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Review

The vesicular monoamine transporter 2: An underexplored pharmacological target

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ABSTRACT

Active transport of neurotransmitters into synaptic vesicles is required for their subsequent exocytotic release. In the monoamine system, this process is carried out by the vesicular monoamine transporters (VMAT1 and VMAT2). These proteins are responsible for vesicular packaging of dopamine, norepinephrine, serotonin, and histamine. These proteins are essential for proper neuronal function; however, compared to their plasma membrane counterparts, there are few drugs available that target these vesicular proteins. This is partly due to the added complexity of crossing the plasma membrane, but also to the technical difficulty of assaying for vesicular uptake in high throughput. Until recently, reagents to enable high throughput screening for function of these vesicular neurotransmitter transporters have not been available. Fortunately, novel compounds and methods are now making such screening possible; thus, a renewed focus on these transporters as potential targets is timely and necessary.

1. Vesicular monoamine transporters: overview

The vesicular monoamine transporters (VMATs) are part of the Major Facilitator Superfamily (MFS) and the solute carrier family of transporters (SLC) subfamily. Like other MFS family members, VMATs contain 12 transmembrane spanning domains, with cytosolic C- and N-terminals and large glycosylated intravesicular loops. Members of the SLC18 subfamily are Drug:H^+ antiporters; these transporters exchange intravesicular protons for extravesicular neurotransmitter.

The vesicular monoamine transporters are essential for proper monoaminergic neurotransmission, which requires the sequestration of transmitter into synaptic vesicles by VMAT for subsequent Ca^{2+}-stimulated exocytotic release (Sudhof, 2004). This critical function is accomplished by the secondary active transport of neurotransmitters against their concentration gradient into synaptic vesicles (Liu and Edwards, 1997). As proton exchangers, VMATs rely on the proton gradient generated by the V-type ATPase across the vesicular membrane and the import of chloride via the ClC-3 chloride channels. The high concentration of intravesicular protons allow for the exchange of two protons for each molecule of neurotransmitter transported (Knoth et al., 1981; Parsons, 2000). VMATs primarily transport monoamines (dopamine, serotonin, norepinephrine and histamine), but also sequester toxicants into vesicles, shunting them away from cytosolic sites of action (Liu et al., 1992a,b; Erickson et al., 1992; Kariya et al., 2005; Sulzer et al., 2005; Caudle et al., 2007; Thomas et al., 2008; Gainetdinov et al., 1998). This is particularly interesting given sequence homology between VMATs and the bacterial toxin extruding antiporters (TEXANs) (Schuldiner, 1995).

In mammals, there are two VMAT isoforms. VMAT1 (SLC18A1) is expressed exclusively in the periphery, with expression in the sympathetic nervous system, adrenal chromaffin cells, and endocrine/paracrine cells of the gut. VMAT2 (SLC18A2) has both peripheral (enteric nervous system, adrenal chromaffin cells, and endocrine cells of the stomach, and platelets) and central nervous system (all monoaminergic neurons of the brain) expression (Weihe et al., 1994). The transporters share common substrates with the exception of histamine, which is believed to be preferentially packaged by VMAT2.

2. Vesicular monoamine transporters in disease

Many neurological and psychiatric disorders can be linked to dysfunction of monoaminergic systems, including Parkinson's disease (PD), Huntington's disease, ADHD, dystonia, schizophrenia, addiction, and depression (Howell and Kimmel, 2008; Picconi et al., 2003; Russell, 2002; Schwartz et al., 2003; Song et al., 2012;

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Taylor et al., 2000). Although the origin of monoaminergic dysfunction varies, manipulation of vesicular function could be a useful target for modulating monoamine homeostasis. Data from our lab and others suggests that direct modification of monoamine vesicular function may be beneficial in a variety of disorders, either in isolation or in conjunction with existing therapies. For purposes of this review, we will focus on dopamine packaging by VMAT2 and PD, which has been the focus of work in our lab. Data from many labs have demonstrated that proper packaging of dopamine into vesicles is critical since cytosolic dopamine is neurotoxic. Cytosolic dopamine is metabolized by enzymatic deamination or broken down by autoxidation, producing reactive, harmful oxidative products (Alter et al., 2013; Eisenhofer et al., 2004; Burke et al., 2004; Rees et al., 2009; Wey et al., 2012; Goldstein et al., 2011; Zahid et al., 2011; Sulzer and Zecca, 1999). Efficient transport of dopamine by VMAT2 prevents accumulation of these toxic byproducts.

2.1. Toxicological disruption of vesicular transport

As explored in our recent review, "Vesicular Integrity in Parkinson's Disease," many insults, both environmental and genetic, that lead to PD converge on vesicle function (Alter et al., 2013). Several classes of environmental toxicants, including pesticides, polychlorinated biphenyls, and brominated flame retardants, have been associated with PD pathology (Alter et al., 2013; Hatcher et al., 2008; Caudle et al., 2012; Chaudhry et al., 2008). Epidemiological evidence linking these toxicants to disease risk is extensive (Tanner and Langston, 1990; Tanner and Aston, 2000; Tanner et al., 2011; Steenland et al., 2006; Semchuk et al., 1991, 1992; Ritz, 2000; Priyadarshi et al., 2000, 2001; Gatto et al., 2009; Elbaz et al., 2009; Ascherio et al., 2006). Additionally, mechanistic studies have demonstrated that these compounds exert selective toxicity to dopaminergic neurons via inhibition of synaptosomal and vesicular uptake of dopamine and resultant oxidative stress (Seegal et al., 1986, 1990, 2000; Sanchez-Ramos et al., 1998; Richardson and Miller, 2004; Richardson et al., 2006; Miller et al., 1999; Mariussen and Fonnum, 2001, 2003; Lee and La, 2004; Kitazawa et al., 2003; Fonnum et al., 2006, 2009; Caudle et al., 2005, 2006; Bradner et al., 2013; Bemis and Seegal, 2004).

