Published in final edited form as:

Eur J Pharmacol. 2007 September 24; 571(1): 25–28. doi:10.1016/j.ejphar.2007.05.044.

Therapeutic doses of amphetamine and methylphenidate selectively redistribute the vesicular monoamine transporter-2

Evan L. Riddle, Glen R. Hanson, and Annette E. Fleckenstein

Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

Abstract

High-dose administration of psychostimulants traffics the vesicular monoamine transporter-2 (VMAT-2), as assessed by subcellular fractionation of rat striatal tissue. This study demonstrates that administration of low doses of amphetamine or methylphenidate differentially traffic VMAT-2 within nerve terminals, with effects similar to those observed after high-dose administration. Trafficking of vesicular glutamate, acetylcholine, or GABA transporters was not altered by high- or low-dose amphetamine or methylphenidate treatment. These data represent the first report that amphetamine redistributes VMAT-2 protein. In addition, these data demonstrate that the trafficking of VMAT-2 after amphetamine or methylphenidate is selective for monoaminergic neurons.

Keywords

vesicular monoamine transporter; VMAT; amphetamine; methylphenidate; synaptic vesicle; ADHD

1. Introduction

Several reports have demonstrated that psychostimulants differentially alter striatal vesicular monoamine transporter-2 (VMAT-2) function and distribution. Specifically, multiple high-dose administrations of the dopamine-releasing agent, methamphetamine (Brown et al., 2000), or its related analog, methylenedioxymethamphetamine (Hansen et al., 2002), rapidly decrease rat striatal vesicular dopamine uptake in a non-membrane-associated (referred to herein as a cytoplasmic) vesicular preparation. The methamphetamine-induced decrease in uptake is associated with a decrease in VMAT-2 immunoreactivity in this preparation (Riddle et al., 2002). Effects of the related stimulant, amphetamine, have not been reported. In contrast, high-dose administration of the dopamine-releasing agents cocaine (Brown et al., 2002), bupropion (Rau et al., 2005) or methylphenidate (Sandoval et al., 2002) increases vesicular dopamine uptake in rats. These effects also represent a redistribution of VMAT-2-containing synaptic vesicles (Riddle et al., 2002; Sandoval et al., 2002, 2003; Rau et al., 2005) within nerve terminals. The specificity, dose-dependency, and mechanisms underlying these phenomena remain to be fully characterized.

Because amphetamine and methylphenidate are used commonly for the treatment of attention deficit disorder, the present study determined if: 1) administration of clinically relevant doses

Corresponding Author: Evan L. Riddle, Ph.D., University of Utah, Department of Pharmacology and Toxicology, 30 South 2000 East, Rm. 201, Salt Lake City, Utah 84112, Phone: 801-587-9200, FAX: 801-585-5111, Email: evan.riddle@pharm.utah.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of amphetamine or methylphenidate redistributes VMAT-2; and 2) psychostimulants alter trafficking of other vesicular transporter proteins. Results demonstrate that low, clinically relevant, doses of amphetamine and methylphenidate (Schiffer et al., 2006) differentially and selectively alter the subcellular distribution of striatal VMAT-2 protein. The implications of these phenomena will be discussed.

2. Materials and Methods

2.1 Animals

All experiments were conducted in accordance with the National Institutes of Health (USA) Guidelines for the Care and Use of Laboratory Animals. Where indicated, male Sprague-Dawley rats (300–350g; Simonsen Laboratories, Gilroy, CA) received a single injection of amphetamine (2 or 15 mg/kg, s.c.), methylphenidate (2 or 40 mg/kg, s.c.), or saline vehicle (1 ml/kg).

2.2 Drugs and chemicals

(±)Methylphenidate hydrochloride and d-amphetamine were supplied by the National Institute on Drug Abuse (Bethesda, MD). The VMAT-2 antibody was purchased from Chemicon (Temecula, CA), and the vesicular glutamate, acetylcholine, and GABA transporter antibodies were generously provided by Dr. Robert Edwards (University of California San Francisco, CA).

