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Methylphenidate-Induced Alterations in Synaptic Vesicle Trafficking and Activity: Functional Consequences and Therapeutic Implications

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Abstract

The psychostimulant, methylphenidate (MPD), is commonly prescribed to treat attention-deficit hyperactivity disorder. MPD binds to the neuronal dopamine (DA) transporter where it blocks the inward transport of DA. The present study expands upon these findings by examining the effects of *in vivo* MPD administration on the vesicular monoamine transporter-2 (VMAT-2) in membrane-associated vesicle and cytoplasmic vesicle subcellular fractions (i.e., those vesicles that do and do not co-fractionate with synaptosomal membranes after osmotic lysis, respectively) isolated from lysates of rat striatal synaptosomes. The results indicate that a single MPD administration redistributes VMAT-2 and associated vesicles within nerve terminals away from the synaptosomal membranes and into the cytoplasm, as assessed 1 hour after treatment. DA transport is also increased by MPD in both vesicle fractions (due to vesicle trafficking in the cytoplasmic vesicles and to kinetic upregulation of the VMAT-2 in the membrane-associated vesicles). This, in turn, leads to an increase in the DA content of both vesicle fractions as well as an increase in the velocity and magnitude of K^+-stimulated DA release from striatal suspensions. Taken together, these data show that the trafficking, DA sequestration function, DA content, and exocytotic DA release function of synaptic vesicles can all be pharmacologically manipulated by *in vivo* MPD treatment. These findings may provide important insights useful for understanding and treating disorders involving abnormal DA transmission including drug abuse, Parkinson's disease, and attention-deficit hyperactivity disorder.

Keywords

dopamine; methylphenidate; methamphetamine; neurotoxicity; Parkinson's disease; vesicular monoamine transporter-2

Introduction

Methylphenidate (MPD) is a ritalinic acid psychostimulant that is commonly used to treat attention-deficit hyperactivity disorder. MPD binds to the neuronal dopamine (DA) transporter where it blocks the inward transport of DA into neuronal cells.[1–3] MPD also indirectly affects DA transport by the vesicular monoamine transporter-2 (VMAT-2), a protein that transports cytoplasmic DA into synaptic vesicles inside neuronal cells for storage and subsequent release.[4, 5] The VMAT-2 is the sole transporter responsible for

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sequestering cytoplasmic DA into vesicles and alterations in VMAT-2 function may thus regulate both intra- and extra-neuronal DA levels and subsequent postsynaptic events.

Recent attention has focused on the pharmacological regulation of cytoplasmic VMAT-2-containing vesicles (i.e., those vesicles that do not co-fractionate with synaptosomal membranes after osmotic lysis) isolated from lysates of rat striatal synaptosomes. *In vi*vo MPD administration increases DA transport in cytoplasmic vesicles purified from the striata of MPD-treated rats.4 This increase occurs concurrent with a redistribution of VMAT-2 protein from the membrane-associated vesicle fraction (i.e., those vesicles that co-fractionate with synaptosomal membranes after osmotic lysis) into the cytoplasmic vesicle fraction, and both phenomena are DA D1 and D2 receptor-mediated.4, 5

The present study examines the effects of MPD administration on the vesicular monoamine transporter-2 (VMAT-2) in both the cytoplasmic and membrane-associated vesicles. The data reviewed here and reported previously6 show that the trafficking, DA sequestration function, DA content, and exocytotic DA release function of both cytoplasmic and membrane-associated synaptic vesicles can be pharmacologically manipulated by *in vivo* MPD treatment. These findings may provide important insights useful for understanding and treating disorders involving abnormal DA transmission including drug abuse, Parkinson's disease, and attention-deficit hyperactivity disorder.

Materials and Methods

Animals and Drug Treatment

Male Sprague-Dawley rats (300 - 360 g from Charles River Laboratories (Raleigh, NC)) were housed in a light- and temperature-controlled room with free access to food and water. (±)-MPD hydrochloride was supplied by the National Institute on Drug Abuse (Bethesda, MD). MPD doses, calculated as the free base, were dissolved in 0.9 % (w/v) saline before being administered at 1 ml/kg as indicated in the table legends. All animal procedures were approved by the University of Utah Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*.

