Role of Monoamine Transporters in Mediating Psychostimulant Effects

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ABSTRACT

Monoamine transporters such as the dopamine (DA) transporter (DAT) and the vesicular monoamine transporter-2 (VMAT-2) are critical regulators of DA disposition within the brain. Alterations in DA disposition can lead to conditions such as drug addiction, Parkinson's disease, and schizophrenia, a fact that underscores the importance of understanding DAergic signaling. Psychostimulants alter DAergic signaling by influencing both DAT and VMAT-2, and although the effects of these drugs result in increased levels of synaptic DA, the mechanisms by which this occurs and the effects that these drugs exert on DAT and VMAT-2 vary. Many psychostimulants can be classified as releasers (ie, amphetamine analogs) or uptake blockers (ie, cocaine-like drugs) based on the mechanism of their acute effects on neurotransmitter flux through the DAT. Releasers and uptake blockers differentially modulate the activity and subcellular distribution of monoamine transporters, a phenomenon likely related to the neurotoxic potential of these drugs to DAergic neurons. This article will review some of the recent findings whereby releasers and uptake blockers alter DAT and VMAT-2 activity and how these alterations may be involved in neurotoxicity, thus providing insight on the neurodegeneration observed in Parkinson's disease.

KEYWORDS: dopamine transporter, vesicular monoamine transporter-2, amphetamine, cocaine, methylphenidate, Parkinson's disease

INTRODUCTION

Dopamine (DA) is a monoamine neurotransmitter important for many physiological processes such as motor movement, reward, motivation, and cognition. Accordingly, DAergic dysfunction can lead to a variety of disease states including drug addiction, Parkinson's disease, and schizophrenia. Consequently, appreciation of regulatory mechanisms relevant to the functioning of DA systems is critical

to the elucidation of the etiology of these disorders and the development of more effective therapeutics.

Under normal physiological conditions, the DA transporter (DAT) and vesicular monoamine transporter-2 (VMAT-2) are key components in the regulation of DA disposition in the synapse and cytosol. Following vesicular DA release into the synapse, the DAT transports DA from the perisynaptic area into the cytosol of the presynaptic nerve terminal of DA neurons. The VMAT-2 transports cytosolic and newly synthesized DA into synaptic vesicles. Sequestration of DA into these vesicles makes the transmitter available for synaptic release in response to appropriate stimulation. It also provides an environment to protect against the intracellular production of reactive oxygen species that can result from DA oxidation.

Psychostimulants are potent modulators of DAergic signaling; therefore, it is not surprising that alterations in DAT and VMAT-2 function are caused by psychostimulant treatment, and that unique effect profiles exist for different drug types in this pharmacological category. It has long been known that psychostimulants alter DAT function; however, more recently, novel responses of DAT to these drugs as well as unexpected effects on VMAT-2 function have been observed. For example, our group and other researchers have recently observed heretofore unreported effects of the psychostimulants on monoamine transporters that help distinguish between 2 principal types of psychostimulants and provide insight into the physiological regulation of these proteins and their potential roles in both etiology of pathology and therapeutic targets. This review will focus on some of the recent findings elucidating mechanisms whereby amphetamine- and cocaine-like drugs alter DAT and VMAT-2 function. In addition, the clinical relevance of such alterations will be discussed.

PSYCHOSTIMULANTS AND MECHANISMS OF ACTION

Psychostimulants promote increased extracellular DA concentrations; however, there are 2 primary mechanisms by which these agents affect the DAT. Psychostimulants can be separated into "uptake blockers" and "releasers" based on the mechanism of their acute effects on neurotransmitter flux through the DAT. Although uptake blockers can have releasing properties^{1,2} and releasers may also have some uptake blocking ability,³ the general separation of drugs into

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these 2 classes helps to functionally distinguish the pharmacological profiles of some of the most commonly used psychostimulants.

In addition to effects on the DAT, other researchers^{4,5} and we⁶⁻⁸ have reported data indicating that uptake blockers and releasers differentially regulate the VMAT-2. These changes are associated with a redistribution of VMAT-2, and presumably VMAT-2-containing vesicles, within striatal pre-synaptic nerve terminals. Through the use of DA receptor agonists and antagonists, it is clear that this psychostimulant-induced vesicular redistribution is mediated by DA receptors. However, DA receptor-mediated vesicular trafficking is not sufficient to explain all of the psychostimulant-induced vesicular trafficking, suggesting that DA receptor-independent pathways also contribute to these psychostimulant effects.

UPTAKE BLOCKERS

Cocaine and methylphenidate (MPD) are among the best-characterized uptake blockers with respect to effects on DAT and VMAT-2. Cocaine and MPD bind common sites on the DAT⁹ and in most cases, their mechanisms of action on DA systems appear to be similar. However, with respect to VMAT-2, differences have been observed and will be discussed below.

