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Review

Differential effects of stimulants on monoaminergic transporters: Pharmacological consequences and implications for neurotoxicity

Annette E. Fleckenstein*, James W. Gibb, Glen R. Hanson

Department of Pharmacology and Toxicology, University of Utah, 30 South 2000 East, Rm 201, Salt Lake City, UT 84112, USA

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Abstract

Many psychostimulants alter plasmalemmal monoaminergic transporter function. Some, such as cocaine, prevent the reuptake of newly released dopamine, serotonin or norepinephrine into their associated neurons. Others, such as the amphetamines, facilitate release of these transmitters into the extraneuronal space by causing a reversal of function of these carrier proteins. An understanding of how psychostimulants regulate the function of not only plasmalemmal, but also vesicular monoamine transporter function is important to appreciate the pharmacological and sometimes neurotoxic consequences of administering these drugs, as well as the physiological regulation of these carrier proteins. Hence, this review will describe recent ex vivo studies investigating the rapid and differential affects of several stimulants on both plasmalemmal and vesicular monoamine transporter function. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Psychostimulant; Dopamine trasporter; Amphetamine

1. The dopamine transporter

1.1. Psychostimulants differentially affect dopamine transporters

It has been established that the reinforcing and behavioral effects of psychostimulants are caused, at least in part, by the ability of these agents to increase extraneuronal dopamine concentrations. Some, such as cocaine, cause this increase by preventing the reuptake of newly released dopamine into neurons via the dopamine transporter (Heikkila et al., 1975; Nicolaysen and Justice, 1988). Other stimulants, such as amphetamine, purportedly increase dopamine release through a reversal of this transporter (Liang and Rutledge, 1982; Jones et al., 1999). An understanding of how drugs such as these alter transporter function is necessary to appreciate the pharmacological consequences of psychostimulant administration, as well as the physiological regulation of this, and related, carrier proteins.

Recent work from this laboratory demonstrates that in vivo administration of psychostimulants differentially alters dopamine transporter function in a heretofore unreported manner (Metzger et al., 1998; Fleckenstein et al., 1999). Specifically, high-dose in vivo administration of phenylethylamines such as amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA), cathinone, or methcathinone rapidly decrease the activity of the dopamine transporter, as assessed in striatal synaptosomes after removal of residual drug. In contrast, in vivo high-dose injections of fenfluramine, cocaine or methylphenidate have little or no effect on the activity of this transporter in synaptosomes after the drug is removed (Table 1).

1.2. Dopamine transporters and the methamphetamine model

Although much remains to be elucidated regarding mechanisms underlying the ability of amphetamine and related analogs to decrease dopamine transporter activity, much has been learned concerning this phenomenon by characterizing the effect of one amphetamine-analog, methamphetamine, on dopamine transporter function. Specifically, it has been demonstrated in a rat model that

* Corresponding author. Tel.: +1-801-585-7474; fax: +1-801-585-5111.

E-mail address: fleckenstein@hsc.utah.edu (A.E. Fleckenstein).

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Table 1
Summary of effects on dopamine transporter function

	IC_{50} (nM)	Single administration (% control)	Multiple administration (% control)
Amphetamine	94 ± 7	80 ± 3[a]	11 ± 1[a]
Methylphenidate	165 ± 38	87 ± 2	107 ± 7
Methamphetamine	291 ± 4	63 ± 8[a]	30 ± 8[a]
Cocaine	337 ± 24	117 ± 4[a]	109 ± 3
Methcathinone	344 ± 68	72 ± 5[a]	50 ± 5[a]
Cathinone	856 ± 85	78 ± 4[a]	27 ± 3[a]
MDMA	1527 ± 137	67 ± 3[a]	58 ± 6[a]
Fenfluramine	13,800 ± 2000	100 ± 5	93 ± 5

IC_{50} values represent means of at least three independent experiments. For multiple administration experiments, drug was administered at the following maximal sublethal doses: amphetamine, 10 mg/kg, s.c.; methylphenidate, 40 mg/kg, s.c.; methamphetamine, 10 mg/kg, s.c.; cocaine, 30 mg/kg, i.p.; cathinone, 40 mg/kg, s.c.; methcathinone, 30 mg/kg, s.c.; MDMA, 15 mg/kg, s.c; and fenfluramine, 40 mg/kg, s.c. The same doses were employed in single administration experiments with the exception of methamphetamine which, for comparison with data published previously from this laboratory, was administered at 15 mg/kg, s.c. In single-administration experiments, rats received drug, or saline vehicle (1 ml/kg, s.c. or i.p.) and were decapitated 1 h later. In multiple administration experiments, rats received four injections of drug (2-h intervals) or saline vehicle (1 ml/kg, s.c. or i.p.) and were decapitated 1 h after the final injection. Values represent means expressed as percentage of saline-treated controls. For individual single and multiple administration experiments, control values ranged from 444 ± 18 to 1020 ± 50 fmol/mg protein, and values represent means ± 1 S.E.M. of determinations in 6–9 rats.

[a]Values for drug-treated rats that differ significantly from saline-treated controls ($P \leq 0.05$). (reproduced from Fleckenstein et al., 1999).

multiple injections of methamphetamine rapidly (within 1 h) reduce striatal dopamine uptake by approximately 75% (Fig. 1); an effect attributable to a reduced V_{max} and an unchanged $K_{m.}$ (Kokoshka et al., 1998b). A similar phenomenon has been described in mice following methamphetamine treatment (Sandoval et al., 1999). This decrease is not due to residual drug introduced by the original subcutaneous injections since drug levels found in synaptosomes prepared from treated rats are much less than those necessary to affect directly dopamine uptake in the striatal preparations. In addition, western blotting indicates that the decrease in striatal transporter function is not associated with a loss of transporter protein (Kokoshka et al., 1998b). However, this decrement is associated with a decrease in B_{max}, and a slight increase in K_d, of binding of the dopamine transporter ligand 2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate (WIN35428). These data suggest that the methamphetamine-induced decrease in transporter function is either due to a modification of the protein per se or a change in a factor regulating transporter function.

