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Localization and Expression of VMAT2 Across Mammalian Species: A Translational Guide for Its Visualization and Targeting in Health and Disease

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Abstract

VMAT2 is the vesicular monoamine transporter that allows DA, NE, Epi, His, and 5-HT uptake into neurons and endocrine cells. A second isoform, VMAT1, has similar structure and function, but does not recognize histamine as a substrate. VMAT1 is absent from neurons, and its major function appears to be in endocrine cells, that is, enterochromaffin cells, which scavenge 5-HT, but not histamine, from dietary sources. This chapter provides an update on the neuroanatomical distribution of VMAT2 across various mammalian species, including human, primate, pig, rat, and mouse. When necessary, VMAT1 expression is provided as a contrast. The main purpose of this chapter is to allow clinicians, in particular endocrinologists and diagnosing neuroradiologists and neuropathologists, an acquaintanceship with the possibilities for VMAT2 as a target for *in vivo* imaging, and drug development, based on this updated information.

1. INTRODUCTION

VMAT1 and VMAT2 (SLC18A1 and SLC18A2, see Eiden, Schafer, Weihe, and Schutz, 2004) are the two vesicular monoamine transporters (VMATs) in mammals (Erickson, Eiden, & Hoffman, 1992; Liu et al., 1992). They are a part of a larger family of transporters, termed, by Schuldiner, TEXANs, for *toxin-extruding antiporter* system (Fig. 15.1; Schuldiner, Shirvan, & Linial, 1995). This designation reflects the ancestral function of exchanging protons for intracellular toxic metabolites in bacteria. It also provides a useful cellular metaphor for the actions of VMATs in mammalian cells, in that these transporters are required for the accumulation of monoamine transporters in secretory vesicles not only to make them available for exocytotic secretion but also to sequester and then extrude them from the cytoplasm, where their oxidation products may cause cumulative damage to the cell (Guillot & Miller, 2009). VMAT2 is responsible for the uptake and storage of the monoamines dopamine (DA), norepinephrine (NE), epinephrine (Epi), histamine (His), and 5-hydroxytryptamine (serotonin) (5-HT) in neurons of the central and peripheral nervous systems, endocrine cells, and tumors deriving from these cells. Several previous assumptions about VMAT2 expression in the brain and periphery have been importantly refined. A major assumption has been that VMAT2 expression in adult rodents accurately models its expression in juvenile and adult humans. Important exceptions are highlighted,

relevant in particular to the use of *in vivo* VMAT2 imaging with the high-affinity ligand tetrabenazine (TBZ) in estimation of beta cell mass in prognosis of human type I diabetes. A second assumption, that VMAT2 is coexpressed with VMAT1 in mammalian brain and therefore that TBZ imaging does not provide an accurate reflection of monoamine uptake capability in human brain, has also been shown to be inaccurate: adult human and rodent brain expresses exclusively VMAT2. A third assumption, that cholinergic neurons of the sympathetic nervous system controlling sweat production have equivalent adrenergic cofunctionality during certain periods of development, has likewise acquired important species-specific caveats. VMAT2 expression also forms the basis for a new categorization of aminergic neurons in the brain that reveals populations of nonclassical monoaminergic neurons of potentially novel function and targeting in pathophysiological conditions. Finally, the assumption that VMAT2 expression is “static” in neurons, and therefore acts as a biomarker for neuronal and endocrine cell number, and not functional status, is critically reviewed, and the implications for imaging of human disease using TBZ ligands are assessed. In summary, detailed neuroanatomical description of VMAT2 expression and localization provides a new basis for the rational use of TBZ in both basic and clinical studies of human disease involving monoamine physiological function, and dysfunction, in disease.