DAT

Cocaine and MPD are classified as DA uptake blockers because their primary mechanism of action is by directly binding and inhibiting the transport of DA through the

DAT.¹⁰ Blockade of DAT activity leads to increased extracellular DA levels and is not associated with selective long-term toxicity to the nigrostriatal DA pathway.¹¹ Of interest, blockade of DAT by cocaine leads to a rapid increase in DA uptake in synaptosomes prepared from treated rats,¹² a preparation from which the drug has been presumably been washed out. Perhaps this occurs via enhanced recruitment of DATs to the plasma membrane.¹³ These acute increases in DA uptake and plasmalemmal surface expression, observed in rodents and cell lines, respectively, after cocaine administration likely represent efforts to maintain normal synaptic DA functions. In humans who have repeatedly increased synaptic DA levels through the use of cocaine, increased DAT function is also observed, as assessed in synaptosomes from cryoprotected human brain.¹⁴ The combination of an initial DAT blockade and a subsequent increase in DA uptake could contribute to the development and expression of cocaine addiction. It is possible that an overabundance of extracellular DA during DAT blockade triggers this compensatory increase in DAT activity, which would ultimately produce a deficit in extracellular DA, perhaps contributing to drug dependence.

VMAT-2

In addition to blocking DAT, cocaine and MPD administrations alter the subcellular localization of VMAT-2-containing synaptic vesicles (Figure 1).^{7,8} Even though distinct patterns are observed with differential centrifugation techniques, suggesting that trafficking is readily regulated and likely has important functional consequences,^{7,8,15} it is unclear precisely which pool(s) of synaptic vesicles

Figure 1. Western blots demonstrating that cocaine redistributes VMAT-2 immunoreactivity from the synaptosomal membrane to the cytoplasm. Rats received a single injection of saline vehicle (1 mL/kg, intraperitoneally [ip]) or cocaine (30 mg/kg, ip) and were killed 1 hour later. Immunoreactivity was assessed as described by Riddle et al.⁷ Columns represent the mean optical density, and error bars represent the SEM of determinations in 6 treated rats. *Values for cocaine-treated rats that are significantly different from saline-treated controls; $P \leq .05$.

are altered as a consequence of exposure to these DAT inhibitors.

Although precise details are lacking, it appears that increased extracellular DA caused by the uptake blockers causes the redistribution of VMAT-2-containing vesicles from a plasmalemmal membrane fraction to a nonmembrane associated (presumably cytosolic) fraction because cocaine-induced vesicular trafficking can be blocked with DA D2 receptor antagonists.¹⁶ In contrast, MPD-induced trafficking involves both D2 and D1 receptors since D1 and D2 antagonists are required to block the effects of MPD.⁸ Consistent with a role for D2 receptors in these effects caused by DAT inhibitors is the finding that administration of D2 agonists causes vesicle trafficking in a manner similar to uptake blockers.^{8,17-19} This finding suggests that in response to DAT blockade, the increased extracellular concentrations of DA in the synapse stimulates DA receptors causing vesicles to move into the cytosol. Implications of vesicular trafficking into the cytosol will be discussed subsequently.

RELEASERS

Amphetamine analogs such as amphetamine, methamphetamine (METH), and 3,4-methylenedioxymethamphetamine (MDMA) are classified as “releasers.” Releasing drugs such as these likely promote DA release by disrupting vesicular pH gradients allowing vesicular DA to redistribute into the cytoplasm.^{20,21} Subsequently, as cytoplasmic DA levels rise, DA exits the neuron via reverse transport and/or channel-like activity of the DAT,^{22,23} leading to a dramatic increase in synaptic DA levels.

DAT

In rats, a single high-dose injection (10 mg/kg) of METH rapidly (within 1 hour) and reversibly decreases the amount of DA taken up into synaptosomes prepared from treated rodents.²⁴ Data from cell lines expressing the DAT demonstrate that exposure to amphetamine rapidly reduces plasma membrane-associated DAT, an effect likely representing a significant shift of this protein to the cytosolic fraction.²⁵ For in vivo relevance, it is difficult to extrapolate the time course of DA release through the DAT and a reduction of DAT on the cell surface; however, releasing drugs probably promote initial DA release followed by a removal of DAT from the cell surface.

The effects of releasing drugs become more complicated with higher doses (such as 4×10 mg/kg/injection of METH at 2-hour intervals) that cause persistent deficits in striatal DA systems. As with a single injection of METH, multiple high-dose administrations cause a rapid (within 1 hour after final METH injection) decrease in DAT activity; however, this decrease in DAT is substantially greater and may be

linked to persistent DAergic deficits. Although the mechanisms of releaser-induced toxicity is not completely understood, DA, hyperthermia, and oxygen radicals contribute to this phenomenon.²⁶

In addition to releaser-induced changes in DAT activity, our laboratory has recently demonstrated physical alterations in DAT caused by high doses of these drugs. More specifically, neurotoxic regimens of METH induce DAT complex formation.²⁷ At present, it is unclear whether these complexes are homomeric or heteromeric at their site of production, but these protein complexes only occur when neurotoxic regimens of METH are administered. These complexes appear to be linked to toxicity as their formation is dependent on DA, hyperthermia, and reactive species,²⁸ requisite factors for the METH-induced persistent DA deficits in the striatum. The functional consequences of METH-induced DAT complex formation remain to be determined.