The methamphetamine-related decrease in striatal dopamine transporter activity is relatively selective, as neither γ-aminobutyric acid (Haughey et al., 2000) nor glutamate (Kokoshka et al., 1998a) transport is affected by this treatment regimen. It is also regionally specific as evidenced by findings that, in contrast to effects on the striatal dopamine transporter, multiple methamphetamine injections have little impact on dopamine transport into synaptosomes prepared from rat nucleus accumbens 1 h after drug treatment (Kokoshka et al., 1998b).

Twenty-four hours after methamphetamine treatment, approximately half of the decrease in striatal dopamine transporter function caused by methamphetamine treatment is restored: the remaining deficit appears to persist for at least 7 days (Fig. 1; Kokoshka et al., 1998b). From these studies, it appears that two distinct elements comprise this apparent biphasic response to methamphetamine: (1) a rapidly reversing acute transporter response (ATR) component (i.e., that which recovers during the first 24 h after treatment); and (2) a persistent ATR component which remains for at least 24 h after drug treatment.

In order to investigate the nature of the ATR components, the role of dopamine in their initiation was assessed. Dopamine was investigated because methamphetamine administration effects a change in neuronal dopamine disposition that increases synaptic dopamine levels (Holson et al., 1996; Nash and Yamamoto, 1992; O'Dell et al., 1991). Dopamine, in turn, can decrease transporter activity (Berman et al., 1996) perhaps by causing formation of reactive oxygen species (Graham, 1978) which compromise dopamine transporter function (Berman et al., 1996; Fleckenstein et al., 1997a). A role for dopamine in the ATR phenomenon caused by multiple methamphetamine injections was suggested by findings that depletion of striatal dopamine levels using the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, attenuates the methamphetamine-induced diminution in striatal dopamine uptake (Metzger et al., in press). Pretreatment with either the dopamine D_1 receptor antagonist, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH-23390), or the dopamine D_2 receptor antagonist, eticlopride, attenuates this deficit as well (Metzger et al., in press). However, it is important to note that neither dopamine depletion nor the dopamine receptor antagonists fully prevents the methamphetamine-induced decrease in dopamine transporter function. Hence, these data underscore the likelihood that there are at least two distinct components of the dopamine transporter-related ATR caused by multiple administrations of methamphetamine: one is dopamine-dependent (ATR_{DA}) and the second is dopamine-independent (ATR_{IND}).

The question as to whether the dopamine-independent ATR_{IND} is associated with the rapidly reversing ATR component or with the ATR component remaining 24 h after multiple methamphetamine injections is not yet resolved; however, insight comes from data involving a single methamphetamine administration. Specifically, a single injection causes a rapid and completely reversible reduction in dopamine transporter activity, as assessed in rat striatal synaptosomes prepared 1 h after metham-

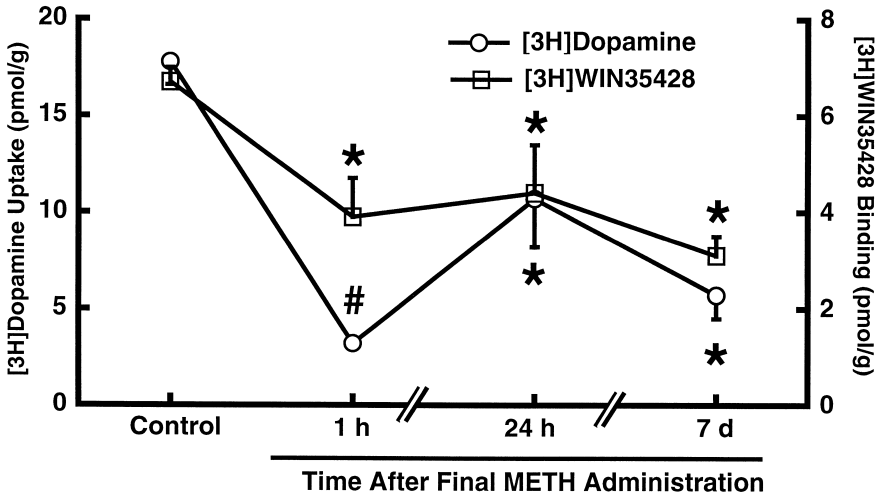

Fig. 1. Time–response effect of multiple methamphetamine (METH) administrations on [^{3}H]dopamine uptake and [^{3}H]WIN35428 binding in striatal synaptosomes. Rats received four injections of methamphetamine (2-h intervals; 10 mg/kg/injection, s.c.) or four injections of saline vehicle (1 ml/kg/injection, s.c.; zero time controls). Rats were decapitated 1 h, 24 h or 7 days after the final injection. Values represent means ± 1 S.E.M. of determinations in 6–14 rats. * Values for methamphetamine-treated rats that differ significantly from controls. #Value for [^{3}H]dopamine uptake that differs significantly from that obtained from rats decapitated 1 h after the final methamphetamine administration ($P \leq 0.05$). Reproduced from Kokoshka et al. (1998b).

phetamine treatment (Fleckenstein et al., 1997b). Like the ATR induced by multiple methamphetamine injections, this change is a decrease in V_{max} with no change in K_m, and is not due to residual drug introduced by the original subcutaneous injection. Interestingly, neither pretreatment with methyl-*p*-tyrosine, SCH23390 nor eticlopride prevents this rapid and reversible decrease in dopamine transporter activity caused by a single methamphetamine injection (Metzger et al., in press). Hence, this single-injection effect has (and, in fact, appears to consist solely of) a dopamine-independent component (ATR_{IND}). Although yet to be demonstrated, it is possible that the ATR_{IND} components resulting from a single and multiple methamphetamine injections represent the same drug-induced phenomenon. Accordingly, since the ATR_{IND} induced by a single drug administration is rapidly reversed by 24 h after methamphetamine treatment, the ATR_{IND} associated with multiple methamphetamine injections likely corresponds to the reversible component of the deficit induced by multiple methamphetamine injections described above.

