Drug Interactions with Vesicular Amine Transport

Doris Peter, Juan Jimenez, Yongjian Liu, Andrew Merickel, David Krantz, and Robert H. Edwards

INTRODUCTION

A large body of evidence has implicated monoamine neurotransmitters in a range of behavioral phenomena including mood. Reserpine depletes monoamines and induces a syndrome resembling depression (Frize 1954), giving rise to the monoamine hypothesis of affective disorders. In addition, many drugs used to treat depression act by inhibiting the reuptake of norepinephrine and serotonin from the synapse (Axelrod et al. 1961; Iversen 1976). The reinforcing properties of cocaine derive from its action to inhibit the reuptake of dopamine. Antipsychotic drugs also interfere with signaling by dopamine by interacting with dopamine receptors. Thus, extensive pharmacologic observations have implicated monoamines in psychiatric disease and drug abuse. In each case, the mechanism of drug action has revealed important features of normal signaling by monoamines, such as the role of specific receptors and transport proteins. In the case of psychostimulants, however, the specific mechanism of action remains unclear. In contrast to cocaine, which blocks the reuptake of dopamine from the synapse, amphetamines induce the release of monoamine stored within the presynaptic cell, apparently through a mechanism fundamentally different from the quantal release of classic synaptic transmission.

Classical synaptic transmission involves the regulated release of neurotransmitter in response to neural activity. Although regulated release can result from the reversal of electrogenic plasma membrane neurotransmitter transporters by depolarization (Attwell et al. 1993; Schwartz 1987), the vast majority of regulated release in the nervous system results from the regulated fusion of vesicles with the plasma membrane. Thus, synaptic transmission usually requires the storage of neurotransmitters in specialized secretory vesicles.

Neural peptides and classical transmitters differ in the mode by which they enter secretory vesicles specialized for regulated release. In the case of neural peptides and hormones, protein precursors translocate

into the lumen of the endoplasmic reticulum during translation. The proteins then sort into large, dense core vesicles (or secretory granules in endocrine cells) and undergo proteolytic processing and other modifications before regulated release. In the case of classical transmitters, however, packaging of both newly synthesized transmitter and transmitter accumulated by plasma membrane reuptake occurs in the cytoplasm. Thus, storage in secretory vesicles depends on specific transport from the cytoplasm into the vesicle.

Classical studies have identified four distinct vesicular transport activities for monoamines: acetylcholine, glutamate, and the inhibitory transmitters gamma-aminobutyric acid (GABA) and glycine. The availability of bovine adrenal chromaffin granules with easily detectable transport activity for monoamines has enabled characterization of the bioenergetic basis for this class of neurotransmitter transport. In contrast to the plasma mem-brane transporters that use the Na gradient across the plasma membrane to remove transmitter from the synapse and so terminate its action, the vesicular transporter expressed by chromaffin granules uses the proton electrochemical gradient produced by a vacuolar H+-ATPase to drive transport (Johnson 1988; Kanner and Schuldiner 1987). Specifically, the transporter exchanges two protons in the lumen of the vesicle for one monoamine in the cytoplasm (Knoth et al. 1981). Since the H+-ATPase usually generates a ΔpH of approximately two (log units) in secretory granules, this active transport system can produce concentration gradients of up to 104 inside the vesicle relative to outside. Other vesicular neuro-transmitter transporters appear to use a similar mechanism. Like the vesicular amine transporter, the transporter for acetylcholine also uses predominantly the ΔpH component of the proton electrochemical gradient (Anderson et al. 1982), whereas the vesicular glutamate transporter uses mainly the electrical component of the gradient (Δy) (Carlson et al. 1989; Maycox et al. 1988) and the GABA transporter uses both ΔpH and Δy (Hell et al. 1990). Interestingly, the bioenergetics of vesicular amine transport appear to have an important role in the action of psychostimulants such as amphetamines.

Amphetamines appear to induce the release of stored monoamines by interfering with plasma membrane transport and vesicular amine storage rather than by inducing vesicle fusion with the plasma membrane. Inhibitors of plasma membrane amine transport block the effects of amphetamines, suggesting that reversal of the transporter mediates the release of monoamines from the cytoplasm into the synapse (Rudnick and Wall 1992a, 1992b, 1992c; Sulzer et al. 1993),

possibly by exchange of extracellular amphetamine for cytoplasmic monoamine. The lipophilic properties of amphetamines may enable them to diffuse rapidly back out of the cell after uptake, providing for virtually unlimited exchange and net efflux of the transmitter. Alternatively, protracted uptake of the amphetamine due to rapid diffusion back across the plasma membrane may eventually run down the Na+ gradient that drives transport, allowing monoamines to equilibrate across the plasma membrane in accord with their concentration and potentially accounting for the associated neural toxicity. In the case of either model, however, emptying the cytoplasmic pool of monoamine into the synapse would have little physiological effect unless the normally low cytoplasmic concentrations are increased by efflux from the storage vesicles.

