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Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter

(amine storage/psychostimulants/neurotoxicity/neuroendocrine lineage)

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ABSTRACT **A second isoform of the human vesicular monoamine transporter (hVMAT) has been cloned from a pheochromocytoma cDNA library. The contribution of the two transporter isoforms to monoamine storage in human neuroendocrine tissues was examined with isoform-specific polyclonal antibodies against hVMAT1 and hVMAT2. Central, peripheral, and enteric neurons express only VMAT2. VMAT1 is expressed exclusively in neuroendocrine, including chromaffin and enterochromaffin, cells. VMAT1 and VMAT2 are coexpressed in all chromaffin cells of the adrenal medulla. VMAT2 alone is expressed in histamine-storing enterochromaffin-like cells of the oxyntic mucosa of the stomach. The transport characteristics and pharmacology of each VMAT isoform have been directly compared after expression in digitonin-permeabilized fibroblastic (CV-1) cells, providing information about substrate feature recognition by each transporter and the role of vesicular monoamine storage in the mechanism of action of psychopharmacologic and neurotoxic agents in human. Serotonin has a similar affinity for both transporters. Catecholamines exhibit a 3-fold higher affinity, and histamine exhibits a 30-fold higher affinity, for VMAT2. Reserpine and ketanserin are slightly more potent inhibitors of VMAT2-mediated transport than of VMAT1-mediated transport, whereas tetrabenazine binds to and inhibits only VMAT2. *N*-methyl-4-phenylpyridinium, phenylethylamine, amphetamine, and methylenedioxymethamphetamine are all more potent inhibitors of VMAT2 than of VMAT1, whereas fenfluramine is a more potent inhibitor of VMAT1-mediated monamine transport than of VMAT2-mediated monoamine transport. The unique distributions of hVMAT1 and hVMAT2 provide new markers for multiple neuroendocrine lineages, and examination of their transport properties provides mechanistic insights into the pharmacology and physiology of amine storage in cardiovascular, endocrine, and central nervous system function.**

Storage of monoamines in secretory organelles of neurons, endocrine/paracrine cells, basophils, blood platelets, and mast cells is critical for their regulated, physiological secretion. Monoamine accumulation from the cytoplasm into storage organelles is mediated by vesicular monoamine transporters (VMATs) with an absolute dependence on a vacuolar ATPase-generated proton gradient to transport the cationic amine substrates into the storage organelle in exchange for protons (1).

Recently, we cloned a VMAT (formerly named MAT) from a rat basophilic leukemia cell line (RBL-2H3) by functional expression of T7 promoter-driven cDNA sublibraries in CV-1 fibroblasts infected with T7 polymerase-expressing recombinant vaccinia virus (2). Permeabilization of the plasma membrane with digitonin provided the first demonstration that monoamine substrates could be directly accumulated by an intracellular compartment of nonneuroendocrine cells expressing this transporter in an ATP-dependent fashion that was sensitive to the specific inhibitors reserpine and tetrabenazine (TBZ). The mRNA for this transporter (now called VMAT2) was shown to be abundantly expressed in monoaminergic cell bodies of the central nervous system and also in the stomach but not in the adrenal gland. Independently, Edwards and colleagues (3) cloned a highly related VMAT (formerly named CGAT) from a rat pheochromocytoma (PC12) cell line based on its ability to confer resistance to *N*-methyl-4-phenylpyridinium (MPP^+) toxicity in transfected Chinese hamster ovary cells by intracellular sequestration of the toxin. CGAT (now called VMAT1) was shown to be abundantly expressed in the adrenal medulla but absent from the brain, and it displayed low sensitivity to inhibition by TBZ. Subsequently, the pharmacology and the tissue distribution of the two isoforms of the rat VMATs have been characterized in detail (4–8).

Somewhat surpisingly, the neuronal VMAT2 isoform has been found to be abundantly expressed in the bovine adrenal medulla (9–11). This observation suggested that important species differences may exist in the expression of the VMAT isoforms in endocrine organs and neural tissues. Because there are in fact two human VMAT isoforms (hVMAT1 and hVMAT2), we compared the distributions of the two isoforms in the human neuroendocrine system and hVMAT1 and hVMAT2 transport properties *in vitro* to determine how differences in the properties of each transporter might explain the differential uptake, action, and toxicity of neuropharmacological and neurotoxic agents in human.

