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Nature of methamphetamine-induced rapid and reversible changes in dopamine transporters

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Abstract

The nature of methamphetamine-induced rapid and transient decreases in dopamine transporter activity was investigated. Regional specificity was demonstrated, since [^{3}H]dopamine uptake was decreased in synaptosomes prepared from the striatum, but not nucleus accumbens, of methamphetamine-treated rats. Differences among effects on dopamine transporter activity and ligand binding were also observed, since a single methamphetamine administration decreased [^{3}H]dopamine uptake without altering [^{3}H]WIN35428 ([^{3}H](−)-2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate) binding in synaptosomes prepared 1 h after injection. Moreover, multiple methamphetamine injections caused a greater decrease in [^{3}H]dopamine uptake than [^{3}H]WIN35428 binding in synaptosomes prepared 1 h after dosing. Finally, decreases in [^{3}H]dopamine uptake, but not [^{3}H]WIN35428 binding, were partially reversed 24 h after multiple methamphetamine injections. Western blotting indicated that saline- and methamphetamine-affected dopamine transporters co-migrated on sodium dodecyl sulfate (SDS) gels at approximately 80 kDa, and that acute, methamphetamine-induced decreases in [^{3}H]dopamine uptake were not due to loss of dopamine transporter protein. These findings demonstrate heretofore-uncharacterized features of the acute effect of methamphetamine on dopamine transporters.

Keywords: Methamphetamine; Dopamine transporter; Dopamine

1. Introduction

Multiple high-dose administrations of the psychostimulant, methamphetamine, cause persistent decreases in central dopamine uptake or binding sites in rodents, non-human primates and perhaps humans (Wagner et al., 1980; Woolverton et al., 1989; Brunswick et al., 1992; Eisch et al., 1992; Wilson et al., 1996; Villemagne et al., 1998) deficits presumably reflecting nerve terminal degeneration. Distinct from these long-term deficits, we reported recently that high dose methamphetamine administration causes a rapid and reversible decrease in striatal dopamine transporter activity (Fleckenstein et al., 1997b). This transient decrease likely does not reflect a loss and subsequent replacement of dopamine transporters, since recovery of transporter function was observed 24 h after methamphetamine treatment; a period less than that is likely necessary to synthesize dopamine transporters de novo (i.e., the $t_{1/2}$ for dopamine transporter turnover is approximately 6 days; Fleckenstein et al., 1996b). We believe the transient effect of methamphetamine on dopamine transporters to be significant, since it provides evidence of a heretofore-uncharacterized mechanism whereby monoamine transporter activity can be modulated rapidly in vivo.

Because study of the rapid and reversible decrease in dopamine transporter activity may facilitate understanding of not only the pharmacology of methamphetamine, but also mechanisms of transporter regulation, the present studies were conducted to elucidate the nature of the acute consequences of methamphetamine administration on dopamine transporters. Specifically, regional selectivity was assessed by comparing effects of methamphetamine on dopamine transporters in the striatum and nucleus accumbens. Moreover, the acute effects of methamphetamine on dopamine transporter activity, ligand binding sites and protein were compared by evaluating synaptosomal [^{3}H]dopamine uptake, [^{3}H]WIN35428 ([^{3}H](−)-2-β-

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carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate) binding, and dopamine transporter Western blots. The binding and blot studies were of particular importance, considering the assertion that the loss of dopamine transporter activity was not associated with a loss of transporter protein (Fleckenstein et al., 1997b). The results indicate that methamphetamine (1) decreases dopamine transporter function in a regionally selective manner; and (2) differentially alters the activity and ligand-binding properties of dopamine transporters. The significance of these findings to the neurochemical effects of methamphetamine is discussed.

2. Materials and methods

2.1. Animals and treatments

Male Sprague–Dawley rats (200–300 g; Simonsen Laboratories, Gilroy, CA) were housed at 23°C with a 12-h alternating light/dark cycle. Food and water were provided ad libitum. Rats received methamphetamine or saline vehicle (s.c.) as indicated in the text, and all drug concentrations were calculated as free base. Animals were sacrificed by decapitation. All experiments were conducted in accordance with National Institutes of Health guidelines.

