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European Journal of Pharmacology 382 (1999) 45–49

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Short Communication

Differential effects of psychostimulants and related agents on dopaminergic and serotonergic transporter function

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Received 3 May 1999; received in revised form 6 August 1999; accepted 10 August 1999

Abstract

High-dose administrations of amphetamine, methamphetamine, cathinone, methcathinone or methylenedioxymethamphetamine rapidly decrease dopamine and serotonin transporter function in vivo, as assessed in striatal synaptosomes obtained from drug-treated rats. In contrast, high-dose injections of fenfluramine, cocaine or methylphenidate had little or no effect on the activity of these transporters. Interestingly, the capacity of these agents to directly alter dopamine and serotonin uptake, as assessed in vitro by direct application to rat striatal synaptosomes, did not predict their potential to modulate transporter activity following in vivo administration. These findings demonstrate heretofore-unreported differences in the effects of these agents on monoamine transporter function, and a distinction between drug effects after direct application in vitro vs. administration in vivo.

Keywords: Amphetamine; Cocaine; Methylphenidate; Fenfluramine; Transporter

1. Introduction

This laboratory has reported that both a single and multiple administrations of methamphetamine cause a rapid and reversible decrease in striatal dopamine transporter activity; effects attributable to decreases in V_{max} of transport (Fleckenstein et al., 1997b; Kokoshka et al., 1998b). These transient decreases do not reflect loss and subsequent replacement of dopamine transporters, since: (1) methamphetamine does not cause an acute loss of transporter protein (Kokoshka et al., 1998b); and (2) the time necessary to recover transporter function (<1 day) is much less than that likely required to synthesize dopamine transporters de novo (the $t_{1/2}$ for dopamine transporter turnover is ≈ 6 days; Fleckenstein et al., 1996). Moreover, these decreases are not due to the direct effects of residual methamphetamine introduced by the original subcutaneous (s.c.) injection (Fleckenstein et al., 1997b; Kokoshka et al., 1998b). Instead, we believe that these rapid and reversible effects of methamphetamine provide evidence of a previously uncharacterized mechanism whereby transporter activity can be modulated rapidly in vivo.

Recent evidence suggests that dopamine contributes to the acute decrease in dopamine transporter function following multiple administrations of methamphetamine since this effect is attenuated by prior dopamine depletion with the tyrosine hydroxylase inhibitor, α-methyl-*para*-tyrosine (Metzger et al., 1998b). Consistent with this finding, direct application of dopamine to striatal synaptosomal preparations decreases dopamine transporter activity (Berman et al., 1996). Since many psychostimulants alter dopamine uptake and release, it might be expected that these, too, might effect the rapid and reversible decrease in dopamine transporter activity caused by methamphetamine. Hence, the purpose of this study was to determine whether other psychostimulants and related agents alter dopamine transporter function. Since methamphetamine effects a rapid and reversible decrease in serotonin transporter function as well (Kokoshka et al., 1998a), effects on serotonin uptake were also assessed. For comparison, effects on dopamine and serotonin uptake after direct application of psychostimulant or related agent of interest to striatal synaptosomes in vitro were also assessed since these assays are employed commonly to predict effects of these agents on transporter function in vivo. The results reveal differential effects of psychostimulants on dopamine and serotonin transporter function, and indicate that the capacity of a

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00588-9

compound to alter monoamine uptake in vitro does not predict its potential to modulate monoamine transporters after administration in vivo.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g; Simonsen Laboratories, Gilroy, CA) were housed at 23°C with a alternating light/dark cycle (lights on 14 h/day). Food and water were provided ad libitum. Rats were sacrificed by decapitation. All experiments were conducted in accordance with National Institute of Health guidelines.

2.2. Drugs and chemicals

[7,8-^{3}H]Dopamine (46 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL). 5-[1,2-^{3}H(*N*)]-hydroxytryptamine (30 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Methamphetamine hydrochloride, methcathinone hydrochloride, methylenedioxymethamphetamine hydrochloride, and cathinone hydrochloride were furnished by the National Institute on Drug Abuse. Fenfluramine hydrochloride was obtained from A.H. Robins (Richmond, VA). D-Amphetamine sulfate was obtained from Sigma (St. Louis, MO). Methylphenidate hydrochloride was obtained from Ciba Geigy (Summit, NJ). Cocaine hydrochloride was obtained from Mallinckrodt (St. Louis, MO), and citalopram was obtained from H. Lundbeck A/S (Copenhagen, Denmark). Pargyline hydrochloride was obtained from Abbott Laboratories (North Chicago, IL). All drug concentrations were calculated as free base. 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.; methylenedioxymethamphetamine, 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.