MATERIALS AND METHODS

Preparation of cDNA Library and Cloning of hVMAT1. An oligo(dT)-primed, size-selected cDNA library from a human pheochromocytoma was constructed in a modified T7 promoter bearing plasmid expression vector, CDM7/amp (12). Initially, subdivisions were screened using a PCR-amplified and random-primed ^{32}P-labeled hVMAT2 coding sequence probe at low stringency as described (13), because it was expected that human pheochromocytoma, like rat PC12 cells, would express only a VMAT1 isoform. Unexpectedly, a hVMAT2 clone was obtained

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Abbreviations: ECL, enterochromaffin-like; MDMA, methylenedioxymethamphetamine (also known as "ecstasy"); MPP^+, *N*-methyl-4-phenylpyridinium; PEA, phenylethylamine; 5HT, serotonin; TBZ, tetrabenazine; TBZOH, dihydrotetrabenazine; VMAT, vesicular monoamine transporter; hVMAT, human VMAT.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U39905).

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from this library. The library was rescreened at low stringency with a rat VMAT1 probe that was cloned from a PC12 cDNA library (5), and a hVMAT1 clone was obtained. DNA sequencing was performed on each strand of the DNA, after subcloning of overlapping fragments into pUC18 as described (13).

Characterization of the Transport Properties of hVMAT1 and hVMAT2. Functional expression of hVMAT1 and hVMAT2 cDNAs was performed using the vaccinia virus/bacteriophage T7 hybrid system (14). The transport of [^{3}H]serotonin (5HT) was performed in digitonin-permeabilized CV-1 cells expressing the cDNAs as described (4). The binding of [^{3}H]dihydrotetrabenazine (TBZOH) was performed by addition of the radioligand to permeabilized fibroblasts exactly as described for [^{3}H]5HT. Cells were washed twice with 1.25 ml of ice-cold intracellular medium, solubilized in 1% SDS, and counted by scintillation counting.

Generation of hVMAT Antibodies. Peptides were synthesized from the predicted C-terminal sequences of hVMAT1 and hVMAT2 (RMYATQKPTKEFPLGEDSDEEPDHEE and TQNNIQSYPIGEDEESESD, respectively) with an additional N-terminal cysteine residue through which coupling to maleimide-activated keyhole limpet hemocyanin (Pierce) was accomplished after peptide purification by HPLC (Peninsula Laboratories). Antibodies were generated by immunization of New Zealand White rabbits as described (5).

Immunohistochemistry. Human tissues were immersion-fixed in Bouin–Hollande fixative, washed free of excess fixative in alcohol, and embedded in paraffin for preparation of 7-μm sections for staining. Primary antibodies were used at 1:3000–10,000 dilution, and binding to antigen was visualized by application of biotinylated goat anti-rabbit anti-IgG antibody, followed by streptavidin-coupled peroxidase and incubation with peroxide and diaminobenzidine as described (15). The primary rabbit antisera used were: anti-hVMAT1 #10 (1:2000–4000), anti-hVMAT2 #80182 (1:2000–4000), anti-serotonin 43H37R (1:10000; Immuno Nuclear/IBL), and anti-chromogranin A (1:3000, Lenny anti-WE-14) (16).

RESULTS

Sequence Analysis of the hVMAT1 cDNA. The 2.7-kb VMAT1 cDNA clone revealed an open reading frame of 1545 bp, predicting a protein of 526 amino acids. The amino acid sequence of hVMAT1 is shown in Fig. 1. hVMAT1 and hVMAT2 display an overall homology of 60%, with greatest identity within the putative 12 transmembrane regions of these proteins. Three potential glycosylation sites are observed for hVMAT1 in contrast to four sites in VMAT2 in a poorly conserved region predicted to reside within the lumen of the storage organelle. The cytoplasmic N and C termini are also poorly conserved between VMAT1 and VMAT2 isoforms.

Functional Expression of hVMAT1 and hVMAT2 in Digitonin-Permeabilized CV-1 Cells. hVMAT1 and hVMAT2 transported [^{3}H]5HT with similar kinetics (Fig. 2*A*). In the presence of ATP, [^{3}H]5HT uptake mediated by either isoform reached similar maximal levels (≈50 times greater than nonspecific uptake into mock-transfected CV-1 cells) by 10 min at 37°C. Uptake was energy dependent (>80% ATP dependent) and was abolished by the proton-translocating ionophore carbonylcyanide *p*-trifluoromethoxyphenylhydrazone or by the vacuolar H^+-ATPase inhibitor tri(*n*-butyl)tin. The transport process was saturable, with apparent affinity (K_m) for 5HT of 1.3 μM and 0.8 μM and V_{max} values of 37 and 43 pmol/min per 450,000 cells for VMAT1 and VMAT2, respectively (Fig. 2*A*, *Inset*).

