenate dihydrotetrabenazine binding. Similar findings have been reported in rat tissue 1 h after multiple methamphetamine administrations (Brown et al., 2000).

Methamphetamine-induced redistribution of vesicles may have important implications regarding the neurotoxic prop-

erties of the stimulant. It is well established that high dose methamphetamine administration causes persistent deficits in dopaminergic neuronal function (for review see Fleckenstein et al., 2000; Nath et al., 2000; Ricaurte and McCann, 1992; Seiden et al., 1988). Several groups have suggested that dopamine per se contributes to this long-term damage, as it is attenuated by pretreatment of rats with the dopamine synthesis inhibitor, α-methyl-*p*-tyrosine (Gibb and Kogan, 1979; Wagner et al., 1983). The precise mechanism whereby dopamine contributes to these long-term decreases is unknown, although dopamine can contribute to the formation of highly reactive oxygen species (Graham et al., 1978; Hastings, 1995). Since cytoplasmic dopamine levels are regulated largely by VMAT-2, deficits in the function of this carrier protein may contribute to the damage. Specifically, it can be hypothesized that by decreasing vesicular dopamine uptake, dopamine may accumulate within nerve terminals promoting the formation of reactive oxygen species and thereby causing neurotoxicity. Support for this hypothesis comes from findings by Fumagalli et al. (1999), who reported that VMAT-2 heterologous knockout mice had worsened methamphetamine-induced deficits compared to wild-type mice. Further studies elucidating the role of VMAT-2 and associated vesicles in affecting methamphetamine-induced neurotoxicity are warranted.

Noteworthy are findings that not all psychostimulants decrease vesicular dopamine uptake in mouse purified vesicular fractions. Specifically, a single injection of methylphenidate or cocaine increased vesicular dopamine uptake, as assessed in vesicles prepared 1 h after treatment. Similar findings have been reported in rats (Brown et al., 2001; Sandoval et al., 2002). The mechanism by which these dopamine reuptake inhibitors increase vesicular dopamine uptake in mice requires further investigation. However, previous findings in rats demonstrate that the increase in vesicular dopamine uptake following methylphenidate treatment is associated with dopamine D1 and D2 receptor activation (Sandoval et al., 2002), whereas dopamine D2 receptor activation contributes to the increase in cocaine-treated rats (Brown et al., 2001). Hence, these data provide additional evidence that VMAT-2 function can be rapidly altered in response to drug treatment. One interesting implication of this phenomenon extends from the hypothesis that a decrease in vesicular dopamine uptake may be neurotoxic. Accordingly, drugs that increase vesicular dopamine uptake may be neuroprotective in these model systems. Interestingly, it has been demonstrated that pre- and/or post-treatment with dopamine reuptake inhibitors, including methylphenidate (Sandoval et al., 2003), can protect against the long-term dopaminergic deficits caused by methamphetamine treatment (Marek et al., 1990; Schmidt and Gibb, 1985). The demonstration that psychostimulants can rapidly and bi-directionally alter VMAT-2 in a mouse as well as a rat provides an additional model for investigating the role of VMAT-2 in affecting stimulant-induced neurotoxicity. Such studies may provide insight not only into the neurotoxicity afforded by methamphetamine, but also deficits resulting from other disorders perhaps involving abnormal intraneuronal dopamine distribution such as Parkinson's disease.

In conclusion, the present study demonstrates that psychostimulants rapidly alter vesicular monoamine transport in a mouse model. In particular, methamphetamine rapidly decreases vesicular dopamine uptake via a mechanism involving both dopamine D2 receptors and hyperthermia. Both similarities and differences in effects on mice and rats are reported. This information not only extends our understanding of potential neurotoxic mechanisms, but also provides a foundation for the future use of genetic knockout animals that will enhance our understanding of mechanisms whereby stimulants alter vesicular localization and neurotransmitter uptake.

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