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

[^{3}H]Dopamine and [^{3}H]serotonin uptake were determined in rat striatal synaptosomal preparations as described previously (Kokoshka et al., 1998a). Briefly, synaptosomes were prepared by homogenizing fresh striatal 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 15 min at 4°C). The resulting pellets (P2) were then washed twice (i.e., resuspended in phosphate-buffered sucrose and centrifuged at $22{,}000 \times g$ for 15 min at 4°C). This washing procedure has been demonstrated to remove virtually all residual methamphetamine from a synaptosomal preparation (Fleckenstein et al., 1997b), and is presumably sufficient to remove any drug residual from the original injections. The final pellet (P4) was 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). Transport of [^{3}H]dopamine or [^{3}H]serotonin was determined in synaptosomes (i.e., resuspended P4) obtained from 1.5 or 7.5 mg striatal tissue (original wet weight), respectively, per reaction tube. Uptake assays were initiated by addition of [^{3}H]dopamine (0.5 nM final concentration) or [^{3}H]serotonin (5 nM final concentration). Samples were incubated for 3 min at 37°C and filtered through Whatman GF/B filters soaked previously in 0.05% polyethylenimine. Filters were washed rapidly three times with 3 ml ice-cold 0.32 M sucrose using a Brandel filtering manifold. Radioactivity trapped in filters was counted using a liquid scintillation counter.

2.4. Data analysis

Statistical analyses between two groups were conducted using a two-tailed Student's *t*-test. IC_{50} values were determined using EBDA (McPherson, 1986) with a minimum of eight data points per curve (each point determined in triplicate), and competing drug concentrations ranging from 1 nM to 500 μM.

3. Results

We reported previously that a single administration of methamphetamine, methylenedioxymethamphetamine or methcathinone decreased striatal [^{3}H]dopamine uptake to 63%–72% of control values in striatal synaptosomes prepared 1 h after drug administration (Metzger et al., 1998a). Results presented in Table 1 demonstrate that high-dose administration of cathinone or amphetamine decreased [^{3}H]dopamine uptake as well (i.e., to 78% and 80% of control values, respectively). In contrast, a single high-dose injection of cocaine, methylphenidate or fenfluramine caused little or no decrease in synaptosomal [^{3}H]dopamine uptake.

Like a single administration, multiple high-dose injections (i.e., four injections at 2-h intervals) of several of the agents under study decreased dopamine transporter function 1 h after treatment. Multiple administrations of amphetamine, cathinone or methamphetamine exerted the greatest effects, decreasing [^{3}H]dopamine uptake to 11%, 27% and 30% of control values, respectively. Methcathinone and methylenedioxymethamphetamine administra-

Table 1
Summary of effects on dopamine transporter function
IC_{50} values represent means of at least three independent experiments, and were obtained as described in Section 2. In single administration experiments, rats received drug (see Section 2 for doses), 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, see Section 2 for doses) 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 percent 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 $\pm$ 1 S.E.M. of determinations in six to nine rats.

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

[a] Values for drug-treated rats that differ significantly from saline-treated controls ($P \leq 0.05$).
[b] Value reported previously (Fleckenstein et al., 1997b).
[c] Values reported previously (Metzger et al., 1998a).

tions decreased [^{3}H]dopamine uptake to 50% and 58% of control values, respectively. In contrast, neither multiple high-dose injections of cocaine, methylphenidate nor fenfluramine altered significantly [^{3}H]dopamine uptake (Table 1).

Results presented in Table 2 demonstrate that a single injection of methamphetamine, methcathinone, or methylenedioxymethamphetamine decreased striatal [^{3}H]serotonin uptake by as much as 21%. In contrast, neither a single high dose injection of cocaine, amphetamine, cathinone, methylphenidate, nor fenfluramine altered [^{3}H]serotonin uptake. Multiple administrations of amphetamine, methamphetamine, cathinone, methcathinone or methylenedioxymethamphetamine decreased [^{3}H]serotonin uptake to 42%–60% of controls. Multiple injections of methylphenidate, cocaine or fenfluramine were without effect.

Besides summarizing the effects of the agents described on dopamine and serotonin transporter function after a single or multiple administrations, Tables 1 and 2 also compare the effects of these same drugs on monoamine uptake (i.e., IC_{50} values) after direct application to striatal synaptosomes in vitro. No correlation was observed between the IC_{50} values and the ability of the agents to alter monoamine uptake after administration in vivo.

4. Discussion

Recent evidence has suggested that dopamine is necessary for the rapid and reversible decrease in dopamine transporter function caused by multiple injections of methamphetamine (Metzger et al., 1998b). This may be due to findings that dopamine promotes the formation of oxygen radicals (Graham, 1978) which can oxidize and inactivate dopamine transporters (Berman et al., 1996; Fleckenstein et al., 1997a). Consistent with this hypothesis, direct application of dopamine to striatal synaptosomal preparations decreases dopamine transporter activity (Ber-

Table 2
Summary of effects on serotonin transporter function
IC_{50} values represent means of at least three independent experiments, and were obtained as described in Section 2. In single administration experiments, rats received drug (see Section 2 for doses), 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, see Section 2 for doses) 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 percent 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 $\pm$ 1 S.E.M. of determinations in six to nine rats.