Inhibition of transport by TBZ is a key feature distinguishing VMAT1 from VMAT2. TBZ inhibited the uptake of [^{3}H]5HT by VMAT2 with a K_i of ≈100 nM, whereas concentrations of TBZ as high as 20 μM did not affect transport mediated by VMAT1 (Fig. 2*B*). Furthermore, only VMAT2 showed significant binding of [^{3}H]TBZOH (Fig. 2*B*, *Inset*). Binding of

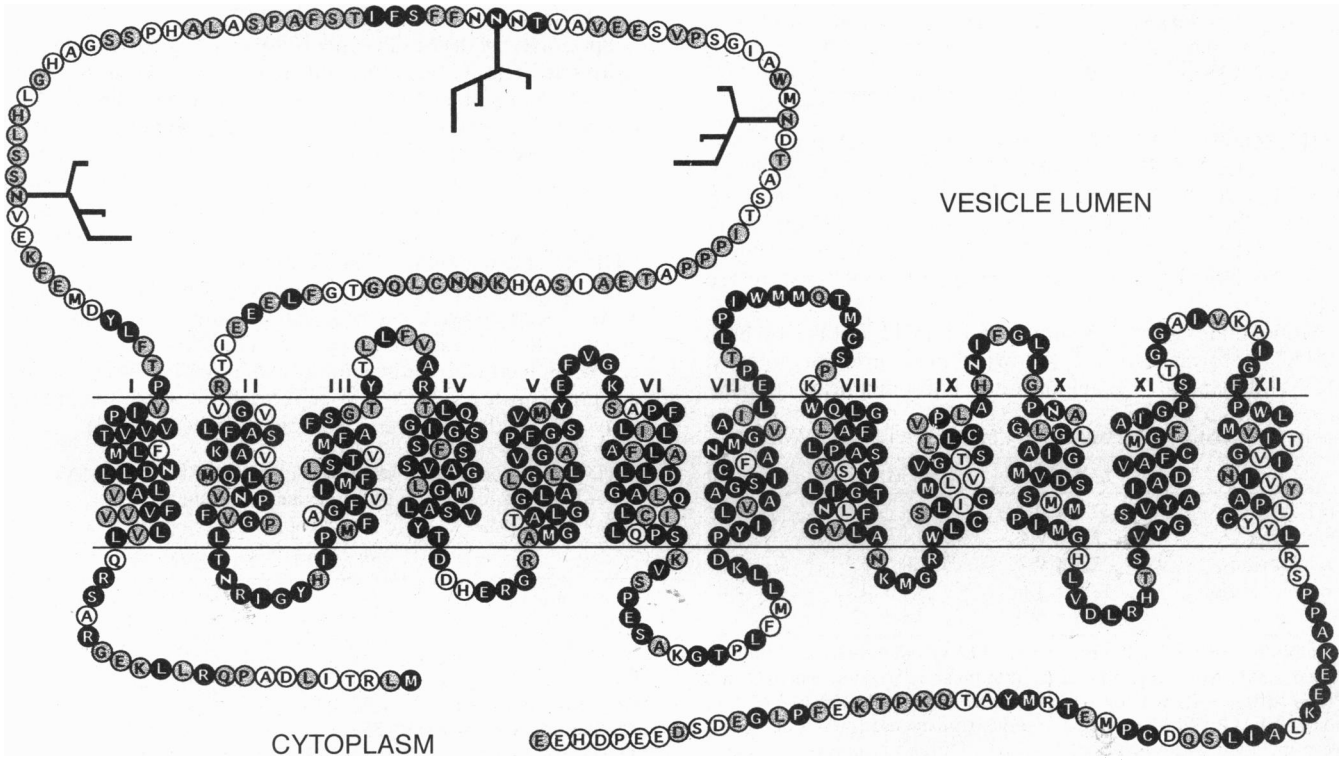

FIG. 1. Primary amino acid sequence and predicted secondary structure of hVMAT1. Twelve putative transmembrane domains (I-XII) and potential sites for N-linked glycosylation are indicated. Black indicates amino acids conserved between rat and human VMAT1 and among rat, human, and bovine VMAT2. Gray indicates amino acids unique to rat and human VMAT1. White indicates amino acids unique to hVMAT1.