2.2. Drugs and chemicals

(±)Methamphetamine hydrochloride and (−)-cocaine hydrochloride were furnished generously by the National Institute on Drug Abuse. Pargyline hydrochloride was obtained from Abbott Laboratories (North Chicago, IL). [7,8-^{3}H] Dopamine (46 Ci/mmol) and [^{125}I] protein A (30 mCi/mg) were purchased from Amersham Life Sciences (Arlington Heights, IL). [*N*-methyl-^{3}H]-WIN35428 ((−)-2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate; 84.5 Ci/mmol) was purchased from New England Nuclear (Boston, MA).

2.3. Synaptosomal [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding

Unless specified, synaptosomal [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding were determined in synaptosomal preparations as described previously (Fleckenstein et al., 1996a). Synaptosomes were prepared by homogenizing fresh striatal or nucleus accumbens tissue in ice-cold 0.32 M phosphate-buffered sucrose (pH 7.4) followed by centrifugation ($800 \times g$ for 12 min at 4°C). Supernatants were then centrifuged ($22{,}000 \times g$ for 12 min at 4°C), and the resulting pellets (P2) resuspended in ice-cold assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 16 sodium phosphate, 1.4 $MgSO_4$, 11 dextrose, 1 ascorbic acid; pH 7.4) or 0.32 M phosphate-buffered sucrose (pH 7.4) for assessment of [^{3}H]dopamine uptake or [^{3}H]WIN35428 binding, respectively. Transport of [^{3}H]dopamine was determined in synaptosomes (i.e., resuspended P2) obtained from 1.5 mg striatal or 2 mg nucleus accumbens tissue (original wet weight) per reaction tube. [^{3}H]dopamine uptake assays were conducted in assay buffer, and initiated by addition of [^{3}H]dopamine (0.5 nM final concentration, except in kinetic experiments wherein 0.1 to 1000 nM was used). Samples were incubated for 3 min at 30°C. [^{3}H]WIN35428 binding (0.5 nM final concentration) was conducted in phosphate-buffered 0.32 M sucrose (pH 7.4) with synaptosomes obtained from 2 mg (original wet weight) of striatal tissue per reaction tube, and samples were incubated on ice for 2 h. Cold saturation experiments were performed with unlabeled WIN35428 concentrations ranging from 0.1 to 5000 nM. Data are expressed per original wet weight of tissue, and all tissues within a given experiment were processed concurrently.

2.4. Western blotting

Western blotting of dopamine transporters were conducted as described previously (Vaughan et al., 1993) using the antiserum 15, generated against amino acids 6–30. Striatal tissue was homogenized in sucrose phosphate buffer and centrifuged at $18{,}500 \times g$ for 12 min. The resulting membranes were solubilized in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis sample buffer at 20 mg/ml original wet weight and 50 μl samples were subjected to electrophoresis on 8% polyacrylamide gels. In two independent experiments, tissue from five or six control or methamphetamine-treated animals was immunoblotted. The separated proteins were transferred to 0.45 μm nitrocellulose, and the blocked membranes were probed with antiserum 15 diluted 1:100, followed by detection with [^{125}I]protein A. Washed and dried membranes were subjected to autoradiography with Kodak Biomax film and computer analysis by Molecular Dynamics Phosphorimager. To determine linearity of response, tissue standard curves were generated by titrating solubilized tissue with SDS sample buffer in 10% increments, ranging from 100% load (standard sample) to a final of 10%. The resulting data (not shown) demonstrated excellent linearity of response, and showed clearly quantifiable differences between the 10% increments. Furthermore, because of the possible involvement of oxidative phenomena in producing the methamphetamine results we also examined the effect of reducing agents on dopamine transporter electrophoretic mobility and western blot appearance. We found no detectable difference between dopamine transporters prepared and blotted entirely in the absence or in the presence of 10 mM dithiothreitol. Nevertheless, the blots performed in these studies were done entirely in the absence of reducing agents.

2.5. Methamphetamine determination

Striatal synaptosomes were prepared as described above and maintained frozen at −70°C until assay. Methamphetamine concentrations were determined using a Finnigan 4500 MAT mass spectrometer operating in positive chemical ionization mode (methane/ammonia reagent gas) coupled to a DB5MS-30M-0.25μ capillary column as described previously (Fleckenstein et al., 1997b; Kokoshka et al., 1998). Methamphetamine levels were determined in P2 synaptosomal preparations, and concentrations were determined per 1 or 0.5 ml assay volume as employed for [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding assays, respectively.