To characterize further the nature of the ATR components, the contribution of hyperthermia to these changes has been determined. Hyperthermia was studied because methamphetamine administration elevates body temperature (Bowyer et al., 1994; Farfel and Seiden, 1995; Albers and Sonsalla, 1995) which, in turn, contributes to reactive oxygen species formation (Fleckenstein et al., 1997c; LaVoie and Hastings, 1999). Moreover, pretreatment with methyl-*p*-tyrosine, SCH23390 or eticlopride attenuates or prevents methamphetamine-induced hyperthermia (Albers and Sonsalla, 1995; Metzger et al., in press); hence, it was important to distinguish if the drug-induced attenuation of the methamphetamine effect was associated with dopamine depletion or receptor blockade and not simply prevention of hyperthermia. It was determined that prevention of methamphetamine-induced hyperthermia attenuates the decrease in dopamine transporter activity induced by multiple administrations of methamphetamine. It was also observed that reinstatement of methamphetamine-induced hyperthermia to methyl-*p*-tyrosine-, SCH23390- or eticlopride-pretreated rats partially restores the decrease in dopamine transporter activity. In contrast, prevention of methamphetamine-related hyperthermia does not alter the decrease in dopamine transporter function induced by a single administration of methamphetamine. These data indicate that both hyperthermia and dopamine contribute to the decrease in dopamine transporter function observed after multiple methamphetamine treatments, and that the ATR_{IND} (i.e., the component associated with a single methamphetamine administration) is not associated with a methamphetamine-induced increase in body temperature (Metzger et al., in press).

Recently, a role for reactive oxygen species in causing the methamphetamine-related ATR (reduction in dopamine transporter activity resulting from multiple methamphetamine injections) was demonstrated since pretreatment with the antioxidant, *N*-*t*-butyl-α-phenylnitrone (PBN), prior to multiple methamphetamine administrations attenuated the methamphetamine-induced acute decrease in dopamine transporter activity without preventing the associated hyperthermia (Metzger et al., in press). In contrast, PBN treatment did not prevent the decrease in dopamine transporter function caused by a single methamphetamine treatment (Metzger et al., in press). These data suggest that oxygen radicals contribute to the ATR_{DA}, but not the ATR_{IND}.

Further evidence indicating the existence of at least two distinct ATR elements induced by methamphetamine treatment comes from a recent finding of a discordance between ligand binding and activity of the dopamine transporter after multiple methamphetamine injections. Specifically, methamphetamine decreases dopamine uptake more than binding of WIN35428, in synaptosomes prepared 1 h after dosing (Fig. 1; Kokoshka et al., 1998b). One interpretation of these data is that the decrease in WIN35428 binding is associated with only one of the ATR elements contributing to the decrease in uptake (i.e., either the ATR_{DA} or the ATR_{IND}). Interestingly, the rapid and reversible decrease in dopamine transporter activity observed after a single methamphetamine injection (an ATR_{IND}) also is not associated with a change in WIN35428 binding (Kokoshka et al., 1998b). Hence, it is possible that if (as postulated above) the ATR_{IND} resulting from a single or multiple methamphetamine injections represents the same phenomenon, then the ATR_{IND} associated with multiple methamphetamine injections is not related to a decrease in WIN35428 binding. Accordingly, the ATR_{DA} resulting from multiple methamphetamine injections would be associated with the decrease in WIN35428 binding depicted in Fig. 1. A summary of the features ascribed to the ATR_{IND} and ATR_{DA} is depicted in Fig. 2.

Mechanisms responsible for the ATR_{IND} remain to be determined. Factors that apparently do not contribute to this effect include dopamine, hyperthermia (see above), glutamate and serotonin (5-hydroxytryptamine; 5-HT) (Metzger et al., in press). However, other factors remain to be examined. One possibility is that the rapid and reversible effect of methamphetamine represents a reversible post-translational modification such as a protein kinase C-mediated transporter phosphorylation. Once phosphorylated, the transporter may be internalized thereby accounting for the apparent decrease in transporter function. This hypothesis is supported by findings that administration of amphetamine, a metabolite of methamphetamine, alters striatal protein kinase C activity (Giambalvo, 1992). Moreover, protein kinase C-induced regulation of dopamine transporter and/or concomitant phosphorylation of dopamine transporter has been reported, both in expression systems (Huff et al., 1996; Kitayama et al., 1994; Zhang et al., 1997) and in mouse and rat synaptosomes (Copeland et al., 1996; Vaughan et al., 1997); in each of these cases, phosphorylation of the dopamine transporter leads to a decrease in function.

Data perhaps inconsistent with a model linking protein kinase C-mediated phosphorylation with transporter internalization are findings that activation protein kinase C enhances dopamine release while inhibition of protein kinase C inhibits amphetamine-mediated dopamine release (Kantor and Gnegy, 1998; Cowell et al., 2000). Still, recent studies by Pristupa et al. (1998) and Melikian and Buckley (1999) support the methamphetamine-phosphorylation–internalization hypothesis by demonstrating dopamine transporter internalization in response to protein kinase activation. Most recently, Saunders et al. (2000) demonstrated that both amphetamine and dopamine cause internalization of human dopamine transporter in EM4 cells (human embryonic kidney cells), likely through a clathrin-mediated pathway. However, an argument against internalization as the explanation for the ATR_{IND} is that this change in transporter activity is not associated with a change in WIN35428 binding; although this discrepancy can be explained if WIN35428, like cocaine (Jackson and Hersey, 1991), is lipid soluble and hence detects both surface and internalized dopamine transporter. Accordingly, Pristupa et al. (1998) demonstrated WIN35428 binding may not distinguish between surface and internalized