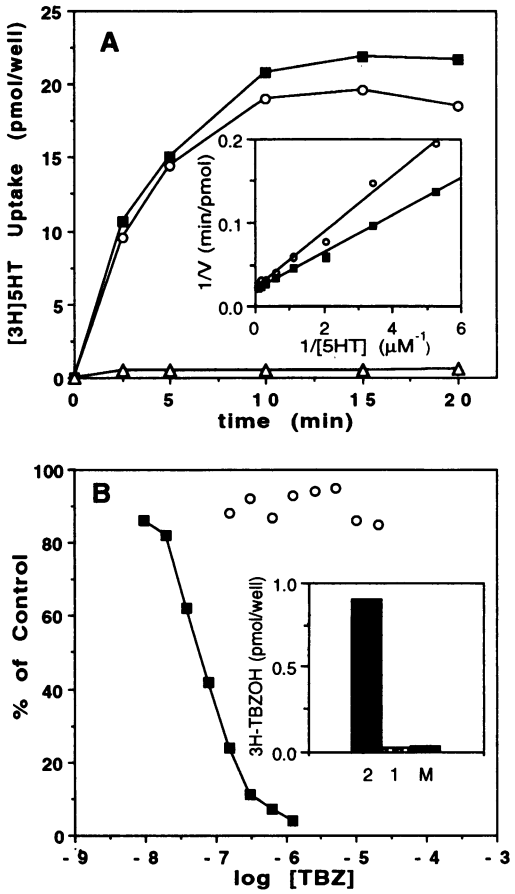

FIG. 2. Comparison of hVMAT1 and hVMAT2 in digitonin-permeabilized CV-1 fibroblasts. (*A*) Time course of [^{3}H]5HT (90 nM) accumulation. ○, hVMAT1; ■, hVMAT2; △, mock-transfected cells. (*Inset*) Lineweaver–Burk analysis of initial uptake velocity (2 min) of [^{3}H]5HT (0.09–12 μM). (*B*) Differential sensitivity of VMAT1 and VMAT2 to inhibition by TBZ (2 min). (*Inset*) [^{3}H]TBZOH (2.5 nM) binding to VMAT2 (2), VMAT1 (1), and mock (M). Data presented are averages of two experiments performed in duplicate.

[^{3}H]TBZOH to VMAT2-transfected, permeabilized CV-1 cells occurred rapidly (saturable by 5 min at 37°C) to levels more than 20-fold higher than background and was completely displaceable by TBZ at 2 μM. In contrast, [^{3}H]TBZOH binding to VMAT1-expressing CV-1 cells under identical conditions was indistinguishable from binding to mock-transfected cells.

Monoamine substrates inhibited [^{3}H]5HT uptake by both VMAT isoforms with the following rank order of potency: 5HT > dopamine > epinephrine > norepinephrine > histamine (Table 1). While the transporters exhibit similar affinity for 5HT (<2-fold difference), catecholamine substrates showed ≈3-fold higher affinity for VMAT2 than for VMAT1, and histamine exhibited a 30-fold higher apparent affinity for VMAT2 than for VMAT1.

Table 1. Relative substrate affinity for hVMAT1 and hVMAT2

	hVMAT1	hVMAT2	X-fold
5HT	1.4 ± 0.2	0.9 ± 0.1	1.6
Dopamine	3.8 ± 0.4	1.4 ± 0.2	2.7
Epinephrine	5.5 ± 0.7	1.9 ± 0.2	2.9
Norepinephrine	13.7 ± 1.6	3.4 ± 0.5	4.0
Histamine	4696 ± 601	143 ± 12	33

Inhibition of [^{3}H]5HT uptake (90 nM) was measured at 2 min. K_i values (μM) were determined by nonlinear regression using Graph Prism software (San Diego, CA). Control uptake for hVMAT1 (≈7 pmol), hVMAT2 (≈8 pmol), and mock-transfected (≈0.4 pmol) cells were included with each dose response. Uptake in mock-transfected cells was subtracted from all points. Data are presented as mean ± SEM from at least three experiments performed in duplicate. X-fold values represent the relative higher affinity of the substrates for VMAT2.

The effect of various compounds to inhibit [^{3}H]5HT uptake by the VMAT isoforms is shown in Table 2. Reserpine and ketanserin were potent inhibitors with the apparent affinity of both compounds for VMAT2 ≈3-fold higher than for VMAT1.