2.6. Data analysis

Statistical analyses between two groups were conducted using a two-tailed Student's *t*-test. Comparisons among three or more groups were conducted using analysis of variance followed by a Fisher's Least Significant Differences test. Differences among groups were considered significant if the probability of error was less than 5%. V_{max} and K_m values were calculated from Eadie–Hofstee plots. B_{max} and K_D values were determined using EBDA and LIGAND (Munson and Rodbard, 1980; McPherson, 1986). One site of binding was statistically preferred in all experiments and hence data were analyzed as such.

3. Results

A single methamphetamine injection (15 mg/kg, s.c.) decreased [^{3}H]dopamine uptake in rat synaptosomes prepared 1 h after drug administration (Fig. 1): an effect attributable to a decreased V_{max} and no change in K_m (Fleckenstein et al., 1997b). This decrease was not associated with a change in [^{3}H]WIN35428 binding (Fig. 1). A

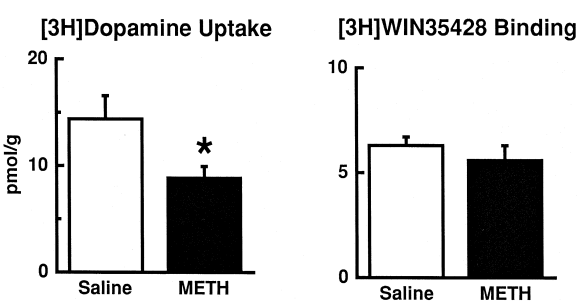

Fig. 1. Effects of a single methamphetamine administration on [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding in striatal synaptosomes. Rats received either methamphetamine (15 mg/kg, s.c.), or saline vehicle (1 ml/kg, s.c.) 1 h prior to decapitation. Assays were conducted using 0.5 nM [^{3}H]dopamine or 0.5 nM [^{3}H]WIN35428 (final concentrations) as described in Section 2. Values represent means (pmol/g tissue [original wet weight]) ±1 SEM of six determinations. *Values for methamphetamine-treated rats that differ significantly from saline-treated controls ($P \leq 0.05$).

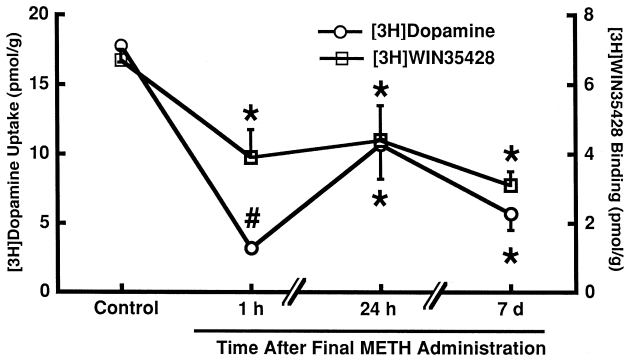

Fig. 2. Time-response effect of multiple methamphetamine administrations on [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding in striatal synaptosomes. Rats received four injections of methamphetamine (2-h intervals; 10 mg/kg/injection, s.c.) or four injections of saline vehicle (1 ml/kg/injection, s.c.; zero time controls). Rats were decapitated 1 h, 24 h or 7 days after the final injection. Assays were conducted using 0.5 nM [^{3}H]dopamine or 0.5 nM [^{3}H]WIN35428 (final concentrations) as described in Section 2. Values represent means (pmol/g tissue [original wet weight]) ±1 SEM of determinations in 6–14 rats. *Values for [^{3}H]dopamine uptake or [^{3}H]WIN35428 binding that differ significantly from saline-treated controls. #Value for [^{3}H]dopamine uptake that differs significantly from values for both saline-treated controls and rats decapitated 24 h after the final methamphetamine administration ($P \leq 0.05$).

single methamphetamine injection did not affect either the B_{max} (114 ± 29 vs. 103 ± 16 pmol/g tissue [original wet weight] for saline- and methamphetamine-treated rats, respectively) or K_D (2.7 ± 0.6 vs. 2.6 ± 0.5 nM for saline- and methamphetamine-treated rats, respectively; $n = 6–7$) of striatal [^{3}H]WIN35428 binding.