The mechanism by which amphetamines induce the release of stored monoamines into the cytoplasm remains uncertain. Amphetamines may exchange for monoamines in the lumen of the vesicle. Recent obser-vations, however, suggest that amphetamines act as weak bases to disrupt the pH gradient across the vesicle membrane (Schuldiner et al. 1993b; Sulzer and Rayport 1990). Previous work has shown that in the presence of ΔpH, chromaffin granules retain loaded monoamines for up to an hour (Maron et al. 1983). In the absence of ΔpH, previously loaded mono-amines rapidly leak from the vesicle, suggesting that ΔpH prevents reversal of the transporter. Even without weak bases such as the amphetamines, efflux could occur under conditions of energy failure that deplete the ATP in nerve terminals and allow ΔpH to dissipate. The studies describing efflux did not, however, clearly indicate a role for the transporter in efflux. To understand these and other questions about the role of vesicular neurotransmitter transport in synaptic transmission, neuropsychiatric disease, and drug abuse, the authors have isolated cDNA clones for several vesicular neurotrans-mitter transporters and developed biochemical assays to characterize their functional properties, including their interaction with psychostimulants.

VESICULAR AMINE TRANSPORT CONFERS RESISTANCE TO MPP+

The potent neurotoxin N-methyl-4-phenyltetrahydropyridine (MPTP) produces a syndrome with remarkable clinical and pathological similarity to idiopathic Parkinson's disease (PD) (Langston et al. 1983). As with PD, MPTP produces clinical bradykinesia and rigidity. Pathologically, MPTP also results in the relatively selective

degeneration of dopaminergic neurons in the substantia nigra. In addition, the MPTP syndrome responds to L-dopa, with the eventual development of typical disabling dyskinesias.

The strong resemblance between MPTP toxicity and idiopathic PD has suggested that the study of MPTP toxicity will reveal mechanisms that also participate in the pathogenesis of PD. As a neutral lipophilic compound, MPTP easily penetrates the blood-brain barrier. Monoamine oxidase B, presumably expressed by glia, then converts MPTP to the active metabolite N-methyl-4-phenylpyridinium (MPP+) (Heikkila et al. 1984; Langston et al. 1984; Markey et al. 1984). Plasma membrane amine transporters recognize and accumulate MPP+ within monoamine cell groups, accounting for the selectivity of degeneration (Javitch et al. 1985). Inside the cell, MPP+ enters mitochondria and inhibits respiration, apparently at the level of complex I in the respiratory chain (Krueger et al. 1990).

A growing body of evidence supports the relevance of MPTP toxicity for idiopathic PD. The drug selegiline hydrochloride prevents the MPTP syndrome by inhibiting monoamine oxidase B and also appears to slow the rate of progression in PD (Parkinson Study Group 1989). Further, defects in complex I of the respiratory chain appear in PD as well as MPTP toxicity (Mizuno et al. 1989; Ozawa et al. 1990; Parker et al. 1989; Shoffner et al. 1991). However, particular features of the MPTP syndrome are still not understood, and these features may play an important role in idiopathic PD.

Adrenal chromaffin cells express a plasma membrane norepinephrine transporter and accumulate large amounts of MPP+ after systemic injection of MPTP, but, in contrast to dopaminergic neurons in the substantia nigra, these cells do not degenerate (Johannessen et al. 1985; Reinhard et al. 1987). Similarly, rat pheochromocytoma PC12 cells (derived from the adrenal medulla) accumulate MPP+ through a plasma membrane amine transporter but show toxicity only to extremely high concentrations (Denton and Howard 1987; Snyder et al. 1986). Although inhibition of plasma membrane amine transport blocks MPP+ toxicity entirely in PC12 cells, the Chinese hamster ovary (CHO) fibroblast cell line lacks plasma membrane amine transport activity and shows more sensitivity to MPP+ than do PC12 cells. Thus, this potent neurotoxin appears to have more toxicity for a fibroblast cell line than for a neural cell line.