The effect of various neuroactive and neurotoxic compounds to inhibit [^{3}H]5HT uptake by hVMAT1 and hVMAT2 indicates that the absence of electron-donating substituents on the aromatic ring of these compounds greatly increases their differential effects (Table 2). The false neurotransmitter phenylethylamine (PEA) showed nearly a 10-fold higher affinity for VMAT2 than for VMAT1. Similarly, amphetamine displayed ≈20-fold higher affinity for VMAT2 than for VMAT1. The effect of amphetamine was stereospecific, with the (+) isoform about five times more potent than the (−) isoform to inhibit uptake mediated by either VMAT1 or VMAT2. The large differential effect of PEA and both amphetamine stereoisomers was due largely to the reduced potency to inhibit [^{3}H]5HT uptake mediated by VMAT1, compared with the other compounds tested. The presence of the methylenedioxy moiety on the aromatic ring in a racemic (±) mixture of methylenedioxymethamphetamine (MDMA) reduced the differential apparent affinity for VMAT1 vs. VMAT2 to that observed with the catecholamines, with an overall 2-fold lower apparent affinity for the transporters. The presence of a trifluoromethyl group on the aromatic ring of (±) fenfluramine caused an opposite effect, in that VMAT1 was about two times more sensitive to inhibition than VMAT2, with an overall apparent affinity of fenfluramine for VMAT1 greater than that of the catecholamines. Like PEA, the neurotoxic MPP^+ showed ≈8-fold higher apparent affinity for VMAT2 than for VMAT1. The biogenic amine substrates were, however, considerably more potent for inhibition of [^{3}H]5HT uptake by either isoform than was MPP^+.

Specificity of hVMAT1 and hVMAT2 C-Terminal Peptide Antisera. The C-terminal anti-peptide antibodies against VMAT1 and VMAT2 were used to differentially stain VMAT1- and VMAT2-expressing CV-1 cells in the presence and absence of the peptides used to generate each antiserum, to ensure their complete specificity before use in mapping the differential expression of each transporter isoform in the human nervous and endocrine systems (Fig. 3). Based upon the characteristics of [^{3}H]5HT uptake by either VMAT transporter isoform, equivalent amounts of each protein can be expressed in CV-1 cells. Only VMAT1-expressing CV-1 cells can be stained with antiserum against VMAT1. Likewise, only VMAT2-expressing CV-1 cells can be stained with antiserum against VMAT2. When antisera are preincubated with 25 μM peptides from which the antisera were produced, specific staining was abolished (Fig. 3).

Table 2. Pharmacologic sensitivity of hVMAT1 and hVMAT2 toward various inhibitors, psychoactive compounds, and neurotoxins

	hVMAT1	hVMAT2	X-fold
Reserpine	0.034 ± 0.005	0.012 ± 0.003	2.8
Tetrabenazine	>20	0.097 ± 0.02	—
Ketanserin	1.7 ± 0.2	0.54 ± 0.07	3.1
PEA	34 ± 5	3.7 ± 0.5	9.2
Amphetamine (+)	47 ± 6	2.1 ± 0.2	22
Amphetamine (−)	259 ± 33	10 ± 1.7	26
MDMA (+/−)	19 ± 3	6.9 ± 1	2.8
Fenfluramine (+/−)	3.1 ± 0.4	5.1 ± 0.5	0.6
MPP^+	69 ± 10	8.9 ± 1.4	7.8

Experiments are presented as described in Table 1.

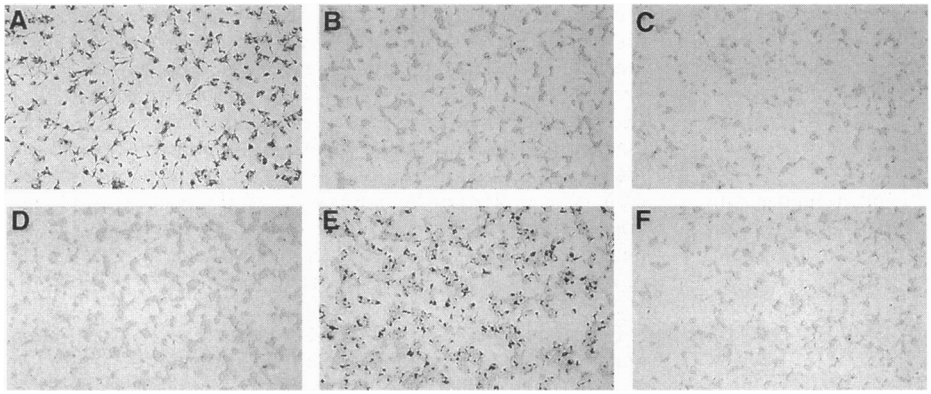

FIG. 3. Specificity of anti-hVMAT1 and anti-hVMAT2 antisera. (*A*) CV-1 cells expressing hVMAT1 and stained with anti-hVMAT1. (*B*) CV-1 cells expressing hVMAT2 and stained with anti-hVMAT1. (*C*) CV-1 cells expressing hVMAT1 and stained with anti-hVMAT1 adsorbed with peptide. (*D*) CV-1 cells expressing hVMAT1 and stained with anti-hVMAT2. (*E*) CV-1 cells expressing hVMAT2 and stained with anti-hVMAT2. (*F*) CV-1 cells expressing hVMAT2 and stained with anti-hVMAT2 adsorbed with peptide.