A dissociation between [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding was also observed after multiple methamphetamine administrations. Methamphetamine (10 mg/kg, s.c.; four injections at 2-h intervals) caused a greater decrease in [^{3}H]dopamine uptake than in [^{3}H]WIN35428 binding (i.e., decreases of 82% vs. 42%, respectively) in synaptosomes prepared 1 h after the final methamphetamine injection (Fig. 2). In separate experiments, it was determined that this acute decrease in [^{3}H]dopamine uptake was attributable to a reduction in V_{max} (2065 ± 467 vs. 939 ± 390 pmol/g tissue [original wet weight]/3 min for saline- and methamphetamine-treated rats, respectively; $p < 0.05$) and not K_m (70.0 ± 1.5 vs. 78.3 ± 6.2 nM for saline- and methamphetamine-treated rats, respectively; $n = 3$). The decrease in [^{3}H]WIN35428 binding appeared to be attributable to a reduction in both ligand affinity (K_D of 6.1 ± 0.6 and 8.5 ± 0.6 nM for saline- and methamphetamine-treated rats, respectively; $p = 0.05$) and B_{max} (156 ± 16 and 111 ± 19 pmol/g tissue [original wet weight] tissue for saline- and methamphetamine-treated rats, respectively; $p = 0.08$; $n = 11–12$). These methamphetamine-induced decreases in uptake and binding were not due to residual methamphetamine introduced by the original subcutaneous injection, since it was determined that the IC_{50} values for [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding (291 nM [Fleckenstein et al.,

1997b] and 4.9 μM, respectively) were substantially greater than methamphetamine concentrations determined to be in the synaptosomal preparations (less than 6 nM [Kokoshka et al., 1998] and 13 nM, in the [^{3}H]dopamine and [^{3}H]WIN35428 assays, respectively). Moreover, the methamphetamine-induced decrease in [^{3}H]dopamine uptake was somewhat selective for striatal dopamine transporters, since multiple methamphetamine administrations did not affect [^{3}H]dopamine uptake into synaptosomes prepared from the nucleus accumbens (Fig. 3).

The decrease in [^{3}H]dopamine uptake induced by a single methamphetamine injection is reversed 24 h after administration (Fleckenstein et al., 1997b). Similarly, results presented in Fig. 2 demonstrate that the effects of multiple administrations on [^{3}H]dopamine uptake were partially recovered by 24 h after the final methamphetamine injection. In contrast, methamphetamine-induced decreases in [^{3}H]WIN35428 binding caused by multiple methamphetamine administrations did not recover, and were equivalent in magnitude 1 and 24 h after the last methamphetamine administration. In separate experiments, it was determined that the methamphetamine-induced decrease in [^{3}H]WIN35428 binding 24 h after multiple administrations was associated with a decrease in B_{max} (77.8 $\pm$ 3.1 and 33.5 $\pm$ 10.8 pmol/g tissue [original wet weight] tissue for saline- and methamphetamine-treated rats, respectively; $p < 0.05$) and no change in K_D (4.1 $\pm$ 0.4 and 3.4 $\pm$ 0.2 nM for saline- and methamphetamine-treated rats, respectively; $n = 5–6$). Seven days after the final methamphetamine injection, both [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding were decreased: similar long-term effects on [^{3}H]dopamine uptake and dopamine transporter ligand binding have been reported previously (Wagner et al., 1980; Ricaurte et al., 1982; Woolverton et al., 1989; Brunswick et al., 1992; Eisch et al., 1992; Wilson et al., 1996; Villemagne et al., 1998).