2. VMAT2 EXPRESSION IN THE DIGESTIVE SYSTEM

The major monoamine-containing cells of the digestive system include the neurons of the enteric nervous system, the enterochromaffin (EC) cells of the intestine, and enterochromaffin-like (ECL) cells of the stomach. EC cells express only VMAT1, and it has been hypothesized (Duerr et al., 1999) that this is related to the fact that VMAT1 does not transport His (Erickson, Eiden, Schäfer, & Weihe, 1995; Merickel & Edwards, 1995) and the need for selective uptake of serotonin, and not His, from the diet by these intestinal endocrine cells. The inability of VMAT1 to recognize and transport His correlates structurally with the inability of VMAT1 to bind TBZ, making the latter a specific ligand for VMAT2 (Varoqui & Erickson, 1997). The region of VMAT2 responsible for TBZ binding has been mapped with VMAT1/VMAT2 chimeric proteins (Peter, Vu, & Edwards, 1996). The ECL cells of the stomach express VMAT2 and do in fact synthesize and accumulate from the diet and store His. The high ratio of His to serotonin in ECL cells provides an experiment of nature as to the likely composition of monoamines in EC cells, where His uptake is excluded via expression of VMAT1. Consistent with this expression pattern, neuroendocrine tumors derived from the gut are found to express VMAT1 and contain 5-HT (Anlauf et al., 2003). In contrast, although human pancreatic beta cells express VMAT2, insulinomas or pancreatic endocrine tumors frequently lose VMAT2 expression (Anlauf et al., 2003). Thus, VMAT1/VMAT2 expression is becoming established as a routine marker for differential diagnosis of NET and PET versus other tumor types found in the gastroenteropancreatic tissues. VMAT2 expression is also high in mast cells, which accumulate both serotonin and His in variable ratios, and in corresponding mastocytomas arising in the gut and throughout the body (Anlauf et al., 2004, 2006).

Frey and coworkers first reported the imaging of VMAT2 via PET scanning (Frey et al., 1996), employing 11C-DHTBZ (dihydrotetrabenazine) as the PET ligand (Fig. 15.2). Earlier, VMAT2 expression had been reported in human pancreatic beta cells (Anlauf et al., 2003). The discovery of VMAT2 expression in endocrine pancreas occasioned considerable efforts to localize VMAT2 in the pancreas and to employ VMAT2 as a marker for beta cell mass in human patients (Bormans, 2010; Ichise & Harris, 2010; Kung et al., 2008; Normandin et al., 2012; Saisho et al., 2008). Unfortunately, some confusion arose as to the appropriateness of TBZ for beta cell imaging when attempts to optimize this procedure in rodent models led to conflicting results (Freeby et al., 2008; Ichise & Harris, 2010; Raffo et

al., 2008; Souza et al., 2006). We have since clarified these, to some extent, by reporting a survey of those mammalian species that as adults do or do not express VMAT2 and/or VMAT2 mRNA in beta cells of the pancreas (Fig. 15.3; Schafer et al., 2013).

VMAT2 neurons are found throughout the enteric nervous system, leading investigators to assume that in all species, these represented the well-known “serotonergic enteric nervous system” (Gershon, 1999). In fact, VMAT2 costaining with TH and tryptophan hydroxylase revealed that in addition to a subpopulation of serotonergic neurons, there is a substantial dopaminergic complement of intrinsic enteric neurons in the human gut (Fig. 15.4).

3. VMAT2 EXPRESSION IN THE RODENT AND PRIMATE BRAIN

VMAT2 is expressed in DA, NE, His, and 5-HT neurons in rodent and human brain and in mast cells resident in the CNS. So far, no obvious species differences in the distributions of VMAT2-positive cells in adult rodents and primates have been observed, except those noted for sudomotor neurons (see succeeding text). One species difference occurring in development should be noted however. VMAT2 is transiently expressed in thalamocortical afferents in rodent from embryonic day approx. E16 to P6 (Lebrand et al., 1998; Schütz, Schäfer, Eiden, & Weihe, 1998a) and has been postulated to act to scavenge 5-HT from the extracellular space (the neurons also possess the serotonin plasma membrane transporter SERT) (Cases et al., 1998; Lebrand et al., 1998). However, the analogous transient expression of the VMAT2-positive neuronal system in primates does not extend to the thalamocortical projections prominent in the rodent (Lebrand, Gaspar, Nicolas, & Hornung, 2006).

Over the past several decades, TH neurons lacking dopamine beta-hydroxylase (DBH) in the brain have been considered to be *de facto* dopaminergic. Closer examination, mainly by the laboratories of Ugrumov, Nagatsu, and our own, has revealed a plethora of neuronal subpopulations with various patterns of monoaminergic traits, including VMAT2 expression, that have allowed both recategorization and species comparison (see Fig. 15.5 and Balan et al., 2000; Ikemoto, Nagatsu, Nishimura, Nishi, & Arai, 1998; Kitahama et al., 1998; Komori, Fujii, & Nagatsu, 1991; Tashiro et al., 1989; Ugrumov, 1997, 2009). The so-called bi- and monoenzymatic neurons of the hypothalamus described by Ugrumov are thought either to synthesize DA directly or to synthesize L-Dopa (type 1 monoenzymatic lacking aromatic amino acid decarboxylase (AADC)) and transfer L-Dopa to type 2 monoenzymatic neurons that lack TH but express AADC. These neuronal populations have not yet been characterized in detail, with respect to their expression of VMAT2.