Differential Distribution of VMAT1 and VMAT2 in the Human Nervous and Endocrine Systems. The anatomical characterization of hVMAT1 compared with hVMAT2 in the human nervous and endocrine systems is shown in Fig. 4. hVMAT2-immunoreactive cells and fibers were observed in the biogenic amine-containing nuclei of the brain stem. hVMAT2-positive cell bodies and their axonal and dendritic processes in the dopaminergic cells of the substantia nigra are shown in Fig. 4*A*. hVMAT2-immunoreactive nerve fibers and terminals were abundant in cerebral cortex (Fig. 4*B*), presumably arising from ascending monoaminergic systems of the brain stem. hVMAT1 was completely absent from neuronal cell bodies and terminal fields in brain, including sections adjacent to those shown in Fig. 4*A* and *B* (data not shown).

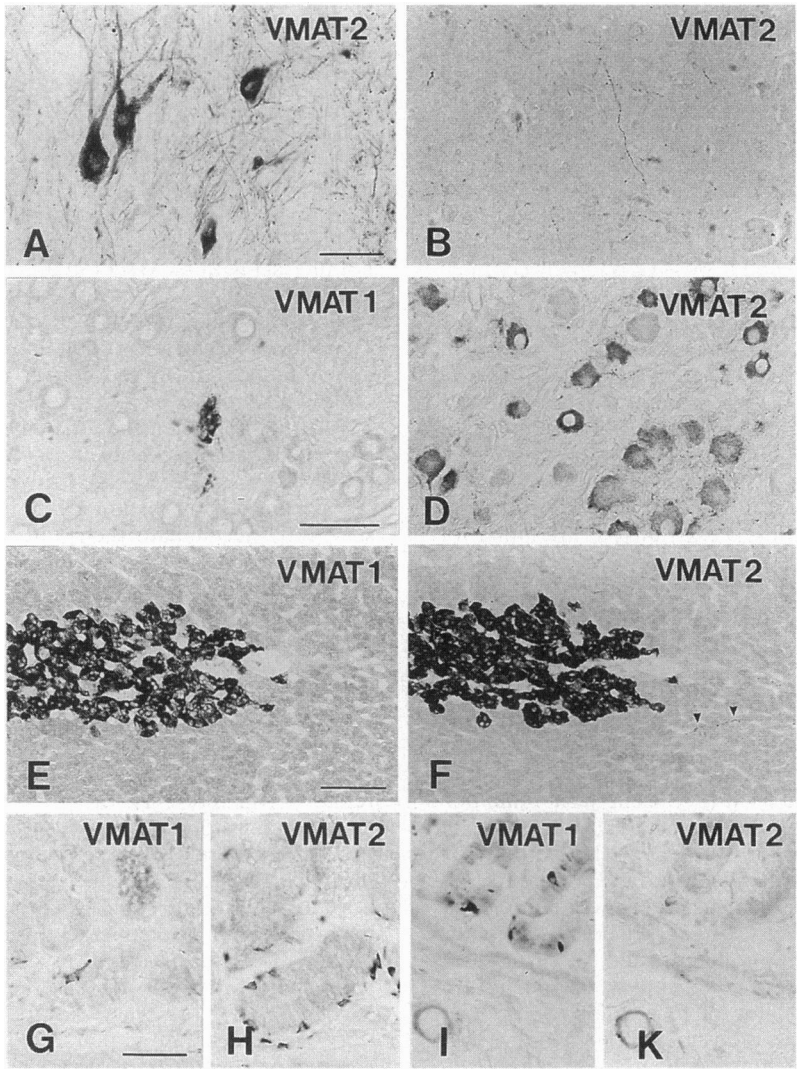

FIG. 4. Immunohistochemical visualization of hVMAT1 and hVMAT2 immunoreactivity in human neuronal and endocrine tissues. (*A*) Substantia nigra. (*B*) Frontal cortex. (*C* and *D*) First thoracic sympathetic ganglion. (*E* and *F*) Adrenal gland. (*G* and *H*) Corpus of the stomach. (*I* and *K*) Duodenum. Paraffin sections were incubated with the antiserum indicated in each panel (VMAT1, rabbit anti-hVMAT1; VMAT2, rabbit anti-hVMAT2). Arrowheads in *F* indicate VMAT2-immunoreactive fibers in adrenal cortex. (*A* and *B*, scale = 50 μm; *C* and *D*, scale = 100 μm; *E* and *F*, scale = 200 μm; *G–K*, scale = 50 μm.)

hVMAT1 staining in sympathetic ganglia was confined to the small intensely fluorescent cells of peripheral sympathetic ganglia (Fig. 4*C*), whereas hVMAT2 staining was observed in the principal ganglion cells of the sympathetic nervous system (Fig. 4*D*). In the enteric nervous system, VMAT2-positive, 5HT-containing cell bodies were detected in the myenteric plexus (data not shown).