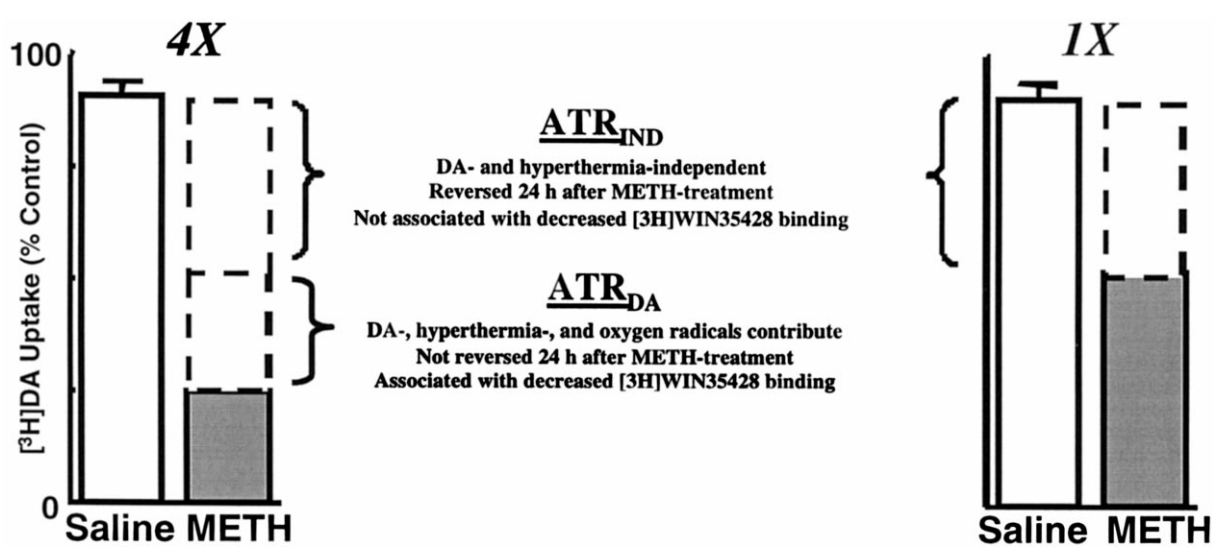

Fig. 2. Schematic representation summarizing the features of the proposed components of the dopamine transporter-associated ATR observed 1 h after multiple (4 ×) or a single (1 ×) methamphetamine (METH) administration(s). See text for details.

dopamine transporter by reporting that acute exposure of human dopamine transporter-expressing Sf9 cells to a protein kinase C activator reduced the V_{max} (but not K_d) for dopamine uptake by approximately 40%; this decrease was not associated with a change in WIN35428 binding, but was shown by confocal microscopy to be due to a rapid sequestration/internalization of human dopamine transporter.

In addition to phosphorylation-related pathways, other signal transduction pathways involving arachidonic acid metabolism (Zhang and Reith, 1996) and nitric oxide (Pogun et al., 1994) have also been implicated in rapid down-regulation of dopamine transporter function. It has also been shown that a nicotinic agonist can depolarize plasma membrane potentials and inhibit dopamine transporter activity in pheochromocytoma PC12 cells (Huang et al., 1999). It is not known currently if these or other mechanisms contribute to the effects of methamphetamine.

1.3. An in vitro model?

To investigate further mechanisms contributing to the ATR phenomena caused by methamphetamine treatment, recent studies have attempted to model these effects in an in vitro preparation. It was determined that, as observed ex vivo in synaptosomes prepared from methamphetamine-treated rats, exposure of synaptosomes prepared from non-treated rats to this stimulant rapidly decreases dopamine transporter activity; an effect that persists even after "washing" methamphetamine from the synaptosomal preparations (Kim et al., 2000; Sandoval et al., 2000). As observed ex vivo, this decrease is attributable to a decrease in V_{max} of uptake (Kim et al., 2000; Sandoval et al., unpublished observation). Also like effects observed after a single methamphetamine injection, this reduction in dopamine transporter activity is not associated with a change in WIN35428 binding (Kim et al., 2000; Sandoval et al., 2000). Hence, this effect, which appears to be due to a direct interaction of methamphetamine with either the dopamine transporter protein or associated second messenger systems, appears to reflect the ATR observed after a single administration of methamphetamine.

The finding that the effects of methamphetamine exposure in vitro reflect the changes in dopamine transporter function observed ex vivo is significant in that it provides a model for studying mechanisms contributing to the rapid and reversible decrease in transporter function occurring after a single methamphetamine injection; a phenomenon apparently distinct from the neurotoxic, irreversible consequences of administering the stimulant. Further evidence supporting the view that the in vitro phenomenon reflects the reversible component of the ATR comes from findings that incubation of synaptosomes under similar conditions with amphetamine, but not cocaine, methylphenidate or fenfluramine, decreased dopamine transporter activity (Kim et al., 2000): phenomena that mimic exactly the effects observed ex vivo after treatment with these agents (Table 1). Moreover, the effect of methamphetamine preincubation on dopamine transporter activity is relatively selective and does not alter striatal glutamate uptake (Kim et al., 2000); again, corresponding with the ex vivo effect after methamphetamine treatment (Kokoshka et al., 1998a). Finally, it is interesting to note that in vitro preincubation with MDMA mimics the effects of preincubation with methamphetamine (Sandoval et al., unpublished observation); the observation that in vivo MDMA treatment rapidly and reversibly decreases dopamine transporter function (Metzger et al., 1998) but causes little or no long-term dopaminergic damage after in vivo administration (Johnson et al., 1988; Insel et al., 1989) confirms that in vitro preincubation does not appear to be a model for neurotoxicity, but rather an independent effect. However, it is possible that while this rapid effect on the dopamine transporter does not necessarily lead to dopamine neurotoxicity (i.e., the MDMA effect), it may still be one of multiple contributing factors.

