Neuropharmacology 56 (2009) 133–138

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Review

Psychostimulant-induced alterations in vesicular monoamine transporter-2 function: Neurotoxic and therapeutic implications

Annette E. Fleckenstein, Trent J. Volz, Glen R. Hanson*

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

ARTICLE INFO

Article history:
Received 29 April 2008
Received in revised form 2 July 2008
Accepted 2 July 2008

Keywords:
VMAT-2
Dopamine transporter
Monoamine transporters
Methylphenidate, Methamphetamine
Dopamine receptors
Drug abuse
Parkinson's disease
Cocaine
Dopamine neurotoxicity
Lobeline
Monoamine vesicles

ABSTRACT

The vesicular monoamine transporter-2 (VMAT-2) is an important regulator of intraneuronal monoamine concentrations and disposition as this protein sequesters critical cytoplasmic monoaminergic transmitters and contributes to their subsequent exocytotic release. This review primarily discusses the impact of psychoactive drugs (including those with abuse potential) on dopamine (DA)-related VMAT-2 and its function. In particular, the different responses by DA-related VMAT-2 and associated vesicles to plasmalemmal uptake blockers like methylphenidate and releasers like methamphetamine are presented. Recent preclinical findings suggest that vesicular transporter systems are highly regulatable, both by changes in localization as well as alterations in the kinetics of the VMAT-2 protein. The capacity for such shifts in VMAT-2 functions suggests the presence of physiological regulation that likely influences the activity of DA systems. In addition, these findings may contribute to our understanding of the pathogenesis of a variety of DA-related disorders such as substance abuse and Parkinson's disease and also suggest new therapeutic targets for treating such diseases.

1. Introduction

The vesicular monoamine transporter-2 (VMAT-2) is an important regulator of intraneuronal monoamine concentrations and disposition as this protein sequesters cytoplasmic dopamine (DA), 5-hydroxytryptamine (5HT, serotonin), and norepinephrine within synaptic vesicles thus contributing to subsequent exocytotic release. This review focuses primarily on VMAT-2 and vesicles found in dopaminergic neurons, as the majority of preclinical studies involving the impact of *in vivo* drug administration on this transporter protein have focused on these neurons.

Historically, many investigators have utilized VMAT-2 levels as a marker of dopaminergic neuronal integrity. The validity of this approach is supported by findings of decreases in VMAT-2 ligand binding site density and/or immunoreactivity in patients with Parkinson's disease (Wilson et al., 1996b; Frey et al., 1996; Miller et al., 1999), a disorder well established to result from a loss of nigrostriatal dopaminergic neurons. Interestingly and perhaps owing to a lesser severity of insult, VMAT-2 density data following chronic abuse of an agent demonstrated pre-clinically to cause persistent dopaminergic deficits, methamphetamine (METH), have been more equivocal. In particular, a loss of VMAT-2 density has been observed in some (Johanson et al., 2006) but not all (Wilson et al., 1996a) human studies.

In addition to the studies involving human subjects described above, numerous preclinical studies have assessed the long-term impact of administering drug treatments demonstrated to cause long-term dopaminergic deficits, including toxins believed to model some aspects of Parkinson's disease (e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, or 6-hydroxydopamine) as well as high-doses of METH. In contrast, more recent preclinical studies have focused on the short-term regulation of VMAT-2 and associated vesicles. These investigations of the acute impact of drugs, particular psychostimulants, on VMAT-2 and associated vesicles have extended our understanding that changes in VMAT-2 activity and levels, especially those occurring rapidly after drug treatment, may reflect factors beyond simply loss of dopaminergic nerve terminals. Instead and like other monoamine transporters, these studies have revealed that the VMAT-2 and associated vesicles are dynamic and highly modifiable targets of pharmacological manipulations and have intriguing therapeutic potential. This paper reviews the recent research that has led to these conclusions.

* Corresponding author. Tel.: +1 801 581 3174; +1 801 585 5111.
E-mail address: glen.hanson@hsc.utah.edu (G.R. Hanson).

0028-3908/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2008.07.002

2. Differences among VMAT-2-containing vesicles

There are likely several populations of VMAT-2-containing vesicles found in neurons, including those of the ready-releasable, recycling, resting, and/or reserve pools (for review, see Rizzoli and Betz, 2005; Sudhof, 2000). For the purpose of this review, VMAT-2-containing vesicles (obtained principally from dopaminergic neurons from rat striatal tissues) have been divided into two types: those that co-fractionate with synaptosomal membranes after osmotic lysis, and those that do not (for details on the osmotic lysis procedure, see Volz et al., 2007 and references contained therein). The VMAT-2 proteins that co-fractionate with synaptosomal membranes as well as the plasmalemmal membrane marker, Na^+/K^+-ATPase, the DA transporter (DAT), and the readily releasable/active zone marker, piccolo (Volz et al., 2007), are defined for purposes of this review as VMAT-2M. Those that do not co-fractionate with these membranes and associated markers are referred to as cytoplasmic VMAT-2 (VMAT-2C). Noteworthy, there is no evidence to date suggesting that the VMAT-2C and VMAT-2M are different proteins; however, recent data demonstrate dramatic differences in the kinetics of these transporters and associated vesicles.

Purified VMAT-2-containing vesicles have been studied by several investigators including Dwoskin and co-workers (Teng et al., 1997), who demonstrated using electron microscopy that the predominant structures in their vesicle preparation (presumably containing the VMAT-2C) were plain spheroid or ellipsoid and approximately 50 nm in diameter. Later studies by Volz et al. (2006a) involving rotating disk electrode voltammetry and using a similar subcellular fractionation procedure containing VMAT-2C demonstrated that uptake into these vesicles is ATP- and temperature-dependent, and that initial velocities of DA uptake display Michaelis–Menten kinetics.