VMAT1- and VMAT2-specific antisera each stained virtually all chromaffin cells of the human adrenal medulla, indicating complete coexpression of both isoforms in the gland (Fig. 4 *E* and *F*). Consistent with this observation, polyadenylated mRNA extracted from human pheochromocytoma demonstrated abundant expression of both VMAT1 (3.0 kb) and VMAT2 (4.2 kb) transcripts upon Northern blot hybridization (data not shown).

VMAT1-positive paracrine cells were found only rarely in the stomach (Fig. 4*G*), whereas the chromogranin A-positive enterochromaffin-like (ECL) cells of the oxyntic mucosa were virtually all VMAT2-positive (Fig. 4*H*). In contrast, the majority of enterochromaffin (chromogranin A-positive) cells of the small and large intestine were VMAT1-positive (and VMAT2-negative), as shown for the duodenum (Fig. 4 *I* and *J*). In both stomach and duodenum the sympathetic nerve terminals innervating blood vessels (Fig. 4*K*) were invariably VMAT2-positive and VMAT1-negative. VMAT2, but not VMAT1, immunoreactivity was also prominent in endocrine pancreas but was absent from exocrine pancreatic tissue (data not shown).

DISCUSSION

The cloning of hVMAT1 and hVMAT2 has allowed characterization of the pharmacology of the transporters and their expression in cells that store biogenic amines in human tissues. The expression of hVMAT1 and hVMAT2 is largely confined to endocrine and neuronal cell populations, respectively. However, hVMAT2 is also expressed in the ECL "histaminocytes" in the oxyntic mucosa of the stomach, consistent with histamine's higher affinity for hVMAT2 than hVMAT1. VMAT2 immunoreactivity is found in the histaminergic tuberomammillary neuronal cell bodies of the rat posterior hypothalamus (7, 8). hVMAT2 mRNA has also previously been observed in the human hypothalamus (17). Furthermore, reserpine-sensitive [^{3}H]histamine transport by rat VMAT2 is greater than by rat VMAT1 (8). Thus, the affinity of histamine for hVMAT2 and the expression of hVMAT2 in neurons and endocrine cells that store and secrete histamine support the notion that hVMAT2 is the histamine vesicular transporter in these cells.

Over 50 years ago, it was shown that histamine was released from the stomach during vagal stimulation and is involved in the regulation of normal gastrosecretory function (18). Identification of histaminocytes of the oxyntic mucosa in stomach has been difficult, however, because of the reliance on antibodies directed to histamine itself (19). Long-term inhibition of gastric secretion by histamine receptor (H2) blockade or proton secretion inhibitors results in ECL cell hyperplasia in the gastric mucosa of the rat (20, 21). Rindi and coworkers (22) have pointed out an apparent increase in the number of cases of chromogranin A-positive gastric ECL carcinomas within the last decade in humans and speculated a link to chronic H2 receptor blocker treatment during this period. The identification of VMAT2 as a "marker" for the ECL cells should greatly facilitate investigation of this important cell population.

Human sympathetic ganglion small intensely fluorescent cells are VMAT1-positive, and principal ganglion cells are VMAT2-positive, whereas chromaffin cells of the adrenal medulla are both VMAT1- and VMAT2-positive. In contrast, rodent chromaffin cells express predominantly VMAT1 with little expression of VMAT2 (5, 7). Because chromaffin cells are believed to represent a non-endstage neuroendocrine differentiation state (23), relatively subtle environmental differences in rodent and human adrenomedullary ontogenesis may affect expression of VMAT2 in chromaffin cells in these two species. VMAT2 expression may thus represent a useful marker in the study of the sympathoadrenal lineage. Likewise, the characterization of the ontogeny and plasticity of the hVMAT2-expressing histaminocytes of the stomach and paracrine cells of the pancreas, and hVMAT1-positive enterochromaffin cells of the digestive tract may provide insight into the developmental and genetic regulation of VMAT1 and VMAT2.

Because VMAT1 and VMAT2 are expressed in all chromaffin cells of human adrenal medulla, both may be present on chromaffin granules in both epinephrine- and norepinephrine-storing cells. Transport into chromaffin granules may be more efficient when VMAT2 is expressed because the catecholamines have somewhat higher affinity for VMAT2 than VMAT1. The possibilities of differential VMAT1/VMAT2 targeting to subpopulations of chromaffin granules, or differential targeting of VMAT1 and VMAT2 to chromaffin granules and synaptic-like microvesicles, in human adrenal medulla remain open for investigation.