1.4. Psychostimulants and acute changes in dopamine transporter function: implications for dopamine neurotoxicity

It is well established that high-dose administration of some psychostimulants causes profound and persisting deficits in central dopamine neurons in rodents (Hotchkiss et al., 1979; Hotchkiss and Gibb, 1980; Wagner et al., 1980; Ricaurte et al., 1982) non-human primates (Woolverton et al., 1989) and humans (Wilson et al., 1996), but with different expressions. For example, methamphetamine treatment causes long-term decreases in tyrosine hydroxylase activity, dopamine transporter function, and/or concentrations of associated neurotransmitters and metabolites (Buening and Gibb, 1974; Seiden et al., 1976; Hotchkiss and Gibb, 1980; Morgan and Gibb, 1980; Eisch et al., 1992). These deficits are thought to be neurotoxic responses that likely reflect destruction of corresponding monoamine axons and/or terminals. In contrast, high-dose amphetamine administration tends to preferentially cause persistent dopamine deficits (Ricaurte et al., 1983), whereas MDMA-induced deficits are typically selective against the serotonin system (Johnson et al., 1988; Insel et al., 1989). High-dose cocaine treatment does not cause long-term dopaminergic deficits (Kleven et al., 1988; Yeh and De Souza, 1991; Benmansour et al., 1992; Cappon et al., 1998).

Changes in dopamine disposition resulting from administration of amphetamine analogs appears necessary for long-term toxicities. For example, dopamine depletion resulting from pretreatment with methyl-*p*-tyrosine attenuates the persistent dopamine deficits caused by methamphetamine treatment (Gibb and Kogan, 1979; Wagner et al., 1983; Schmidt et al., 1985). Still, it is unclear whether

increases in extraneuronal dopamine levels or a redistribution of intraneuronal dopamine are responsible for these effects. Early data suggested that extraneuronal dopamine might be of importance; for instance, pretreatment with dopamine post-synaptic receptor antagonists prevents the long-term dopaminergic deficits resulting from methamphetamine treatment (Sonsalla et al., 1986). In contrast to these findings, other evidence implicates changes in the disposition of intraneuronal dopamine as being important. For instance, LaVoie and Hastings (1999) demonstrated a dissociation between extracellular dopamine concentrations, formation of dopamine oxidation products and long-term dopaminergic deficits suggesting that the toxicity of methamphetamine is not exclusively the result of increases in extracellular dopamine concentrations. Moreover, Cubells et al. (1994) employing 2,7-dichlorofluoresceine diacetate as an indicator of intracellular hydrogen peroxide production, demonstrated methamphetamine-induced oxygen radical formation in ventral midbrain cultures containing dopamine neurons. Based on these findings, it is suggested that methamphetamine might redistribute dopamine from the reducing environment within synaptic vesicles to extravesicular intracellular oxidizing environments, thus generating oxygen radicals and reactive metabolites within dopamine neurons that trigger selective dopamine terminal loss.

Although the question of whether intra- or extra-neuronal dopamine mediates persistent deficits in monoaminergic systems remains unresolved, it is possible that differences in the ability of psychostimulants to affect dopamine disposition may underlie the differential long-term monoaminergic responses to administration of these stimulants. Accordingly, we hypothesize that the magnitude and features of the ATR induced by multiple injections of methamphetamine, and perhaps other amphetamine-analogs, are associated with the magnitude of the persistent dopamine deficits caused by these agents. In particular, we propose that the ATR caused by treatment with these drugs reflects a disruption or "closing" of the transporter such that dopamine can also no longer move from the inside to the outside of the synaptosomes. The net effect of the latter would be that the decrease in transporter activity effectively plugs the dopamine transporter and thereby attenuates dopamine efflux and traps the dopamine in intraneuronal regions where it can damage the terminal. Support for this possibility comes from recent work by Yamamoto and Zhu (1998) who employed microdialysis to measure the effects of multiple methamphetamine injections. These investigators found that less dopamine is released from neurons following the fourth drug injection than after the first. This successive decrease may reflect a depletion of dopamine stores or, alternatively, a disruption of the dopamine transporter such that methamphetamine can no longer effect dopamine release through the transporter.

It is noteworthy that both the ATR_{DA} and the long-term deficits caused by methamphetamine treatment are dopamine-dependent (Gibb and Kogan, 1979; Wagner et al., 1983; Schmidt et al., 1985), and require hyperthermia (Bowyer et al., 1994; Farfel and Seiden, 1995; Albers and Sonsalla, 1995; Metzger et al., in press) and reactive oxygen species (DeVito and Wagner, 1989; Cadet et al., 1994; Cappon et al., 1996; Metzger et al., in press). These similarities support the hypothesis that the ATR_{DA} contributes to the persistent dopaminergic deficits, although this hypothesis remains to be tested directly. Accordingly, it is interesting to note that the amphetamine-analog that caused the smallest ATR after multiple injections (MDMA) is also the least likely to cause long-term striatal dopamine deficits. Moreover and as described above, cocaine, fenfluramine and methylphenidate, which do not cause an ATR, generally do not cause long-term dopaminergic deficits (Kleven et al., 1988; Yeh and De Souza, 1991; Zaczek et al., 1989, 1990; Appel et al., 1990; Yuan et al., 1997).

2. The vesicular monoamine transporter-2 (VMAT-2)

2.1. Psychostimulants differentially alter VMAT-2 function

The dopamine transporter-associated ATR may be only one of multiple factors contributing to an increase in intraneuronal dopamine concentrations and eventually neurotoxicity. For instance, it was reported recently that multiple administrations of methamphetamine rapidly (within 1 h) decrease rat striatal VMAT-2 function (Fig. 3; Brown et al., 2000a). This phenomenon may diminish vesicular dopamine storage thereby promoting the accumulation of