Tissue Preparation and Analysis

The cytoplasmic and membrane-associated vesicle fractions were isolated from the striata of treated rats as described previously.6 SDS-polyacrylamide gel electrophoresis and western blot analysis were performed on the cytoplasmic and membrane-associated vesicle fractions to quantify VMAT-2 immunoreactivity.6, 7 Binding of the [^{3}H]-labeled VMAT-2 ligand, dihydrotetrabenazine ([^{3}H]DHTBZ), in the cytoplasmic and membrane-associated vesicle fractions was performed as described previously.4 Rotating disk electrode voltammetry8, 9 was used to measure the initial velocities of inwardly directed vesicular DA transport in the cytoplasmic and membrane-associated vesicle fractions.6, 9, 10 DA content in the vesicle fractions and in whole striatal tissue was measured *ex vivo* using high performance liquid chromatography with electrochemical detection.6, 11, 12 The initial velocity and magnitude of K^+-stimulated DA release in rat striatal suspensions were measured using rotating disk electrode voltammetry.6, 13 Complete details for tissue preparation and the assays are described elsewhere.4, 6, 9

Results

The results presented in Table 1 demonstrate that synaptic vesicles containing the VMAT-2 are unequally distributed among the two vesicle populations isolated from lysates of rat striatal synaptosomes. The data from both VMAT-2 immunoreactivity and [^{3}H]DHTBZ

binding experiments indicate that approximately 70 % of the VMAT-2 isolated from rat striatal synaptosomes is contained in the membrane-associated vesicle fraction and that approximately 30 % of the VMAT-2 is contained in the cytoplasmic vesicle fraction.

We then examined the effects of MPD on the two vesicle populations. As shown in Table 2, a single injection of MPD (40 mg/kg, s.c.; a dose used previously to investigate MPD-induced vesicular trafficking[4, 6]) increased and decreased VMAT-2 immunoreactivity in the cytoplasmic and membrane-associated vesicles, respectively. These MPD-induced changes in VMAT-2 immunoreactivity occurred concurrent with an increase in DA transport velocities in each vesicular fraction (Table 2). In addition to increasing DA transport velocities, MPD administration increased the DA content in both the cytoplasmic and membrane-associated vesicle fractions without changing whole striatal tissue DA content (Table 3). As shown in Table 4, MPD administration also increased the magnitude and initial velocity of K^+-stimulated DA release from rat striatal suspensions.

Discussion

Administration of the widely prescribed psychostimulant, MPD, increases DA transport in cytoplasmic vesicles purified from the striata of MPD-treated rats.[4] This increase occurs concurrent with a redistribution of VMAT-2 protein from the membrane-associated vesicle fraction into the cytoplasmic vesicle fraction. These phenomena can be manipulated pharmacologically, as a DA D2 receptor agonist mimics the effects of MPD and *in vivo* pretreatment with either a D1 or a D2 receptor antagonist attenuates the MPD-induced changes.[4, 5]

The present study expands upon these findings by examining the effects of MPD on both vesicle populations isolated from lysates of rat striatal synaptosomes. Synaptic vesicles containing the VMAT-2 are unequally distributed among the two vesicle populations with the membrane-associated vesicle fraction containing the majority of the VMAT-2 isolated from the synaptosome (Table 1). The results presented in Table 2 confirmed previous findings that a single injection of MPD (40 mg/kg, s.c.,; a dose used previously to investigate MPD-induced vesicular trafficking[4, 6]) redistributes VMAT-2 within nerve terminals away from the synaptosomal membranes and to the cytoplasm, as assessed 1 h after treatment. This MPD-induced redistribution of vesicles to the cytoplasm results in an increase in DA transport in the cytoplasmic vesicle fraction, as shown in Table 2. Interestingly, MPD also kinetically upregulates the decreased number of VMAT-2 remaining in the membrane-associated vesicle fraction such that DA transport in this vesicle fraction is increased as well (Table 2).

These MPD-induced increases in DA transport in the cytoplasmic and membrane-associated vesicle fractions have several interesting functional consequences. The increase in DA transport (caused by vesicle trafficking in the cytoplasmic vesicles and by kinetic upregulation of VMAT-2 in the membrane-associated vesicles) results in an increase in the DA content of both vesicle fractions with no change in whole striatal tissue DA content (Table 3). By increasing vesicular DA transport velocities, MPD administration thus results in a redistribution of DA within the striatum from the cytoplasm and into the vesicles.