Pioneering work in visualizing VMAT2-positive nerve terminals with 11C-DHTBZ in the brains of Parkinson's, Huntington's, bipolar, and schizophrenic patients and normal aged and normal humans has been performed by Frey and Kilbourn and coworkers (Bohnen et al., 2006; Frey et al., 1996; Suzuki, Desmond, Albin, & Frey, 2001; Zubieta et al., 2000, 2001). These studies originally rested on the assumption that, unlike TH and other catecholaminergic enzymes shown to be dynamically regulated in monoamine terminals, VMAT2 was a relatively static protein, whose abundance should therefore be directly proportional to the number of monoaminergic nerve terminals in a given region of the brain. While this is probably largely the case, as shown by decreased TBZ image intensity in progressed PD, other workers have generated models that imply that TBZ labeling may be somewhat more complex and indeed potentially exploitable for study of drug abuse. Thus, Fleckenstein and coworkers have shown in animal models that VMAT2 may redistribute rather dramatically from the nerve terminal/storage vesicle under certain conditions (Brown, Hanson, & Fleckenstein, 2001). On the other hand, even though TBZ has a much higher affinity for VMAT2 than catecholamines (CAs), the high concentrations of the latter in

nerve terminals may potentially alter TBZ binding depending on amine concentrations in the vesicle (Kilbourn et al., 2010). The most dramatic example of CA/TBZ “interference” may be the adrenal medulla—although adrenomedullary VMAT2 protein levels are among the highest in the body, it is imaged poorly if at all by TBZ (Frey, personal communication). The high CA levels (Winkler, Apps, & Fischer-Colbrie, 1986) in chromaffin granules may be the explanation for this paradoxical observation.

It has been reported that VMAT1 is expressed in neurons of the human central nervous system (Lohoff, 2010); however, we have not been able to confirm these observations (Eiden & Weihe, 2011; Erickson, Schäfer, Bonner, Eiden, & Weihe, 1996). A similar situation is found in rodent brain—essentially all reserpine-sensitive biogenic amine accumulation in the mouse brain is abolished by deletion of the VMAT2 gene in the mouse (Fon et al., 1997), and nonneuronal VMAT expression in the brain seems to be confined to VMAT2 expression in occasional mast cells, as in peripheral tissues (Schütz et al., 1998a; Schütz, Schäfer, Eiden, & Weihe, 1998b).

4. VMAT2 EXPRESSION IN THE PERIPHERAL NERVOUS SYSTEM: SPECIES-SPECIFIC AND DEVELOPMENTAL PATTERNS

The presence or absence of VMAT2 in neurons with both noradrenergic and cholinergic traits can have profound effects on their transmitter functionality, and this can change dramatically in development and be dramatically different among mammalian species. As an example, Figs. 15.6 and 15.7 depict in schematic form data obtained via immunohistochemical staining for TH, AADC, DBH, VMAT2, choline acetyltransferase (ChAT), and vesicular acetylcholine transporter (VACHT) in sympathetic sudomotor neurons and at effector junctions in the heart and skin of rat, mouse, and primate (human and rhesus monkey) (Weihe et al., 2005). VACHT expression occurs early (prior to innervation of target organs such as the sweat gland) in stellate ganglion of all species (Schäfer, Schütz, Weihe, & Eiden, 1997; Weihe et al., 2005). This makes it unlikely that changes in the expression patterns in VACHT and TH and the species-specific expression of VMAT2 at the genomic level are determined by target tissue, albeit changes in protein/secretory vesicle transport, stability, or mRNA translation may well be determined by neuronal interaction with the sweat glands (see Francis & Landis, 1999, and references therein). Since changes in cholinergic/noradrenergic properties in cultured sympathetic neurons can be readily demonstrated to occur at the transcriptional level, caution in extrapolation from cell culture to *in situ* is highly appropriate (Apostolova & Dechant, 2009; Apostolova, Loy, Dorn, & Dechant, 2010). Differences in VMAT2 expression in adult mammalian tissues are even more dramatic and likely of great importance in the reassessment of human autonomic physiology and pharmacology, for example, in cardiac disease, hyperhidrosis (“adrenergic sweating”), and possibly altered thermo-regulation in human dysautonomias.

