

## Rapid Communication

# Methamphetamine Rapidly Decreases Vesicular Dopamine Uptake

Jeffrey M. Brown, Glen R. Hanson, and Annette E. Fleckenstein

Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah, U.S.A.

**Abstract:** Vesicular sequestration is important in the regulation of cytoplasmic concentrations of monoamines such as dopamine. Moreover, recent evidence suggests that increases in cytoplasmic dopamine levels, perhaps attributable to changes in vesicular monoamine transporter function, contribute to methamphetamine-induced dopaminergic deficits. Hence, we examined whether striatal vesicular uptake is altered following methamphetamine treatment. Multiple administrations of methamphetamine rapidly (within 1 h) decreased vesicular dopamine uptake and dihydrotetrabenazine binding, an effect that (a) persisted at least 24 h, (b) was associated with dopamine and not serotonin neurons, and (c) was unrelated to residual drug introduced by the original methamphetamine treatment. These data suggest that methamphetamine rapidly decreases vesicular monoamine transporter function in dopaminergic neurons, a phenomenon that may be associated with the long-term damage caused by this stimulant. **Key Words:** Vesicular monoamine transporter—Methamphetamine—Synaptic vesicles—Dopamine uptake—Striatum. *J. Neurochem.* **74**, 2221–2223 (2000).

High-dose methamphetamine administration produces long-term deficits in dopaminergic systems (for review, see Gibb et al., 1994). Depletion of dopamine by a tyrosine hydroxylase inhibitor,  $\alpha$ -methyl-*p*-tyrosine, attenuates this damage, suggesting a pivotal role for dopamine in this persistent decrement (for review, see Fleckenstein and Hanson, 2000). Although the mechanism by which dopamine contributes to these deficits is unknown, it is established that dopamine can cause formation of highly toxic reactive oxygen species (Graham, 1978). In addition, treatment with methamphetamine increases the formation of reactive oxygen species (Giovanni et al., 1995; Fleckenstein et al., 1997c) and thereby contributes to dopaminergic deficits caused by administering this psychostimulant (Wagner et al., 1985; Cadet et al., 1994).

Recent studies suggest that autoxidation of intraneuronal dopamine contributes to the persistent effects of methamphetamine. For instance, Cubells et al. (1994) suggested that methamphetamine-induced neurotoxicity results from a disruption of vesicular dopamine storage and generation of intracellular reactive oxygen species that overwhelm antioxidant systems.

Cytosolic levels of catecholamines, including dopamine, are regulated largely by the vesicular monoamine transporter-2 (VMAT-2), which transports catecholamines into synaptic vesicles. Accordingly, a methamphetamine-induced decrease in VMAT-2 function may increase intraneuronal dopamine concentrations and thereby contribute to the neurotoxic effects of this stimulant. Hence, the purpose of the present study was to examine whether high-dose methamphetamine treatment alters vesicular dopamine transport.

## MATERIALS AND METHODS

All experiments were conducted in accordance with the NIH *Guidelines for the Care and Use of Laboratory Animals*. Where indicated, male Sprague–Dawley rats (weighing 280–330 g) received multiple high-dose injections of methamphetamine (4  $\times$  10 mg/kg per injection, calculated as free base, s.c., 2-h intervals) or saline vehicle (1 ml/kg per injection, s.c.). Striatal synaptic vesicles were prepared from rats decapitated 1 or 24 h after methamphetamine or saline treatment. Vesicular [ $^3$ H]dopamine uptake was determined as described by Teng et al. (1997) with the following modifications: (a) Synaptic vesicles were isolated in two ultracentrifugation steps (20,000 g, 20 min and 100,000 g, 45 min); (b) vesicles were incubated at 30°C for 3 min in the presence of [ $^3$ H]dopamine (final concentration, 30 nM); and (c) nonspecific uptake was determined by incubating synaptic vesicles at 4°C in the absence of ATP. Protein concentrations were determined by a Bio-Rad protein assay.

Binding of dihydrotetrabenazine (DHTBZ) was performed as described by Teng et al. (1998). In brief,  $\sim$ 2.5  $\mu$ g of protein was incubated in the presence of 2 nM [ $^3$ H]DHTBZ (final concentration) for 10 min at 25°C. The reaction was terminated by addition of 4 ml of ice-cold wash buffer. Nonspecific binding was determined by coinubation with 20  $\mu$ M tetrabenazine.