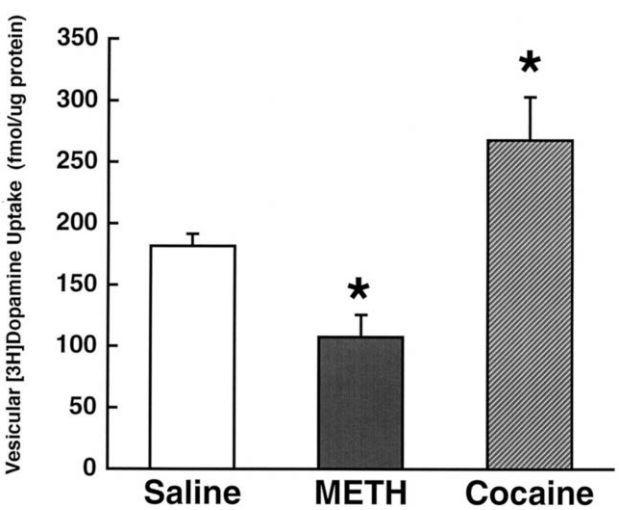

Fig. 3. Methamphetamine (METH) and cocaine administration rapidly decrease and increase, respectively, VMAT-2 activity. Rats received four injections (2-h intervals) of methamphetamine (10 mg/kg, s.c.), cocaine (30 mg/kg, i.p.) or saline vehicle (1 ml/kg), and were decapitated 1 h after treatment. VMAT-2 activity was assessed in synaptosomes prepared from drug- or saline-treated rats as described previously (Brown et al., 2000a). Values represent means ± 1 S.E.M. of determinations in 5–7 rats. * Values for treated rats that differ significantly from controls.

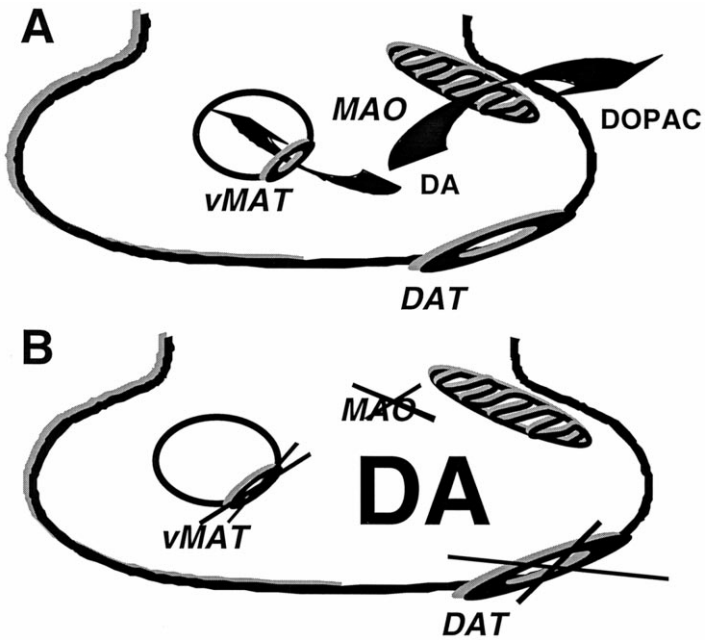

Fig. 4. A schematic representation of the mechanism whereby methamphetamine may increase intraneuronal dopamine (DA) concentrations. Panel A: Under physiological conditions, cytosolic dopamine levels remain low because: (1) dopamine is metabolized by mitochondrial monoamine oxidase (MAO) to form dihydroxyphenylacetic acid (DOPAC); and (2) dopamine is sequestered within vesicles after having been taken up via the vesicular monoamine transporter-2 (vMAT). Panel B: Methamphetamine may increase intraneuronal dopamine concentrations because: (1) dopamine is not sequestered within vesicles since vMAT activity is decreased; (2) dopamine cannot be released via exchange diffusion since dopamine transporter (DAT) activity is decreased; and (3) dopamine is not metabolized because monoamine oxidase is inhibited.

cytoplasmic dopamine. A similar finding was reported by Hogan et al. (1999, 2000) who demonstrated a decrease in VMAT-2 activity in mice as early as 24 h after methamphetamine treatment (an earlier time was not evaluated). Such a cytoplasmic accumulation would be augmented by the methamphetamine-induced decrease in dopamine transporter function described above, as well as by the ability of this stimulant to inhibit monoamine oxidase (Suzuki et al., 1980). This possibility is depicted in Fig. 4.

Interestingly, a role for VMAT-2 in dopamine-related neurotoxicity caused by methamphetamine treatment has been suggested by findings of enhanced dopaminergic deficits in heterozygotic transgenic mice lacking 50% of their VMAT-2 (Fumagalli et al., 1999). Moreover, other investigators have suggested that the ratio of dopamine to vesicular monoamine transporters may dictate the sensitivity of a neuron to dopaminergic toxins (Miller et al., 1999); thus, an increase in VMAT-2 activity would tend to be neuroprotective by sequestering dopamine and perhaps other potentially cytotoxic species. Consistent with this hypothesis, it has been demonstrated that the dopaminergic neurotoxin, 1-methyl-4-phenylpyridinium (MPP^+), is sequestered within neurons that contain the vesicular monoamine transporter (Speciale et al., 1998). Moreover, a cDNA that encodes a vesicular amine transporter prevents toxicity caused by MPP^+ (Liu et al., 1992). Finally, it was recently reported that inhibition of VMAT-2 enhances striatal MPP^+ toxicity in the rat, again suggesting that the sequestration afforded by this carrier can serve a neuroprotective function (Staal and Sonsalla, 2000).

Based on the model proposed above, any pharmacological or physiological treatment that increases VMAT-2 activity would tend to lower cytosolic dopamine concentrations, and thereby be less likely to cause long-term dopaminergic damage. Accordingly, it is interesting to note that cocaine administration rapidly increases VMAT-2 activity (Fig. 3; Brown et al., 2000b) and, as noted above, is predictably not toxic to dopamine systems. Interestingly, amfonelic acid, a dopamine uptake blocker like cocaine, administered as much as 8 h after a neurotoxic methamphetamine treatment, prevents the persistent dopamine deficits caused by the stimulant (Marek et al., 1990): this protection may be due to a cocaine-like induction of VMAT-2 activity that promotes sequestration of the elevated intraneuronal dopamine caused by methamphetamine treatment.