2.2. In vitro and animal models of modified vesicular transport

Many studies in both in vitro and animal models have also demonstrated that unregulated cytosolic dopamine is neurotoxic (Graham et al., 1978; Benshachar et al., 1995; Hastings et al., 1996; Asanuma et al., 2003). In vitro experiments suggest that the relative vulnerability of dopamine neurons in PD may be mediated by cytosolic dopamine (Mosharov et al., 2009). Furthermore, mice that express DAT on non-dopaminergic striatal neurons, which lack VMAT2, take up dopamine into those neurons, but do not store it in vesicles, producing motor deficits and profound striatal neurodegeneration, accompanied by markers of increased dopamine oxidation (Chen et al., 2008). Additionally, transgenic mice with altered expression of VMAT2 have illustrated the critical nature of vesicular storage of dopamine for the integrity of the nigrostriatal system. VMAT2 knockout mice die soon after birth, while heterozygotes develop normally, but display increased sensitivity to amphetamine, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Gainetdinov et al., 1998; Takahashi et al., 1997; Wang et al., 1997. Mice that are hypomorphic for VMAT2 (~5% wild type expression) have been developed as a mouse model of PD (Caudle et al., 2007; Colebrooke et al., 2006; Taylor et al., 2009; Ulusoy et al., 2012). These mice develop normally, but undergo progressive nigrostriatal degeneration, α-synuclein accumulation, show markers of oxidative stress, and develop motor and nonmotor symptoms of PD when they express alpha-synuclein (Caudle et al., 2007; Colebrooke et al., 2006; Taylor et al., 2009).

In addition, genetic mutations linked to PD often affect synaptic vesicle function, leading to deficits in trafficking, transmitter storage and release. Alpha-synuclein has long been known to bind to phospholipids on the vesicle membrane (Jensen, 1998; Davidson et al., 1998; Jo et al., 2000; Perrin et al., 2000). While its function is unknown, genetic ablation of the synuclein genes increases dopamine release (Chandra et al., 2004; Senior et al., 2008). In addition, dopamine influences the utilization of alternate alpha-synuclein transcripts, resulting in changes in localization of alpha-synuclein (Rhinn et al., 2012). Fibrillization of alpha-synuclein is promoted by oxidized dopamine; in turn, fibrillar alpha-synuclein can permeabilize the vesicular membrane, leading to further increases in cytosolic dopamine and thus more oxidative stress, creating a positive feedback loop (Volles et al., 2001). Deficiency of PINK1 function reduces synaptic efficiency by immobilizing synaptic vesicles of the reserve pool (Morais et al., 2009). DJ-1 interacts with synaptic vesicle proteins such as synaptophysin and Rab3A and influences the expression of VMAT2 (Lev et al., 2013; Usami et al., 2011). Finally, DJ-1, PINK1 and parkin knockout mouse models all show substantial presynaptic deficits in dopamine release (Goldberg et al., 2005; Kitada et al., 2007, 2009a,b). Together, these data suggest that vesicular dysfunction is a convergence point for both genetic and environmental risk factors of PD.

3. Current therapeutics targeting VMAT2

Despite the recognized importance of the vesicle in dopaminergic disease, few FDA approved drugs directly and specifically target the vesicle. Two VMAT2 inhibitors, reserpine and tetrabenazine (TBZ), have demonstrated efficacy in the treatment of disease. Although other drugs, such as amphetamine and methylphenidate, are known to affect VMAT2 function, these drugs have a complicated pharmacology due to their interaction with plasmalemmal transporters and inhibition of neurotransmitter metabolism (Sulzer et al., 2005; Fleckenstein et al., 2007). As they do not exclusively act at VMAT2, we have focused on reserpine and TBZ, which specifically target the vesicle.

3.1. Reserpine

Reserpine, an alkaloid isolated from the Indian snakeroot *Rauwolfia serpentine*, was introduced to Western medicine in 1952, and was widely prescribed for its antihypertensive and antipsychotic properties (Furlenmeier et al., 1953). Despite these beneficial effects of reserpine, side effects were described as resembling a parkinsonian syndrome, with symptoms including depression, gastric dysmotility, and extrapyramidal symptoms (Richman and Tyhurst, 1955). Although the molecular target of reserpine was not identified for decades, researchers observed that reserpine depleted dopamine in biological tissue and caused parkinsonism in rats (Brodie et al., 1955; Pletscher et al., 1955). The later discovery that reserpine is an irreversible and non-specific VMAT1/2 inhibitor provided a mechanistic explanation for the effects of this compound (Liu et al., 1992a,b; Erickson et al., 1996). The anti-hypertensive effect results from VMAT2 inhibition in the sympathetic nervous system and chromaffin cells, reducing sympathetic tone and, in turn, reduced blood pressure (Chernow et al., 1984; Mahata et al., 1996). Despite its effectiveness for treating hypertension, the inhibition of VMAT2 within the CNS causes the aforementioned deleterious symptoms, including severe depression (Richman and Tyhurst, 1955; Freis, 1954). These effects are particularly problematic given the irreversible nature of the drug, since washout requires new

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