In contrast to the effects presented for VMAT2C, DA transport via the VMAT-2M does not obey Michaelis–Menten kinetics as is common for monoaminergic transporters. Instead, the uptake profile for VMAT-2M is sigmoidal, with a Hill coefficient of approximately 4.5 (Volz et al., 2007). The sigmoidal nature of this profile has the unique potential of permitting substantial adjustment in transport rate over the concentration range spanning its "steepest" portion. Noteworthy, DA sequestration into VMAT-2M-associated vesicles in the presence of high concentrations of DA is greater in total capacity than in VMAT-2C-associated vesicles. This capacity permits speculation that at least a subpopulation of the VMAT-2M-associated vesicles may serve as a "DA sink" to capture DA and thus prevent its cytoplasmic accumulation. This is relevant, as aberrant DA sequestration leads to reactive species generation and likely contributes to the long-term damage caused by high-dose METH treatment (see discussion below). Thus, the differential kinetic profiles of these two populations of VMAT-2 and related vesicles not only suggest unique physiological roles for these systems, but that these transporters might also provide distinct therapeutic opportunities.

3. VMAT-2 as an index of neurotoxicity following psychostimulant treatment

It is well established in preclinical models that multiple high-dose injections of METH, administered in patterns that mimic "bingeing" in humans, causes long-lasting decreases in the activity of the rate-limiting enzyme in DA synthesis, tyrosine hydroxylase (TH) (Kogan et al., 1976; Hotchkiss et al., 1979; Hotchkiss and Gibb, 1980; Morgan and Gibb, 1980), the function and binding of the DAT (Wagner et al., 1980; Eisch et al., 1992; Guilarte et al., 2003) and levels of associated transmitters (Seiden et al., 1976; Wagner et al., 1980). As these changes can persist for months, these deficits likely reflect destruction of corresponding DA axons and/or terminals.

In addition to the persistent alterations noted above, preclinical studies have focused on the long-term impact of METH on VMAT-2. For example, Guilarte et al. (2003) have demonstrated that METH administration decreases striatal VMAT-2 ligand binding, as assessed 14 days after treatment. Similarly and as noted above, Johanson et al. (2006) demonstrated modest decreases in VMAT-2 binding in positron emission tomography studies. In contrast, Kish and co-workers (Wilson et al., 1996a) found diminished levels of three DA terminal markers (DA, TH and the DAT) in post-mortem striatum of chronic METH users with total VMAT-2 levels being normal. While there may be multiple explanations for this discrepancy, it is possible that total VMAT-2 levels, as detected in this latter study, may not necessarily reflect the integrity of the system and that other factors may contribute to unexpected alterations in its expression. Although the specific explanation for this finding is unclear, the ability to pharmacologically manipulate the state of VMAT-2 and its associated function, as described below, may contribute.

4. Acute effects of DA releasers on VMAT-2C

Early evidence that alterations in VMAT-2 levels may reflect processes unrelated to DA terminal loss comes from studies wherein VMAT-2-containing vesicles are separated by subcellular fractionation (vs. assessing the total content or density of VMAT-2 as illustrated in studies cited above). In particular, Sonsalla and co-workers (Hogan et al., 2000) reported that METH treatment decreased both binding of the VMAT-2 ligand, dihydrotetrabenazine (DHTBZ), and DA uptake, as assessed in a purified cytoplasmic vesicle preparation 24 h after drug treatment. However and importantly, no significant loss of DHTBZ binding was observed in whole striatal homogenates at this time point. These data were the first to highlight the "disparity between homogenates and vesicle preparations" with regard to VMAT-2 after METH treatment.

One possible explanation for this disparity reported by Hogan et al. (2000) is that VMAT-2C was redistributed within nerve terminals after treatment with the METH. This possibility is supported by the work of Brown et al. (2000) who observed that repeated, high-dose administrations of METH to rats rapidly (within 1 h) decreased vesicular DA uptake, as assessed in VMAT-2C-associated vesicles purified from striata of treated rats. This effect on uptake was largely attributable to effects on dopaminergic (vs. serotonergic) nerve terminals, because destruction of the serotonergic projections to the striatum did not alter the effect on vesicular DA transport in this study. Later, Riddle et al. (2002) reported that repeated, high-dose METH injections rapidly redistribute rat striatal VMAT-2C immunoreactivity, and presumably associated vesicles, to a location not retained in the preparation of the synaptosomes. This decrease occurs concurrent with a METH-induced reduction in vesicular DA content in the VMAT-2C-associated fraction (Sandoval et al., 2003). Subsequently, Yamamoto and co-workers (Eyerman and Yamamoto, 2005; Tata et al., 2007) also demonstrated that METH administration decreased VMAT-2 immunoreactivity 1 and 24 h after treatment in a similar preparation.

Of interest are findings that exposure to chronic unpredictable stress can impact both VMAT-2 levels and the persistent dopaminergic deficits caused by METH treatment (Tata et al., 2007). In particular, pre-exposure to the stressors augmented METH-induced decreases in VMAT-2 immunoreactivity, as assessed in synaptosomal, purified vesicular (presumably cytoplasmic) and membrane-associated tissue fractions prepared from rat striata. Unpredictable stress also worsened both METH-induced