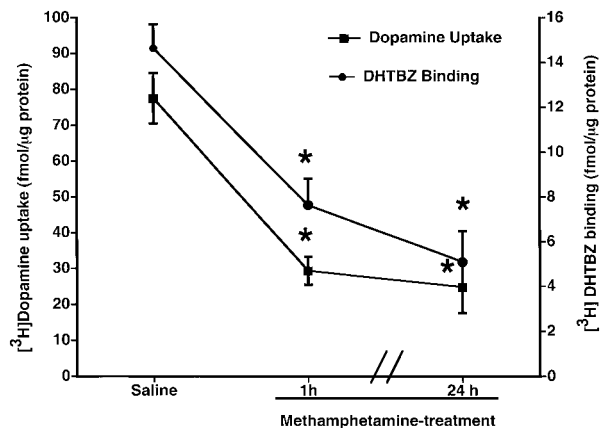
To assess the contribution of vesicles associated with serotonin neurons to the uptake of [ $^3$ H]dopamine in our assay, a

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Address correspondence and reprint requests to Dr. A. E. Fleckenstein at Department of Pharmacology and Toxicology, University of Utah, 30 South 2000 East, Room 201, Salt Lake City, UT 84112, U.S.A. E-mail: Annette.Fleckenstein@hsc.utah.edu

*Abbreviations used:* DHTBZ, dihydrotetrabenazine; PCA, *p*-chloroamphetamine; VMAT-2, vesicular monoamine transporter-2.



**Fig. 1.** Rats received methamphetamine (four injections, 10 mg/kg per injection, s.c., 2-h intervals) or saline vehicle (1 ml/kg per injection, s.c.) and were killed by decapitation 1 or 24 h later. Data are mean  $\pm$  SEM (bars) values for determinations in seven or eight treated rats of vesicular [<sup>3</sup>H]dopamine uptake (■) and [<sup>3</sup>H]DHTBZ binding (●). \* $p < 0.001$ , values for methamphetamine-treated rats that are significantly different from saline-treated controls.

single dose (7.5 mg/kg, s.c., 1 week before the animal was killed) of the serotonin neurotoxin *p*-chloroamphetamine (PCA) was administered where indicated. To demonstrate the extent of the resulting serotonergic deficits, serotonin concentrations were determined in striatal samples using HPLC with electrochemical detection as described previously (Chapin et al., 1986). Protein concentrations in these samples were determined as described by Lowry et al. (1951).

Statistical analyses used an ANOVA or Student's *t* test. Statistical significance was set at  $p \leq 0.05$ .

## RESULTS

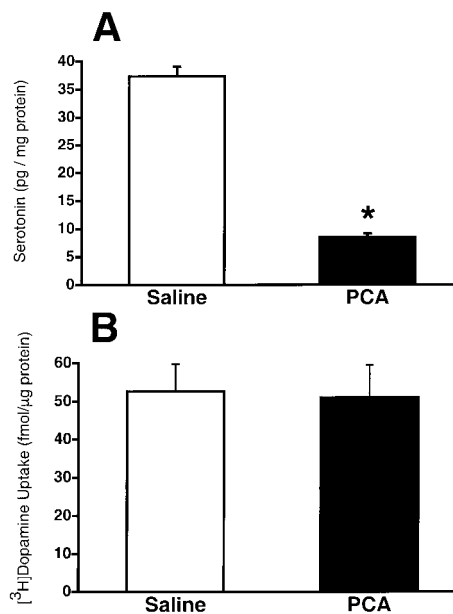
One hour after methamphetamine treatment, vesicular dopamine uptake was decreased by 65%, a deficit that persisted 24 h after treatment. This decrease in vesicular uptake was accompanied by a similar decrease in [<sup>3</sup>H]DHTBZ binding at 1 and 24 h following methamphetamine administration (Fig. 1). The decrease in vesicular uptake was not due to residual methamphetamine because isolation of the vesicles "washes" methamphetamine from the preparation to levels of  $<1$  nM (Fleckenstein et al., 1997b; Kokoshka et al., 1998), a concentration well below that necessary to alter [<sup>3</sup>H]dopamine uptake in our preparation, i.e., in separate experiments, it was determined that vesicular dopamine uptake was unaffected by direct application of methamphetamine at concentrations up to 0.5  $\mu$ M.