3. The 5-HT transporter

3.1. Psychostimulants differentially affect 5-HT transporters

Like dopamine transporters, 5-HT transporters are also rapidly and reversibly affected by treatment with some psychostimulants. Hence, the ATR associated with 5-HT

was assessed. Similar to effects on dopamine transporters, a single injection of methamphetamine, methcathinone or MDMA rapidly (within 1 h) decreases striatal 5-HT transporter function by as much as 20%, as measured in synaptosomes prepared from drug-treated rats. Also, like effects on dopamine transporters, 5-HT transporter activity is not altered by a single injection of cocaine, methylphenidate or fenfluramine (Tables 1 and 2). Parallels among the effects of the agents on dopamine and 5-HT transporters are also observed after multiple injections: thus, treatment with all of the agents examined, except cocaine, methylphenidate and fenfluramine rapidly decreases 5-HT transporter activity. These decreases appear not to be associated with residual drug introduced by the original injection (Fleckenstein et al. 1997b). It should be noted, however, that the magnitude of the drug-induced decrease in dopamine transporter activity is typically greater in magnitude than in 5-HT transporter activity. Still, the parallels among the decreases suggest that common mechanisms may contribute to the effects of the drugs on transporter activity (Table 2; Fleckenstein et al., 1999).

3.2. 5-HT transporters and the methamphetamine model

To ascertain the nature of the changes in 5-HT transporter function caused by amphetamine-analog treatment, effects of methamphetamine were characterized. Multiple injections of this stimulant rapidly decreased 5-HT transporter function as assessed in synaptosomes prepared from the striatum of treated rats; an effect attributable to a decrease in V_{max} and no effect on K_m (Kokoshka et al., 1998a). This ATR occurred without altering binding of the 5-HT transporter ligand, paroxetine (Haughey et al., in press).

Table 2
Summary of effects on serotonin transporter function

	IC_{50} (μM)	Single administration (% control)	Multiple administrations (% control)
Amphetamine	8±3	92±5	60±3[a]
Methylphenidate	26±3	87±5	100±5
Methamphetamine	9±3	83±4[a]	49±2[a]
Cocaine	0.5±0.05	115±7	89±4
Methcathinone	21.2±4.2	79±3[a]	62±7[a]
Cathinone	14±4	93±4	42±6[a]
MDMA	2.6±0.3	80±3[a]	62±3[a]
Fenfluramine	5±2	99±5	94±4

IC_{50} values represent means of at least three independent experiments. Rats received drug or saline vehicle as described for Table 1. Values represent means expressed as percentage of saline-treated controls. For individual single and multiple administration experiments, control values ranged from 228±13 to 1000±41 fmol/mg protein, and values represent means±1 S.E.M. of determinations in 6–9 rats.

[a]Values for drug-treated rats that differ significantly from saline-treated controls ($P \leq 0.05$). (reproduced from Fleckenstein et al., 1999).

As noted above, similarities in the dopamine- and 5-HT-associated ATR after psychostimulant administration have been observed. Moreover, recent studies indicate that the decreases in 5-HT transporter and dopamine transporter function observed after methamphetamine application in vivo are similar. In addition, incubation of synaptosomes with methamphetamine decreases 5-HT transporter function even after washing methamphetamine from the synaptosomal preparation (Sandoval et al., 2000). Given these similarities, the role of factors demonstrated to alter dopamine transporter function has been studied in the response of 5-HT transporters to methamphetamine. Like its role in altering dopamine transporter function, hyperthermia contributes to the methamphetamine-induced ATR for 5-HT transporters resulting from multiple drug treatments in vivo since its prevention attenuates the decrease in transporter activity. A role for dopamine is also evidenced by findings that pretreatment with methyl-*p*-tyrosine, SCH-23390 or eticlopride attenuates the 5-HT ATR associated with multiple methamphetamine injections, effects independent of the ability of these drugs to prevent methamphetamine-induced hyperthermia. In contrast, the 5-HT-associated ATR after a single administration of methamphetamine was dopamine- and hyperthermia-independent (Haughey et al., in press). Hence, it appears that as with the dopamine transporter, the 5-HT ATR consists of at least two distinct components. The precise nature and function of these components remains to be elucidated.

Although several similarities exist between the DA- and 5-HT-assciated striatal ATR caused by methamphetamine administration, differences do exist. For instance, the temporal effects of multiple methamphetamine injections on dopamine- and 5-HT-ligand binding are dissimilar; with ligand binding to the dopamine, but not the 5-HT, transporter decreased 1 and 24 h after treatment. Moreover, pretreatment with the antioxidant/spin-trapping reagent, PBN, attenuates the methamphetamine-induced decrease in dopamine, but not 5-HT, transporter function. This latter finding was unexpected, since it has been demonstrated that the serotonin transporter is vulnerable to oxidative inactivation (Kokoshka et al., 1998a). These data, in addition to findings that ascorbate pretreatment does not prevent the decrease in 5-HT transporter function, suggest that reactive oxygen species do not contribute to this methamphetamine-induced deficit (Haughey et al., in press).

The factors other than dopamine and hyperthermia that contribute to the decrease in 5-HT transporter function remain to be determined. However, as was the case with the dopamine transporter, stimulant-induced phosphorylation and subsequent internalization of the transporter protein are possible explanations for this drug-related transporter effect. Recent studies in 5-HT transporter-transfected cells seemingly argue against this possibility, since amphetamine application prevents protein kinase C-dependent 5-HT transporter phosphorylation (Ramamoorthy and Blakely, 1999). However, as noted above, amphetamine

treatment may activate protein kinase C. Furthermore, studies utilizing enzyme activators and inhibitors in 5-HT transporter-transfected cells have demonstrated protein kinase C-mediated transporter phosphorylation in parallel with transporter internalization and loss of functional uptake capacity (for review, see Blakely et al., 1998). Further inquiry into the role of phosphorylation in mediating the effects of stimulants on 5-HT transporters is warranted.