Evaluation of the interactions of various amine-depleting agents and neurotoxins with VMATs provides insights into the role of vesicular monoamine storage in the pharmacological actions of these agents and structural features required for their differential effects. Reserpine and ketanserin potently inhibit both hVMAT1 and hVMAT2 and define a high-affinity monoamine uptake recognition site and a low-affinity monoamine binding site that may release amines into the vesicle lumen, respectively (24, 25). Like ketanserin, [^{3}H]TBZOH binds to a low-affinity monoamine binding site of human and bovine VMAT2 (17, 26). In contrast, TBZ and [^{3}H]TBZOH do not interact with hVMAT1 at all. The selective effect of TBZ on VMAT2 may account for its efficacy in depleting central monoamine stores in dyskinetic movement disorders (27).

Indirectly acting sympathomimetic amines such as amphetamine and its substituted derivatives, MDMA and fenfluramine, are known to be potent amine-releasing agents (28) and particularly neurotoxic to dopaminergic and serotonergic neurons of the central nervous system in rodents and primates (29–32). The neuronal specificity of the amphetamines relies on specific plasma membrane re-uptake transporters, which selectively accumulate them into particular monoaminergic nerve terminals. Once inside the cell, these substrates compete with monoamines for vesicular sequestration and ultimately disrupt vesicular monoamine storage and H^+ electrochemical gradients (30, 32–36). The potency of these compounds (μM range) and, in particular, the stereospecificity of the action of amphetamine reported here, support a direct interaction with VMAT2 *in vivo*. Although the potency of PEA for VMAT2 is similar to that of the substituted amphetamines, the behavioral and neurotoxic differences between these compounds may relate to the presence of an α-methyl substituent on the amphetamines that prevents degradation by monoamine oxidase (37). Therefore, these compounds persist in the nerve terminal and are more likely to release monoamines from vesicular storage sites and exhaust metabolic energy in futile maintenance of storage vesicle proton gradients. Thus, their intraneuronal actions, both psychopharmacologic and toxic, may include the specific interaction of each agent with VMAT2.

The large decrease in potency (10- to 20-fold) of PEA and amphetamine compared with the moderate decrease seen with the catecholamines and substituted amphetamines (2- to 4-fold) on [^{3}H]5HT uptake mediated by VMAT1 compared with VMAT2 suggest that the lack of strong electron-donating substituents on the aromatic ring play an important role in the differential apparent affinity for hVMAT1 vs. hVMAT2.

Decreased apparent affinity for VMAT1 of substrates that lack aromatic substituents is also exemplified by histamine, which has a 30-fold lower apparent affinity for VMAT1 than for VMAT2. Interestingly, the presence of a trifluoromethyl moiety on the aromatic ring of (±) fenfluramine may impart the selective increase in affinity for hVMAT1 compared with hVMAT2 of this compound. The more potent stereoisomer (+) dexfenfluramine, indicated as an anorectic drug, has recently been shown to produce persistent deficits in serotonergic neuronal markers in the monkey central nervous system when administered at relatively high doses (38). It may be important to examine the effect of this drug on the hVMAT1-expressing enterochromaffin cells in the digestive tract characterized here.

MPP^+ neurotoxicity is thought to be a useful model for idiopathic Parkinson's disease (39, 40). The transport of the neurotoxin MPP^+ into catecholaminergic neurons, in particular dopaminergic neurons of the substantia nigra, by high-affinity plasma membrane re-uptake transporters results in their degeneration (41). Cell death is a result of accumulation of MPP^+ by mitochondria and ultimately inhibition of ATP synthesis (42). It has been demonstrated that sequestration of MPP^+ by chromaffin granules of adrenal medulla and PC12 cells protects these cells against the metabolic damage (42, 43). The affinity of MPP^+ for hVMAT2 and the high level of expression of hVMAT2 in dopaminergic neurons of the substantia nigra would suggest these neurons would also be protected from MPP^+. However, the metabolic and functional compartmentation of the monoamines between vesicular and cytoplasmic pools in dopaminergic vs. noradrenergic neurons differ significantly (44). Hence, a limited vesicular storage capacity of dopaminergic synaptic vesicles, compared with chromaffin granules and noradrenergic dense-core granules, may be insufficient to clear the cytoplasm of MPP^+ in human dopaminergic neurons and, therefore, render them more susceptible to neurotoxin damage.

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