	IC_{50} (μM)	Single administration	Multiple administrations
Amphetamine	8 ± 3	92 ± 5	60 ± 3[a]
Methylphenidate	26 ± 3	87 ± 5	100 ± 5
Methamphetamine	9 ± 3[b]	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]
Methylenedioxymethamphetamine	2.6 ± 0.3	80 ± 3[a]	62 ± 3[a]
Fenfluramine	5 ± 2	99 ± 5	94 ± 4

[a] Values for drug-treated rats that differ significantly from saline-treated controls ($P \leq 0.05$).
[b] Values reported previously (Kokoshka et al., 1998a).

man et al., 1996). Because of our interest in the role of dopamine in affecting transporter function, the effects of agents with varying abilities to increase dopamine release or block its reuptake into nerve terminals were examined.

Results presented in Table 1 support the hypothesis that dopamine can rapidly decrease dopamine transporter function, since drugs such as amphetamine and methamphetamine that cause large quantities of dopamine release (Kuczenski et al., 1991; Stephans and Yamamoto, 1994; Melegra et al., 1995) caused the greatest decrease in transporter function. Furthermore, drugs that cause significantly less increase in extracellular dopamine concentrations, such methylphenidate (Hurd and Ungerstedt, 1989; Hiramatsu and Cho, 1990; Butcher et al., 1991), fenfluramine (De Deurwaerdere et al., 1995) and cocaine (Kuczenski et al., 1991), although administered at high doses, were without effect on the transporter after multiple in vivo administrations. Hence, drug-induced alterations in extracellular dopamine levels may contribute to diminished transporter activity observed after multiple drug injections. Interestingly, the magnitude of decrease in transporter activity observed after a single drug administration did not necessarily predict the ability of a given agent to decrease transporter activity after multiple administrations. Hence, factors besides dopamine such as pharmacokinetics, phosphorylation of the transporter (Copeland et al., 1996; Vaughan et al., 1997) or non-dopamine-dependent reactive species formation (Wrona and Dryhurst, 1998) may contribute to the effects of a single administration. Further inquiry is warranted into the mechanism(s) resulting in the single- and multiple-injection effects.

Like dopamine transporters, serotonin transporters are also rapidly and reversibly affected by methamphetamine (Kokoshka et al., 1998a). Hence, the response of these uptake carriers was assessed. Consistent with effects on dopamine transporters, a single injection of methamphetamine, methcathinone or methylenedioxymethamphetamine decreased serotonin transport by as much as 21%. Also like dopamine transporters, serotonin transporter activity was not altered by a single injection of cocaine, methylphenidate or fenfluramine. Parallels among the effects of the agents on dopamine and serotonin transporters were also observed after multiple injections: thus, treatment by all of the agents examined except methylphenidate, cocaine and fenfluramine rapidly decreased serotonin transporter activity. It should be noted, however, that the magnitude of the drug-induced decrease in dopamine transporter activity was greater in magnitude than in serotonin transporter activity. Still, the parallels among the decreases suggest that common mechanisms may contribute to the effects of the drugs on transporter activity: these mechanisms remain to be determined.

In vitro uptake studies are often used to predict drug effects after in vivo administration. In such studies, drug is applied directly to synaptosomal preparations, and its ability to prevent monoamine uptake is assessed. Results presented in Table 1 include IC_{50} values and demonstrate that the agents decreased dopamine uptake with rank order potency of amphetamine > methylphenidate > methamphetamine > cocaine ≥ methcathinone > cathinone > methylenedioxymethamphetamine > fenfluramine. Consistent with the notion that these in vitro data predict the effects of drug after in vivo administration, agents with low IC_{50} values such as amphetamine and methamphetamine profoundly altered dopamine exchange at its transporter as assessed in striatal synaptosomes prepared from drug-treated rats. Moreover, application of fenfluramine, the drug with the highest IC_{50} value, was predictably without effect on synaptosomal dopamine transport after administration in vivo. In contrast, a single or multiple injections of high doses of cocaine or methylphenidate, compounds which potently diminish synaptosomal dopamine exchange in vitro, had little or no acute effect on associated transporter function in synaptosomes prepared from treated rats. These data indicate that the capacity of a compound to alter dopamine uptake in vitro does not necessarily predict its potential to modulate dopamine transporter after injection in vivo. Similar conclusions can be drawn for the serotonin transporter: IC_{50} values did not predict the effects of the various agents after their injection in vivo. The explanation for these discrepancies likely involve a myriad of factors including differences in metabolism and in the second messenger systems activated by the agents. Such discrepancies must be considered when employing IC_{50} data to predict the effects of drugs in vivo.

In conclusion, the results presented above demonstrate that psychostimulants differentially affect dopamine and serotonin transporter function. The data support the hypothesis that dopamine release may contribute to the ability of multiple administrations of stimulants such as methamphetamine and amphetamine to decrease dopamine transporter function. Finally, these findings demonstrate that the capacity of a compound to alter monoamine uptake in vitro does not predict its potential to modulate monoamine transporters after administration in vivo.

Acknowledgements

This research was supported by PHS grants DA 00869, DA 04222, DA 11389 and DA 00378.

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