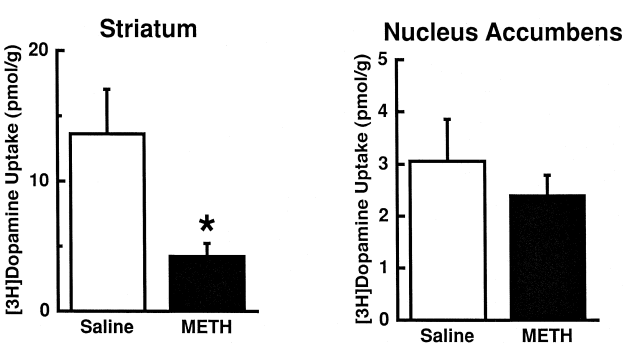

Fig. 3. Effects of multiple methamphetamine administrations on [^{3}H]dopamine uptake in synaptosomes obtained from the striatum (left panel) and nucleus accumbens (right panel). Rats received four injections of methamphetamine (10 mg/kg/injection, s.c.) or saline vehicle (1 ml/kg/injection, s.c.) and were decapitated 1 h after the final injection. Assays were conducted using 0.5 nM [^{3}H]dopamine (final concentration) as described in Section 2. Columns represent means (pmol/g tissue [original wet weight]) and vertical lines 1 SEM of determinations in six rats. * Values for methamphetamine-treated rats that differ significantly from saline-treated rats ($P \leq 0.05$).

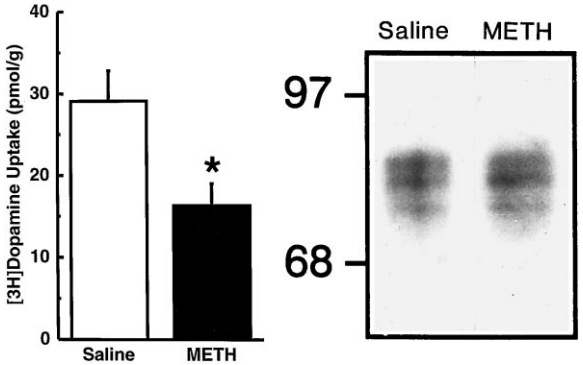

Fig. 4. Effects of multiple methamphetamine administrations on [^{3}H]dopamine uptake and dopamine transporter immunoblots. Rats received four injections of methamphetamine (10 mg/kg/injection, s.c.) or saline vehicle (1 ml/kg/injection, s.c.). Rats were decapitated 1 h after the final injection. The uptake assay (left panel) was conducted using 0.5 nM [^{3}H]dopamine (final concentration) as described in Section 2. Western blotting (right panel) was conducted as described in Section 2. Columns represent means (pmol/g tissue [original wet weight]) $\pm$1 SEM of determinations in five rats. The immunoblot is a representative comparison obtained from one of two independent experiments wherein tissue from five to six control or methamphetamine-treated animals was examined. * Value for methamphetamine-treated rats that differs significantly from saline-treated rats ($P \leq 0.05$).

To determine if the acute loss of [^{3}H]dopamine uptake activity could be attributed to loss of dopamine transporter protein, immunoblotting was utilized to compare striatal tissue from rats treated with multiple methamphetamine administrations to that of control rats (Fig. 4). Tissue was harvested 1 h after the final methamphetamine injection, and the striatum from one hemisphere was used for the immunoblot while that from the opposite side was used for [^{3}H]dopamine uptake analysis. As demonstrated in the representative blot presented in Fig. 4 (right panel), the control tissues showed the robust dopamine transporter immunoreactivity at 80 kDa that has been characterized previously (Vaughan et al., 1993). Tissue from the methamphetamine-treated rats displayed a signal indistinguishable from the controls in overall appearance and intensity (26,719 $\pm$ 2436 and 32,004 $\pm$ 6218 intensity units for saline- and methamphetamine-treated rats, respectively; $n = 5$/group), although tissue from the same animals showed a 44% reduction in striatal dopamine transport activity. This indicates that the methamphetamine treatment produced no gross structural alterations in the protein, such as dimerization or proteolysis, which would be detectable by this technique. Given the sensitivity of our immunoblot technique (i.e., tissue dose curves performed in parallel during these experiments showed clearly distinguishable reductions in signal intensity with incremental 10% reductions in tissue loading), these data strongly suggest that the reduction in transport activity produced by multiple methamphetamine does not involve a loss of dopamine transporter protein.