It has been mentioned that TBZ imaging of VMAT2 in the adrenal medulla *in vivo* is poor, perhaps because of the very high concentrations of CAs in adrenomedullary chromaffin granules (approximately 0.5 M) in the vicinity of the TBZ-binding site of VMAT2 in these storage vesicles. The chromaffin cell of the adrenal medulla also exemplifies an endocrine cell type in which VMAT1 and VMAT2 expression varies markedly among mammalian species. Rodent chromaffin cells express almost exclusively VMAT1, with VMAT2 apparently confined to occasional “paraganglionic”-type cells (Weihe, Schafer, Erickson, & Eiden, 1994), while primate adrenal medulla express both VMAT1 and VMAT2, apparently in all (both Epi- and NE-containing) chromaffin cells. Chronic immobilization stress in the rat causes a prompt and dramatically selective upregulation of VMAT2 protein expression in Epi-containing (PNMT-expressing) cells (Tillinger, Sollas, Serova, Kvetnansky, & Sabban,

2010). This VMAT1/VMAT2 “plasticity” across species and under various physiological conditions contrasts with VMAT1/VMAT2 expression in the digestive system. VMAT1 is invariably expressed in intestinal EC cells, and VMAT2 is invariably expressed in ECL cells of the oxyntic mucosa of the stomach, in all species examined (Erickson et al., 1996; Weihe et al., 1994).

5. TBZ AS A PHARMACOTHERAPEUTIC AGENT

Thus far in this chapter, we have focused on VMAT2 expression as a biomarker and TBZ as an exogenous agent for *in vivo* detection of that biomarker in diagnosis and understanding of human disease. However, VMAT2 has itself long been a drug target, for example, of the VMAT2 ligand and inhibitor, reserpine, in treatment of hypertension. In fact, the correlation of depression with reserpine treatment leading to discontinuation of its clinical use was at the same time one of the clinical mainstays of the monoamine hypothesis of depression leading to the development of monoamine reuptake inhibitors to treat depression (see Axelrod, 1986). TBZ itself has more recently become an FDA-approved therapeutic agent for symptomatic treatment of Huntington’s disease, which is characterized in part by striatal imbalance of dopaminergic and glutamatergic neurotransmission (Frank & Jankovic, 2010).

The levels of occupancy of VMAT2 by labeled TBZ at concentrations used in imaging studies are probably far too low to have clinically meaningful effects during imaging/diagnosis. Nevertheless, it is worth considering that at least in some disease states, imaging with ¹¹C- or ¹⁸F-TBZ analogues to determine the levels and state of activity of VMAT2 may provide specific indications for pharmacodynamic “fine-tuning” of its subsequent therapeutic use at doses that provide greater percent occupancy of sites occluding vesicular monoamine transport. A human genetic disease caused by a single amino acid substitution in VMAT2 (P→L in the linker region between transmembrane domains 9 and 10) has recently been reported (Rilstone, Alkhater, & Minassian, 2013). Pediatric patients homozygous for P→L allele exhibit developmental delays and profound hypokinesia similar to those with genetically determined AADC deficiency, while heterozygous carriers exhibit increased risk for depression. Amelioration of the movement disorder with the D3-selective DA agonist pramipexole suggests impairment of VMAT2 function manifests progressively as altered serotonergic/noradrenergic, and then dopaminergic, neurotransmission. Whether this presumptive gene dosage effect could be recapitulated by carefully modulated pharmacological dosing with TBZ in disorders associated with monoaminergic hypertransmission may be worthy of further consideration.

6. CONCLUSION

If VMAT2 is neither highly dynamic nor static in neurons, where does this leave the field in terms of its use in determining the erosion of VMAT2-expressing tissue mass in diseases such as Parkinson’s and type I diabetes? Certainly, the potential for dynamic transcriptional and posttranslational regulation of VMAT2 in otherwise healthy cells needs to be accounted for in some fashion to interpret potentially pathophysiological findings in VMAT2 imaging studies. Especially as regards the estimation of human beta cell mass in diabetes, the need for truly appropriate animal models to optimize imaging in humans is of paramount concern. Hopefully, a combination of VMAT2 postmortem Westerns, intelligent animal modeling, and DHTBZ imaging will optimize TBZ translation to human disease treatment and prognosis.

In retrospect, our original designation of VMAT2 as the “neuronal” and VMAT1 as the “endocrine” version of the VMAT was a simplification based on examination of rodent tissues (Weihe et al., 1994) and does not survive as a full accounting of mammals from mice

to humans. Rather, VMAT2 is frequently found in the endocrine tissues including the adrenal medulla, ECL cells, and in many, if not all, mast cells. VMAT1 could be more properly referred to as “restricted” in its distribution, especially from cells, such as EC cells, that require preferential accumulation of serotonin but reside in areas that may have high ambient levels of His, which would otherwise be accumulated by a transporter with a VMAT2-like substrate specificity profile.