VMAT-2

Like the uptake blockers, releaser psychostimulants appear to alter the subcellular localization of VMAT-2-containing synaptic vesicles. As with uptake blockers, it remains unclear which precise pool(s) of synaptic vesicles are altered and to what cellular location(s) they traffic; however, releaser drugs at high doses appear to redistribute vesicles from the cytoplasm to a subcellular region not retained in a synaptosomal preparation (Figure 2).⁷ Of interest, similar to uptake blockers, these VMAT-2-related effects are also prevented by pretreatment with D2 antagonists,⁶ which demonstrates that D2 activation is critical for releaser-induced vesicular trafficking. Thus D2 activation is required, but releasers employ heretofore unidentified mechanisms that result in vesicular trafficking in a manner different (and perhaps “opposite” with respect to the cytoplasmic pool) than that observed with D2 receptor agonists.

The effect of releaser psychostimulants to cause neurotoxicity may be related to the effect of these drugs on vesicular trafficking. The basis for this possibility is that neurotoxic effects of releasers likely require buildup of DA in the cytosol.²⁹ Under normal circumstances, VMAT-2 sequesters cytosolic DA, thus preventing the cytotoxic effects of oxidized DA. However, when exposed to releasers, the ability of VMAT-2 to sequester DA is compromised, allowing DA to be redistributed from inside the synaptic vesicle into the cytoplasm.²¹ In addition, it is possible that after redistribution of DA into the cytosol, releasers cause vesicles to be moved far from the site of leakage, increasing the duration of cytosolic DA buildup. With this in mind, experiments were designed to determine if the effect of the uptake blocker, MPD, could protect against METH-induced toxicity.¹⁵ Theoretically, if METH caused DA to leak into the cytoplasm and then removed VMAT-2-containing

Figure 2. Western blots demonstrating that METH redistributes VMAT-2 immunoreactivity from the cytoplasm to a location not retained in a synaptosomal preparation. Rats received multiple high-dose injections of METH (4×10 mg/kg, subcutaneously [sc], at 2-hour intervals) or saline vehicle (1 mL/kg, sc per injection) and were killed 1 hour after the final injection. Immunoreactivity was assessed as described by Riddle et al.⁷ Columns represent the mean optical density, and error bars represent the SEM of determinations in 6 treated rats. *Values for METH-treated rats that are significantly different from saline-treated controls; $P \leq .05$.

vesicles from the cytoplasm, MPD could protect against toxicity by trafficking vesicles into the cytoplasm to sequester the cytosolic DA. Indeed, posttreatment with methylphenidate of animals that received a neurotoxic dosing regimen of METH protected against METH-induced neurotoxicity. These data are consistent with previous findings that posttreatment with another uptake inhibitor, amfonelic acid, protects against the persistent DA deficits caused by METH.³⁰

DISCUSSION

During normal DAergic neurotransmission, the DAT regulates extracellular concentrations of DA, while VMAT-2 directly regulates cytosolic concentrations of DA and indirectly regulates extracellular DA by determining how much DA is released from each vesicle in response to action potential-linked release. It is likely that DA receptors allow the cell to monitor the levels of extracellular DA and provide a feedback mechanism to regulate DAT and VMAT-2 function, permitting the cell to respond to varying conditions. However, because of the reactive nature of DA, abnormal modifications in normal DAergic signaling and regulations may cause drastic consequences that can lead to damage of DA cells.

A common feature of most psychostimulants is that their administration increases synaptic DA concentrations. Psychostimulants achieve this primarily by their effects on DAT and/or VMAT-2 function; however, the mechanisms by which subclasses of psychostimulants (ie, uptake blockers and releasers) act are distinct and as a result produce impor-

tant physiological and functional differences. For example, uptake blockers cause little or no persistent DA deficits, whereas releasers (at high doses) can cause persistent deficits in monoaminergic neurons. The mechanistic differences between uptake blockers and releasers provide clues on how neurotoxicity occurs and also suggest potential targets for therapeutic interventions. This is of great importance for degenerative diseases such as Parkinson's disease, since the cause of the age-related decline in nigrostriatal DAergic nerve terminals associated with this degenerative condition has not been fully elucidated. The selective toxicity of some releasers to specific DAergic neurons allows researchers the means to discover why and how DAergic neurodegeneration in vulnerable systems occurs and provides models to test ways to prevent it. Insights into DAergic neurotoxicity have been gained by advances in our understanding of the "opposite" nature of uptake blockers versus releasers. The releaser, METH, is neurotoxic to nigrostriatal DA neurons when administered at high doses, the same neurons that are lost in Parkinson's disease. Perhaps treatments that prevent METH toxicity have the potential to be useful in the treatment of Parkinson's disease; thus uptake blockers (and perhaps D2 agonists) are protective against METH-induced DA neurodegeneration and therefore should be considered as candidates for slowing the progression of Parkinson's disease.

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