One exciting therapeutic implication of these data relates to the neurotoxic effects of the psychostimulant, methamphetamine. Methamphetamine administration produces aberrant cytoplasmic DA accumulation and the subsequent formation of DA-associated reactive oxygen species, thus leading to long-term damage in both humans and animal models.[14-20] The data presented here reveal that MPD administration promotes the sequestration of cytoplasmic DA into both cytoplasmic and membrane-associated synaptic vesicles. These

findings likely underlie the ability of *in vivo* MPD post-treatments to protect against methamphetamine-induced neurotoxicity in an animal model.11 Abnormal DA disposition also likely contributes to the development of Parkinson's disease,14, 21 and MPD-induced increases in vesicular DA transport and DA sequestration may afford protection in this disease state as well. Considering this, it is noteworthy that MPD treatment improves gait and motor function22–24 as well as cognitive function23, 25 in humans with Parkinson's disease.

Another functional consequence of the MPD-induced increases in vesicular DA transport and DA content is an MPD-induced increase in the speed and extent of stimulated DA release from striatal suspensions (Table 4). Because both the amount of vesicular DA content and the speed of neurotransmitter release can influence receptor activation,26 these findings suggest the important therapeutic implication that MPD treatment influences quantal synaptic transmission in the striatum by increasing the rate at which DA receptors are exposed to DA, and perhaps the magnitude and/or duration of this effect. Taken together, these data show that the trafficking, DA sequestration function, DA content, and exocytotic DA release function of synaptic vesicles can all be pharmacologically manipulated by *in vivo* MPD treatment. These findings may provide important insights useful for understanding and treating disorders involving abnormal DA transmission including drug abuse, Parkinson's disease, and attention-deficit hyperactivity disorder.

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TABLE 1

Relative distribution of striatal VMAT-2 protein in the cytoplasmic and membrane-associated vesicle subcellular fractions as measured by VMAT-2 immunoreactivity and [^{3}H]DHTBZ binding.[a]

	VMAT-2 immunoreactivity[b]	[^{3}H]DHTBZ binding[c]
cytoplasmic vesicles	32 ± 3%	25 ± 6%
membrane-associated vesicles	68 ± 8%	75 ± 6%

[a] Values are expressed as the mean ± SEM of the percent of total striatal VMAT-2 protein isolated from rat striatal synaptosomes.

[b] N = 4.

[c] N = 6.

TABLE 2

MPD administration alters VMAT-2 immunoreactivity and DA transport velocities in the cytoplasmic and membrane-associated vesicle subcellular fractions.[a]

	VMAT-2 immunoreactivity[b]		DA transport velocity[c]	
	saline	MPD	saline	MPD
cytoplasmic vesicles	174 ± 9	304 ± 9*	2.4 ± 0.3	3.6 ± 0.2*
membrane-associated vesicles	222 ± 13	126 ± 6*	0.20 ± 0.03	0.52 + 0.02*

[a] Rats received a single administration of MPD (40 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) and were killed 1 h later.

[b] Mean ± SEM of band density in arbitrary units, N = 4.

[c] Mean ± SEM of DA transport velocity in units of fmol of DA/(s × μg protein), N = 4. Transport velocities were measured at 600 and 2000 nM DA (i.e., DA concentrations that approximate the K_m in each vesicular fraction6) in the cytoplasmic and membrane-associated vesicle fractions, respectively.

* Indicates a statistical difference, $P < 0.05$ via a *t*-test, between saline and MPD-treated groups.

TABLE 3

MPD administration alters DA content in the cytoplasmic and membrane-associated vesicle subcellular fractions.[a]

	DA content[b]	
	saline	**MPD**
cytoplasmic vesicles	15.0 ± 0.7	46 ± 2*
membrane-associated vesicles	42 ± 2	81 ± 2*
whole striatum	8826 ± 1312	9240 ± 621

[a] Rats received a single administration of MPD (40 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) and were killed 1 h later.

[b] Mean ± SEM of DA content in units of ng of DA/striatal g wet weight, N = 6.

* Indicates a statistical difference, $P < 0.05$ via a *t*-test, between saline and MPD-treated groups.

TABLE 4

MPD administration alters K^+-stimulated DA release from striatal suspensions.[a]

DA release velocity[b]		magnitude of DA release[c]	
saline	**MPD**	**saline**	**MPD**
0.9 ± 0.2	2.0 ± 0.2*	6 ± 1	14 ± 2*

[a] Rats received a single administration of MPD (40 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) and were killed 1 h later.

[b] Mean ± SEM of DA release velocity in units of nmol of DA/(s × striatal g wet weight), N = 3.

[c] Mean ± SEM of DA release magnitude in units of nmol of DA/striatal g wet weight, N = 3.

* Indicates a statistical difference, $P < 0.05$ via a *t*-test, between saline and MPD-treated groups.