4. Discussion

The intent of this study was to elucidate the nature of the acute and reversible decrease in [^{3}H]dopamine uptake into striatal synaptosomes which occurs after methamphetamine administration. Results presented in Fig. 3 demonstrate the regionally selectivity of this phenomenon. Specifically, high-dose methamphetamine treatment decreased [^{3}H]dopamine uptake in synaptosomes prepared from the striatum, but not nucleus accumbens. The disparity among the effect of methamphetamine on dopamine transporters in the nucleus accumbens and striatum is consistent with previous demonstrations of regional selectivity in the effects of methamphetamine on dopaminergic systems. For example, the nucleus accumbens is less susceptible than the striatum to long-term decreases in tyrosine hydroxylase activity, dopamine content and [^{3}H]mazindol binding after multiple methamphetamine administrations (Morgan and Gibb, 1980; Eisch et al., 1992). Although dopamine transporters from the striatum and nucleus accumbens in many studies appear pharmacologically and structurally similar (Boja and Kuhar, 1989; Lew et al., 1992; Vaughan, 1995), subtle differences in these proteins may contribute to their differential response to methamphetamine. Furthermore, differences in the environment surrounding the transporters (i.e., differences in antioxidant levels) or in post-translational regulation also may be of importance.

Methamphetamine-induced reductions in striatal [^{3}H]-dopamine uptake after multiple administrations resulted from a decrease in transporter V_{max}. These decreases were not attributable to residual methamphetamine introduced by the original subcutaneous injections since residual methamphetamine levels found in synaptosomes prepared from treated rats were much less than those necessary to affect directly [^{3}H]dopamine uptake in the striatal preparations. The acute decrease in transporter activity was also not due to a loss of dopamine transporter protein since multiple methamphetamine injections decreased [^{3}H]dopamine uptake without altering the intensity of transporter immunoreactivity on sodium dodecyl sulfate gels (Fig. 4). Furthermore, the decrease in [^{3}H]dopamine uptake caused by multiple administrations of methamphetamine recovers by 24 h to an extent greater than can be explained by synthesis of dopamine transporters *de novo* considering that the $t_{1/2}$ for dopamine transporter turnover is approximately 6 days (Fleckenstein et al., 1996b). These observations indicate that the methamphetamine-induced decrease in transporter activity may be caused by a discrete and reversible modification of its structure. Such a modification would likely be subtle, since no difference in migration properties on a sodium dodecyl sulfate gel were detected between control and methamphetamine-affected transporters.

A methamphetamine-induced modification of dopamine transporter structure could occur via several mechanisms. One possibility stems from observations that methamphetamine administration causes oxygen radical formation (Kondo et al., 1994; Giovanni et al., 1995; Fleckenstein et al., 1997c), and that oxygen radicals rapidly decrease transporter activity (Berman et al., 1996; Fleckenstein et al., 1997a). Hence, methamphetamine-induced oxidation of dopamine transporters may confer subtle conformational changes in the protein which decrease activity. A second possibility is that the rapid and reversible effect of methamphetamine represents a reversible post-translational modification such as transporter phosphorylation. This hypothesis is supported by findings that (1) administration of amphetamine, a metabolite of methamphetamine, alters striatal protein kinase C activity (Giambalvo, 1992); and (2) protein kinase C-induced regulation of dopamine transport and/or concomitant phosphorylation of dopamine transporters has been reported, both in heterologous expression systems (Kitayama et al., 1994; Huff et al., 1997; Zhang et al., 1997) and in mouse and rat synaptosomes (Copeland et al., 1996; Vaughan et al., 1997). Other signal transduction pathways involving arachidonic acid metabolism and nitric oxide have also been implicated in rapid down-regulation of dopamine transporter function (Pogun et al., 1994; Zhang and Reith, 1996), although it is not known currently if these mechanisms contribute to the effects of methamphetamine. Further studies are necessary to address these possibilities.