3.3. Psychostimulants and acute changes in 5-HT transporter function: implications for 5-HT neurotoxicity

It is established that, in addition to causing persistent dopaminergic deficits, high-dose methamphetamine treatment causes long-term damage to 5-HT neurons. These deficits include long-term deficits in tryptophan hydroxylase activity as well as decreases in 5-HT concentrations, transporter uptake and ligand binding sites (Hotchkiss et al., 1979; Hotchkiss and Gibb, 1980; Hirata et al., 1995; Kovachich et al., 1989). These neurochemical changes correlate with the histological destruction of serotonergic neurons in several brain regions (Axt and Molliver, 1991). High-dose administration of other amphetamine analogs such as MDMA (Ricaurte et al., 1985; Stone et al., 1986; Battaglia et al., 1987; Insel et al., 1989) and methcathinone (Gygi et al., 1996; McCann et al., 1998) cause similar persistent 5-HT deficits. As with the methamphetamine-induced neurotoxicity to dopaminergic pathways, oxygen radicals contribute to the long-term 5-HT deficits induced by these stimulants administration as evidenced by findings that: 1) antioxidants prevent long-term deficits in 5-HT neuronal function caused by multiple administrations of methamphetamine (DeVito and Wagner, 1989) or MDMA (Gudelsky, 1996); and 2) methamphetamine-induced 5-HT deficits are attenuated in transgenic mice that express the human CuZn-superoxide dismutase enzyme (Hirata et al., 1995). In addition to oxygen radicals, the 5-HT deficits caused by methamphetamine (Schmidt et al., 1985; Hotchkiss and Gibb, 1980), MDMA (Stone et al., 1988) or methcathinone (Gygi et al., 1997) treatment require the presence of dopamine since these are attenuated by pretreatment with methyl-*p*-tyrosine.

Findings that dopamine and oxygen radicals are each associated with both the acute and long-term 5-HT deficits caused by stimulants such as methamphetamine suggest that the acute deficit in transporter function may contribute to the persistent deficit. Interestingly, drugs that do not cause a decrease in 5-HT transporter function (Table 2) such as cocaine and methylphenidate cause little or no long-term 5-HT deficits (Kleven et al., 1988; Yeh and De Souza, 1991; Zaczek et al., 1989). This correlation is not, however, perfect since amphetamine causes a 5-HT ATR but has relatively little long-term neurotoxic effect on 5-HT neurons (Peat et al., 1985; Ricaurte et al., 1983). Moreover, fenfluramine does not cause an ATR, but does cause long-term 5-HT deficits including decreases in transporter number, 5-HT concentrations (Scheffel et al., 1992; McCann et al., 1997). In addition, 5-HT uptake inhibitors such as fluoxetine prevent long-term deficits caused by methamphetamine administration (Hotchkiss and Gibb, 1980). Hence, the association between the 5-HT ATR and long-term 5-HT consequences remains to be determined.

4. The norepinephrine transporter

4.1. Methamphetamine and the norepinephrine transporter

Like dopamine and 5-HT transporters, the norepinephrine transporter is a member of the Na^+–Cl^- dependent transporter family and as such shares many structural similarities with these carrier proteins. For instance, molecular biology techniques have revealed that like the dopamine and 5-HT transporters, the norepinephrine transporter contains two conserved cysteine residues located in its large, extracellular loop (Pacholczyk et al., 1991), which in the dopamine transporter is critical for proper function (Wang et al., 1995). Given the similarities in structure and ion conductances, it might be anticipated that this transporter would be similarly affected by methamphetamine treatment. However, recent studies indicate that the norepinephrine transporter is less vulnerable than other Na^+–Cl^- dependent transporters to oxidative inactivation (Pogun et al., 1994; Haughey et al., 1999), despite its cysteine residues that seemingly would make this protein vulnerable to oxidative inactivation. Moreover, in contrast to effects on striatal dopamine and 5-HT transporters, methamphetamine-induced decreases in norepinephrine uptake in hippocampal synaptosomes, are attributable to increases in K_m with no change in V_{max}; changes attributable to methamphetamine and/or its metabolites directly inhibiting transport activity; an effect readily prevented by washing residual drug away (Haughey et al., 2000). Hence, it appears that despite structural similarities, norepinephrine transporters are affected by methamphetamine very differently than 5-HT and dopamine transporters. This difference is confirmed by the observation that methamphetamine does not cause the in vitro reduction in norepinephrine transporter activity observed for dopamine and 5-HT transporters (Sandoval et al., 2000). It is interesting to note that precedence for such differences exists: for instance, in contrast to the dopamine and 5-HT deficits caused by methamphetamine treatment, others (Wagner et al., 1980; Ricaurte et al., 1984; Preston et al., 1985) and we (unpublished observations) have observed little or no long-term damage to norepinephrine systems caused by this stimulant. Hence, it is possible that a correlation exists between the absence of an indirect methamphetamine effect on norepinephrine transporters and the lack of persistent neurotoxicity to norepinephrine systems reported by some investigators.

5. Summary

Based on the discussion above, psychostimulants differentially alter aminergic transporter function. There are also strong indications that components of the acute effects of these agents on plasmalemmal transporters may contribute to, or at least be predictive of, their long-term neurochemical consequences. For instance, stimulants that cause a rapid dopamine- or 5-HT-associated ATR generally cause long-term dopamine and 5-HT neuronal damage. Stimulants that do not cause these changes do not cause persistent monoaminergic deficits. Of special interest is the contribution that the rapid response of VMAT-2 to stimulant treatment may play in contributing to or preventing long-term neuronal damage. Much research needs to be conducted in order to characterize these phenomena, and how interactions between the VMAT-2 and the plasmalemmal transporters influence the physiology and pathophysiology of monoaminergic neurons and the reaction of these systems to psychostimulants.

Finally it is important to note that the capacity of psychostimulants to directly inhibit dopamine reuptake, as assessed in vitro in competition assays wherein drug is present throughout the experiment, does not appear to correlate with their ability to modulate transporter activity following in vivo administration (Tables 1 and 2), or likely with indirect effects after in vitro incubation followed by washing (i.e, as described in Section 1.3). The explanation for this divergence likely involves a myriad of factors including differences in the second messenger systems activated by the drugs. These discrepancies underscore the need for investigating effects of psychostimulants ex vivo and mechanisms underlying these changes. Moreover, these data illustrate the importance of considering these differences when employing in vitro data to predict the effects of drugs in vivo.

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