As stated above, the VMAT-2 transports intracellular dopamine into synaptic vesicles. In addition to dopamine neurons, the VMAT-2 is also found in serotonin neurons. To determine whether methamphetamine-induced decreases in vesicular dopamine uptake were a result of disruption of the VMAT-2 in dopaminergic or serotonergic neurons, we examined the contribution of serotonergic VMAT-2 in our assay. Previous studies have demonstrated that the serotonin neurotoxin PCA substantially reduces several markers for serotonergic neurons (Zhou et al., 1996), suggesting a loss of associated terminals. Using a dose of PCA similar to that of Zhou et al. (1996), we found that PCA administration decreased striatal serotonin content by 78% (Fig. 2A). The PCA-induced destruction of sero-

tonin neurons, as reflected by a loss of serotonin, had no significant effect on striatal vesicular [<sup>3</sup>H]dopamine uptake (Fig. 2B), suggesting that vesicles associated with striatal serotonin neurons are not a major component of vesicular uptake in our assay. The finding that most of the VMAT-2 is associated with dopamine neurons is consistent with results from Darchen et al. (1989) demonstrating that unilateral 6-hydroxydopamine (a dopamine neurotoxin) lesion dramatically decreases VMAT-2 (as assessed by DHTBZ binding) in the rat striatum.

## DISCUSSION

Studies by Frey et al. (1997) have demonstrated that multiple high-dose administrations of methamphetamine cause a persistent, i.e., at least 7-day, loss of DHTBZ binding to the VMAT-2. Distinct from this long-term effect, we determined that high-dose methamphetamine treatment rapidly disrupts vesicular dopamine uptake and DHTBZ binding in dopaminergic neurons, suggesting a significant alteration in the VMAT-2 protein. Reminiscent of the effects of high-dose methamphetamine administration on striatal dopamine transporter function (Fleckenstein et al., 1997b), the methamphetamine-induced decrease in vesicular dopamine uptake occurred within 1 h. Unlike dopamine transporter activity, which partially recovers following multiple high-dose methamphetamine administrations (Fleckenstein et al., 1997b), the deficit in vesicular dopamine uptake persisted 24 h after treatment. This later finding is consistent with results from Hogan et al. (1999, 2000), who have also reported a significant decrease in vesicular dopamine uptake 24 h after multiple high-dose methamphetamine injections.



**Fig. 2.** Rats received a single administration of PCA (7.5 mg/kg, s.c.; ■) or saline vehicle (1 ml/kg; □) and were killed 1 week postinjection. Data are mean  $\pm$  SEM (bars) values for determinations in six or seven PCA- or saline-treated rats. Serotonin concentrations (A) and vesicular [<sup>3</sup>H]dopamine uptake (B) were determined as described in Materials and Methods. \* $p < 0.001$ , value for PCA-treated rats that is significantly different from saline-treated controls.

The mechanism(s) whereby methamphetamine rapidly alters vesicular uptake and DHTBZ binding remains unknown. Even though the activity of VMAT-2 and DHTBZ binding is comparably decreased 1 and 24 h after methamphetamine treatment, it is not clear that the changes at these two time points are due to the same mechanisms, or how these two effects relate to the persistent VMAT-2 deficits days later. One or both of these short-term decreases may result from exposure to methamphetamine-induced reactive oxygen species. This statement is based on the fact that the VMAT-2 contains cysteine residues (Lesch et al., 1993) that may make this protein, like other transporters (Berman and Hastings, 1997; Fleckenstein et al., 1997a), vulnerable to oxidative inactivation. Alternatively, it has been reported that methamphetamine treatment causes hydrogen peroxide formation in dopaminergic cells in vitro (Cubells et al., 1994) and that hydrogen peroxide inhibits vesicular H<sup>+</sup>-ATPase (Wang and Floor, 1998). Therefore, methamphetamine may decrease vesicular uptake by disrupting the proton pump that is responsible for generating the electrochemical gradient that drives dopamine uptake into synaptic vesicles. Further studies are necessary to distinguish between these possibilities.

It is interesting to speculate as to the functional significance of the rapid decrease in vesicular dopamine uptake and DHTBZ binding induced by methamphetamine. As noted above, an inability of vesicles to sequester dopamine may lead to its accumulation in the cytoplasm and the formation of neurotoxic species. This possibility is supported by work from Fumagalli et al. (1999), which demonstrated enhanced methamphetamine-induced dopaminergic deficits in heterozygotic transgenic mice lacking 50% of their VMAT-2. Hence, the finding that methamphetamine causes a rapid decrease in vesicular uptake may present an important link among dopamine, oxygen radicals, and methamphetamine-induced deficits.

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