Reduction in [^{3}H]dopamine uptake is commonly associated with a decrease in [^{3}H]WIN35428 binding. Hence, experiments were conducted to determine the effects of methamphetamine treatment on the binding of this transporter ligand. It was anticipated that [^{3}H]WIN35428 binding would be altered in a manner that reflects the decrease in [^{3}H]dopamine transport, and that these data might indicate a physical site at which methamphetamine treatment altered the dopamine transporter protein, thereby decreasing its activity. Unexpectedly, our results demonstrated a clear dissociation between the acute effects of methamphetamine on dopamine transporter activity and ligand binding. For instance, a single methamphetamine injection decreased [^{3}H]dopamine uptake without altering [^{3}H]WIN35428 binding in synaptosomes prepared 1 h after dosing. Further, multiple methamphetamine injections caused a greater reduction in [^{3}H]dopamine uptake (reduced 82%) than [^{3}H]WIN35428 binding (reduced 42%) in synaptosomes prepared 1 h after treatment (Fig. 2). These latter experiments employed only a single concentration of [^{3}H]dopamine or [^{3}H]WIN35428 ('single point analysis'), and were confirmed by saturation studies (i.e., a 55% decrease in V_{max} without changing K_m vs. a 29% decrease in B_{max} with a slight increase in K_D). Finally, decreases in [^{3}H]WIN35428 binding, unlike decreases in [^{3}H]dopamine uptake, were not reversed 24 h after multiple methamphetamine injections (Fig. 2). Hence, the lack of similarity between the loss of dopamine transporter activity and [^{3}H]WIN35428 binding indicate that the metham-

phetamine-induced changes in transporter function cannot be explained simply by modification of the dopamine transporter at the WIN35428 binding site. Instead, these data contribute to an increasing body of evidence suggesting a functional independence of the dopamine translocation process from the characteristics of [^{3}H]WIN35428 binding (see, Kitayama et al., 1992; Lee et al., 1996).

The reversal of decrease in uptake after multiple methamphetamine administrations was partially transient: despite a partial recovery by 24 h, [^{3}H]dopamine uptake was still decreased 6 days later. Interestingly, the discordance between the acute (1-h post-multiple administrations) changes in [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding noted above was not observed 7 days after multiple methamphetamine administrations; i.e., [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding were reduced comparably at this time point. The long-term decrease in [^{3}H]dopamine uptake and number of [^{3}H]WIN35428 binding sites likely reflects degeneration of nerve terminals: similar conclusions have been reported by several investigators (Wagner et al., 1980; Eisch et al., 1992). Thus, the effects of multiple methamphetamine administration on dopamine systems appear to be comprised of two distinct phases: (1) a rapid and reversible phase where decreases in [^{3}H]dopamine uptake are not attributable to a loss of binding sites and protein; and (2) a latter and persistent phase, presumably associated with a loss of dopaminergic terminals, where binding and uptake are similarly decreased.

The biphasic effect of multiple methamphetamine injections appears different than the effects of a single administration. Specifically, a single injection causes a rapid and completely reversible decrease in dopamine transporter activity (Fleckenstein et al., 1997b) and, at doses employed in this study, is apparently without long-term effect on dopaminergic systems (Hotchkiss and Gibb, 1980; Peat et al., 1983). Hence, the reductions in dopamine transporter activity after a single and multiple injections may be mechanistically distinct. The significance of this distinction remains to be elucidated.

It is interesting to speculate about the implications and functional consequences of a rapid methamphetamine-induced change in dopamine transporter function. Because the dopamine transporter is the primary means whereby dopamine is cleared from the synaptic cleft, a decrease in its activity could increase extracellular dopamine. Since extracellular dopamine, perhaps by causing formation of highly toxic quinones (Graham, 1978), is a major effector of methamphetamine-induced neurotoxicity (Gibb and Kogan, 1979; Wagner et al., 1983 Schmidt et al., 1985; Axt et al., 1990), a loss of transporter function could be detrimental. On the other hand, reduction in dopamine transporter activity may be neuroprotective, as evidenced by findings that monoamine transport inhibitors protect against damage to dopamine nerve terminals following high-dose administration of methamphetamine (Schmidt and Gibb, 1985). An investigation into the functional significance of this phenomenon is required.

In conclusion, the present study demonstrates that both a single and multiple administrations of methamphetamine differentially alter the activity and ligand binding properties of the dopamine transporter. Furthermore, multiple administrations of methamphetamine induce a rapid, selective and reversible decrease in dopamine transporter function distinct from the long-term deficits in transport caused by multiple administrations of the stimulant. The fact that the methamphetamine-induced inactivation of dopamine transporters was reversible suggests the presence of a mechanism for regulating transporter function under both drug-affected and normal physiological conditions. These findings may have important implications regarding the regulation of dopaminergic systems.

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