2.3 Preparation of subcellular fractions

Striatal synaptosomes were prepared from rats decapitated 1 h after treatment. Striatal tissue was homogenized in cold 0.32 M sucrose and centrifuged ($1000 \times g$ for 10 min; 4°C). The supernatant (S1) was then centrifuged ($10,000 \times g$ for 15 min; 4°C) and the resulting pellet (P2, whole synaptosome fraction) was lysed at 50 mg original wet weight/ml in cold water and a portion saved for western blot analysis. The remainder of the lysed synaptosomal sample was centrifuged for 20 min at $25,000 \times g$ (4°C) to pellet synaptosomal membranes (P3, synaptosomal membrane fraction), which were then resuspended in water at 50 mg original wet weight/ml and saved for western blot analysis. Prior to resuspension of the synaptosomal membrane fraction (P3), the supernatant (S3 – non-membrane, “cytoplasmic” fraction) was removed and saved for western blot analysis.

2.4 Western blot analysis

Binding of VMAT-2 antibody was performed using aliquots containing 50 μg protein of whole synaptosomes (P2), 30 μg protein synaptosomal membrane (P3), or 20 μg protein cytoplasmic (S3) fraction. Each aliquot was added to loading buffer (final concentration: 2.25% sodium dodecyl sulfate, 18% glycerol, 180 mM Tris base (pH 6.8), 10% β -mercapto-ethanol and bromophenol blue), boiled for 10 min, and loaded on a 10% sodium dodecyl sulfate-polyacrylamide gel. Following electrophoresis, samples were transferred to polyvinylidene difluoride membrane, blocked with 5% nonfat dry milk in Tris-buffered saline with tween (TBST; 250 mM NaCl, 50 mM Tris pH 7.4 and 0.05% Tween 20), and probed with the VMAT-2 antibody. Bound antibody was visualized with horseradish peroxidase-conjugated goat anti-rabbit antibody (Biosource International, Camarillo, CA), and antigen-antibody complexes were visualized by chemiluminescence. Multiple exposures of blots were obtained to ensure development within the linear range of the film. Bands on blots were quantified by densitometry using Kodak 1D image-analysis software. All protein concentrations were determined by a Bio-Rad (Hercules, CA) protein assay.

2.4 Data analysis

Statistical analyses between two groups was conducted by a two-tailed Student's *t*-test. Differences between groups were considered significant if the probability of error was less than 5%.

3. Results

Fig. 1A demonstrates that a single, low-dose injection of methylphenidate (2 mg/kg, s.c.) reduced VMAT-2 immunoreactivity in the synaptosomal membrane (P3) fraction while increasing it in a non-membrane-associated (referred to as cytoplasmic, S3) fraction. In contrast, a single low-dose administration of amphetamine (2 mg/kg, s.c.) decreased VMAT-2 immunoreactivity in the cytoplasmic (S3) fraction (Fig. 1B). Neither treatment altered immunoreactivity in the whole synaptosome (P2) fraction. Effects of methylphenidate appeared maximal at 1 h and returned to control values by 2 h while effects of amphetamine appeared maximal at 1 h, but returned to control values by 4 h (data not shown). The redistribution produced by methylphenidate or amphetamine was specific for VMAT-2 in that neither cholinergic, GABAergic, nor glutamatergic vesicular transporter immunoreactivity was altered by drug treatment, even when administered at high doses (40 mg/kg s.c. and 15 mg/kg s.c. for methylphenidate and amphetamine, respectively; Table 1).

4. Discussion

Both amphetamine and methylphenidate are used clinically to treat attention deficit disorder, perhaps because both drugs increase synaptic dopamine concentrations. However, the mechanism whereby amphetamine and methylphenidate increase extracellular dopamine concentrations differs. Amphetamine causes presynaptic dopamine release, presumably through reverse transport of the dopamine transporter (Sulzer et al., 1995), whereas methylphenidate prevents the re-uptake of dopamine into the presynaptic nerve terminal (Ritz et al., 1987).

The present study demonstrates an additional difference between amphetamine and methylphenidate. Specifically, this is the first demonstration that amphetamine causes VMAT-2 trafficking. This extends previous findings that another dopamine-releasing agent, methamphetamine, redistributes VMAT-2 similarly. Importantly, this study demonstrates that amphetamine-induced trafficking occurs at 2 mg/kg, a dose that causes hyperlocomotive behavior (Scheel-Kruger, 1971), and is presumed clinically relevant (Schiffer et al., 2006). In contrast to amphetamine, results confirm a previous study that methylphenidate redistributes vesicles from the plasmalemmal membrane-associated to the cytoplasmic, non-membrane-associated fraction. This represents the first report that this phenomenon occurs at a dose presumed clinically relevant (Schiffer et al., 2006). Noteworthy are findings that neither vesicular glutamate, acetylcholine, nor GABA transporter immunoreactivity was altered by even high-dose amphetamine or methylphenidate treatment, demonstrating that these phenomena are selective for the vesicular transporter found in monoaminergic neurons.