ABBREVIATIONS

AADC	aromatic amino acid decarboxylase
CA	catecholamine
ChAT	choline acetyltransferase
DA	dopamine
DBH	dopamine beta-hydroxylase
DHTBZ	dihydrotetrabenazine
EC	enterochromaffin
ECL	enterochromaffin-like
Epi	epinephrine
His	histamine
5-HT	5-hydroxytryptamine (serotonin)
NE	norepinephrine
PET	positron emission tomography
SLC	solute carrier family
TBZ	tetrabenazine
TEXAN	toxin-extruding antiporter
VACht	vesicular acetylcholine transporter
VMAT	vesicular monoamine transporter

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Figure 15.1.

The TEXAN (toxin-extruding antiporter) gene family. Evolutionary relationship among transporters of the TEXAN family (monoamine or antibiotic/proton antiporters) as proposed by Schuldiner. Adapted from Schuldiner (1994).

Figure 15.2.

Structure of VMAT2, DHTBZ, and the TBZ-binding site on VMAT2 deduced from chimeric protein and bioinformatic considerations. The reduced form of tetrabenazine (TBZ) used as the ^{11}C -labeled congener for PET imaging studies in humans and other mammals. The affinity of TBZ for human VMAT2 is approximately 10 nM, about tenfold greater than DA, NE, Epi, or 5-HT and 300-fold greater than His. The actual binding site for TBZ binding to VMAT2 has not been determined—studies with VMAT1/VMAT2 chimeric proteins suggest the involvement of several regions (highlighted with gray boxes). Adapted from Peter et al. (1996).

Figure 15.3.

VMAT2 is expressed in human but not in rodent pancreas. (A–C) RT-PCR analysis of laser capture microdissected islets of human (A), pig (B), and mouse pancreas (C) demonstrating expression of VMAT2 mRNA in human and pig pancreatic islets but absence of VMAT2 from mouse pancreatic islets. (D) Coimmunostaining for VMAT2 and insulin in human pancreas demonstrating full expression of VMAT2 in beta cells in all islets of the entire human pancreas. *From Schafer et al. (2013).*

Figure 15.4.

VMAT2-positive monoaminergic neurons of the human enteric nervous system. Summary of chemical phenotypes of the extrinsic sympathetic and parasympathetic innervation of the human gut (input) and the chemical phenotypes of intrinsic gut neurons (enteric ganglia) with respect to cholinergic and catecholaminergic phenotypes based on our observations and previously published data. Major targets of intrinsic neurons are summarized. Both vesicular monoamine transporter 1 (VMAT1, minor) and VMAT2 (abundant) were found in separate sympathetic neurons of the prevertebral ganglia, which are extrinsic noradrenergic (copositive for TH and DBH). The efferent vagal cholinergic innervation of the gut (copositive for ChAT and VachT) is sparse. The large number of intrinsic neurons of the enteric nervous system (ENS) mainly functions autonomously. Intrinsic monoaminergic neurons of the gut express VMAT2 and TH but lack DBH and are, therefore, presumably dopaminergic. A minor population of VMAT2-positive neurons is serotonergic, as characterized by their content of TpH. Targets of intrinsic innervation include other enteric neurons and cell body-free parts of the plexus, diverse compartments of the gut wall, and extraintestinal targets such as projections to the prevertebral ganglia and the plexus of gallbladder and pancreas. *Adapted from Anlauf et al. (2003).*

Figure 15.5.

VMAT2-positive and VMAT2-negative monoaminergic neurons in mammalian brain. Categorization of classical, nonexocytotic (lacking VMAT2 expression), and DOPAergic proposed by the authors previously (Weihe et al., 2006). TH+/AADC–neurons should not be termed “DOPAergic,” however, unless L-DOPA production is actually demonstrated (see text and Ugrumov, 2009).

Figure 15.6.

Species-specific phenotypes of sympathetic sudomotor neurons. Diagram illustrating sympathetic sudomotor innervation during development at late prenatal (A), postnatal (B), and adult (C) stages based on staining for VACHT, VMAT2, and TH. ChAT, TH, DBH, and AADC present in all species are not depicted. *Adapted from Weihe et al. (2005).*

Figure 15.7.

Chemical coding of the human autonomic nervous system. Diagram of noradrenergic/cholinergic cotransmission in parasympathetic and sympathetic innervation of the human heart and skin, deduced from VACHT and VMAT2 costaining (ChAT, TH, and AADC are not depicted). AVA: arteriovenous anastomosis; SG: sweat glands. Adapted from Weihe et al. (2005).