The significance of the dissimilar effect of amphetamine and methylphenidate on VMAT-2 in terms of treatment of attention deficit disorder is uncertain. However, the observed trafficking events presented in this study may be an important feature of the clinical efficacy of these drugs. It has been suggested that synaptic vesicles released by osmotic lysis of synaptosomes constitutes the reserve pool of synaptic vesicles whereas vesicles that remain associated with the synaptosomal membranes belong to the recycling and/or readily-releasable pool (Morciano et al., 2005). Trafficking of VMAT-2-containing synaptic vesicles has the potential to alter the pools of vesicles that are available for physiologically and/or pharmacologically stimulated release (i.e. methylphenidate-induced reduction of vesicles in the recycling and/or readily-

releasable pool), thus altering dopaminergic transmission via a mechanism distinct from effects on the dopamine transporter. Future studies are necessary to address these issues.

In addition to acute changes in dopaminergic transmission, high-dose administrations of amphetamine and methylphenidate are differentially neurotoxic to monoaminergic neurons. Specifically, high-dose amphetamine (Nwanze and Jonsson, 1981) but not methylphenidate (Zaczek et al., 1989) causes persistent dopaminergic deficits in rodent models. This differential impact may be linked to differential effects on the distribution and activity of VMAT-2. Briefly, amphetamine may redistribute VMAT-2 and associated vesicles such that cytoplasmic dopamine accumulates within nerve terminals thereby promoting neurotoxic reactive oxygen species formation. In contrast, methylphenidate may redistribute vesicles into cytoplasmic pools thereby promoting vesicular dopamine sequestration and protecting the neuron from oxidative insult (for review, see Fleckenstein and Hanson, 2003). Ongoing studies are investigating these phenomena.

References

- Brown JM, Hanson GR, Fleckenstein AE. Methamphetamine rapidly decreases vesicular dopamine uptake. *J. Neurochem* 2000;74:2221–2223. [PubMed: 10800970]
- Brown JM, Riddle EL, Sandoval V, Weston RK, Hanson JE, Crosby MJ, Ugarte YV, Gibb JW, Hanson GR, Fleckenstein AE. A single methamphetamine administration rapidly decreases vesicular dopamine uptake. *J. Pharmacol. Exp. Ther* 2002;302:497–501. [PubMed: 12130707]
- Fleckenstein AE, Hanson GR. Impact of psychostimulants on vesicular monoamine transporter function. *Eur. J. Pharmacol* 2003;479:283–289. [PubMed: 14612158]
- Hansen JP, Riddle EL, Sandoval V, Brown JM, Gibb JW, Hanson GR, Fleckenstein AE. Methylenedioxymethamphetamine decreases plasmalemmal and vesicular dopamine transport: mechanisms and implications for neurotoxicity. *J. Pharmacol. Exp. Ther* 2002;300:1093–1100. [PubMed: 11861820]
- Morciano M, Burre J, Corvey C, Karas M, Zimmermann H, Volkandt W. Immunolocalization of two synaptic pools from synaptosomes: a proteomic analysis. *J. Neurochem* 2005;95:1732–1745. [PubMed: 16269012]
- Nwanze E, Jonsson G. Amphetamine neurotoxicity on dopamine nerve terminals in the caudate nucleus of mice. *Neurosci. Lett* 1981;26:163–168. [PubMed: 7301203]
- Rau KS, Birdsall E, Hanson JE, Johnson-Davis KL, Carroll FI, Wilkins DG, Gibb JW, Hanson GR, Fleckenstein AE. Bupropion increases striatal vesicular monoamine transport. *Neuropharmacology* 2005;49:820–830. [PubMed: 16005476]
- Riddle EL, Topham MK, Haycock JW, Hanson GR, Fleckenstein AE. Differential trafficking of the vesicular monoamine transporter-2 by methamphetamine and cocaine. *Eur. J. Pharmacol* 2002;449:71–74. [PubMed: 12163108]
- Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 1987;237:1219–1223. [PubMed: 2820058]
- Sandoval V, Riddle EL, Hanson GR, Fleckenstein AE. Methylphenidate redistributes vesicular monoamine transporter-2: role of dopamine receptors. *J. Neurosci* 2002;22:8705–8710. [PubMed: 12351745]
- Sandoval V, Riddle EL, Hanson GR, Fleckenstein AE. Methylphenidate alters vesicular monoamine transport and prevents methamphetamine-induced dopaminergic deficits. *J. Pharm. Exp. Ther* 2003;304:1181–1187.
- Scheel-Kruger J. Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. *Eur. J. Pharmacol* 1971;14:47–59. [PubMed: 4396660]
- Schiffer WK, Volkow ND, Fowler JS, Alexoff DL, Logan J, Dewey SL. Therapeutic doses of amphetamine or methylphenidate differentially increase synaptic and extracellular dopamine. *Synapse* 2006;59:243–251. [PubMed: 16385551]

- Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J. Neurosci* 1995;15:4102–4108. [PubMed: 7751968]
- Sulzer D, Rayport S. Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. *Neuron* 1990;5:797–808. [PubMed: 2268433]
- Zaczek R, Battaglia G, Contrerra JF, Culp S, De Souza EB. Methylphenidate and pemoline do not cause depletion of rat brain monoamine markers similar to that observed with methamphetamine. *Toxicol. Appl. Pharmacol* 1989;100:227–233. [PubMed: 2551071]

Acknowledgements

Support for these studies was provided by DA11389, DA04222, DA00869 and DA13367.

Figure 1.

Methylphenidate (MPD) or amphetamine (AMPH) alters VMAT-2 immunoreactivity in subcellular fractions. Rats received a single administration of methylphenidate (Fig. 1A; 2 mg/kg, s.c.), amphetamine (Fig. 1B; 2 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) and were sacrificed 1 h later. Columns represent the mean optic density, and error bars represent the S.E.M. of determinations in six treated rats. *Values for methylphenidate- or amphetamine-treated rats that are significantly different from control ($P \leq 0.05$).

Table 1

A single low- or high-dose administration of methylphenidate (MPD) or amphetamine (AMPH) did not alter redistribution of vesicular glutamate (VGLUT-1,2), acetylcholine (VACHT), or GABA (VGAT) transporters in the whole synaptosome (P2) or cytoplasm (S3) fraction. Rats received a single administration of methylphenidate (2 or 40 mg/kg, s.c.), amphetamine (2 or 15 mg/kg, s.c.), or saline vehicle (1 ml/kg, s.c.) and were sacrificed 1 h later. Values represent the mean optic density (in arbitrary units) $\pm$ the S.E.M. of determinations in six treated rats.

	MPD (2 mg/kg)		MPD (40 mg/kg)	
	Whole Synaptosome	Cytoplasm	Whole Synaptosome	Cytoplasm
VGLUT-1	89.7 $\pm$ 6.4	108.7 $\pm$ 11.0	104.8 $\pm$ 6.1	104.9 $\pm$ 11.8
VGLUT-2	92.3 $\pm$ 4.4	82.5 $\pm$ 14.4	95.6 $\pm$ 11.9	113.8 $\pm$ 10.0
VACHT	90.5 $\pm$ 5.1	104.7 $\pm$ 11.9	99.8 $\pm$ 6.5	106.0 $\pm$ 12.4
VGAT	100.6 $\pm$ 15.5	112.7 $\pm$ 9.6	116.8 $\pm$ 6.0	106.6 $\pm$ 7.0
	AMPH (2 mg/kg)		AMPH (15 mg/kg)	
	Whole Synaptosome	Cytoplasm	Whole Synaptosome	Cytoplasm
VGLUT-1	130.3 $\pm$ 10.4	87.9 $\pm$ 3.8	109.6 $\pm$ 19.3	92.3 $\pm$ 2.8
VGLUT-2	116.6 $\pm$ 7.7	97.5 $\pm$ 9.4	96.4 $\pm$ 14.6	106.4 $\pm$ 9.3
VACHT	107.3 $\pm$ 4.8	93.1 $\pm$ 8.6	110.1 $\pm$ 8.9	98.6 $\pm$ 5.9
VGAT	100.1 $\pm$ 6.2	102.4 $\pm$ 9.6	113.2 $\pm$ 14.7	99.4 $\pm$ 5.3