To understand the basis of resistance to MPP+, the authors transferred DNA sequences from the relatively MPP+-resistant PC12 cells into the

relatively MPP+-sensitive CHO fibroblasts and selected the transformants in MPP+. After selection for several weeks, one colony of resistant cells appeared. To dissect the mechanism of their resistance to MPP+, the authors first determined whether the toxin affected respiration (Liu et al. 1992a). In contrast to wild-type CHO cells, which show the rapid inhibition of respiration by MPP+, the resistant cells showed no inhibition, indicating that resistance did not derive from a compensatory mechanism but rather from a primary failure of toxin action. The authors also determined that the resistant cells showed wild-type sensitivity to the other complex I inhibitor rotenone, indicating specificity of the resistance mechanism for MPP+. The authors then found that reserpine completely abolished resistance to the toxin and did not affect the sensitivity of wild-type cells. This suggested that sequences encoding vesicular amine transport had transferred from PC12 cells to CHO fibroblasts. It was presumed that the transporter protects against the toxin by sequestering it in vesicles, away from its primary site of action in mitochondria. To confirm this mechanism of resistance, CHO cells were loaded with large amounts of exogenous dopamine and the intrinsic fluorescence of this transmitter was used to determine its intracellular localization. Whereas wild-type CHO cells showed diffuse staining, MPP+-resistant cells showed a striking, particulate pattern that reverted to wild type in the presence of reserpine, strongly supporting the hypothesis that vesicular amine transport protected the resistant cells by sequestering the toxin in vesicles.

To isolate the sequences responsible for resistance to MPP+, the authors used plasmid rescue (Liu et al. 1992b). Retransfection of the rescued plasmids led to the eventual isolation of a single clone that conferred resistance to MPP+. This clone also conferred vesicular amine transport as determined by dopamine-loaded fluorescence, even in transfected cells not selected in MPP+. Also developed was a quantitative assay for vesicular amine transport using membrane vesicles from the transfected cells. Briefly, the cells are disrupted at narrow clearance (10 micromolars (μm)), the debris sedimented by centrifugation at low speed, and the supernatant incubated in the presence of tritiated monoamine for varying intervals, then rapidly diluted, filtered, and the bound radioactivity measured. As expected, transport activity depends on the presence of ΔpH generated by the vacuolar H+-ATPase; it also shows an affinity for monoamine substrates in the low micromolar range and inhibition by low nanomolar concentrations of reserpine. Surprisingly, tetrabenazine inhibited transport only at high concentrations, but cocaine and tricyclic antidepressant did not inhibit the activity at all. Thus, the

cDNA conferred virtually all of the physiological and pharmacological characteristics expected for the vesicular amine transporter.

The sequence of the cDNA conferring MPP+ resistance and vesicular amine transport predicted a novel protein with 12 transmembrane domains (Liu et al. 1992b). Although many transport proteins are predicted to have 12 transmembrane domains, the primary amino acid sequence of the vesicular amine transporter showed no similarity to the plasma membrane neurotransmitter transporters, or other mammalian transporters and thus appeared to define a novel mammalian gene family that is now known to include the vesicular transporters for other neurotransmitters such as acetylcholine (Erickson et al. 1994; Roghani et al. 1994; Varoqui et al. 1994) (figure 1). However, the first six transmembrane domains of the vesicular amine transporter show weak but definite homology to a class of bacterial antibiotic resistance proteins (Liu et al. 1992b). Interestingly, these proteins transport antibiotics out of bacteria, a phenomenon topo-logically equivalent to the transport of MPP+ into vesicles. Further, the bacterial transporters also act by proton exchange (Kaneko et al. 1985). In the case of the bacterial multidrug resistance transporter, reserpine inhibits its activity (Neyfakh et al. 1991). Thus, the vesicular amine transporter shows functional as well as structural similarity to these bacterial proteins. The relationship suggests that vesicular neurotransmitter transport evolved from these ancient detoxifying systems. Together with cloning of the vesicular amine transporter by selection in MPP+, the relationship raises the possibility that vesicular transport plays two roles in the nervous system: one in packaging transmitter for regulated release and the other in neural protection. However, MPTP or another exogenous toxin has not been identified in idiopathic PD (Tanner and Langston 1990).

Vesicular transport may protect against the normal transmitter dopamine itself. Monoamines and dopamine in particular oxidize very easily, producing free radicals that injure neural cells by a mechanism that does not involve interaction with a specific receptor (Cohen 1990; Michel and Hefti 1990; Rosenberg 1988). Vesicular amine transport would clearly protect against this form of endogenous toxicity as well as against the toxicity of MPP+. Thus, a defect in vesicular amine transport could contribute to the pathogenesis of idiopathic PD. The toxicity associated with amphetamines may also result from the efflux of monoamine stores into the cytoplasm. Indeed, recent imaging studies of primary dopaminergic neuronal cultures show localization of free-radical injury induced by amphetamines to the sites of vesicle accumulation (Cubells et al. 1994).

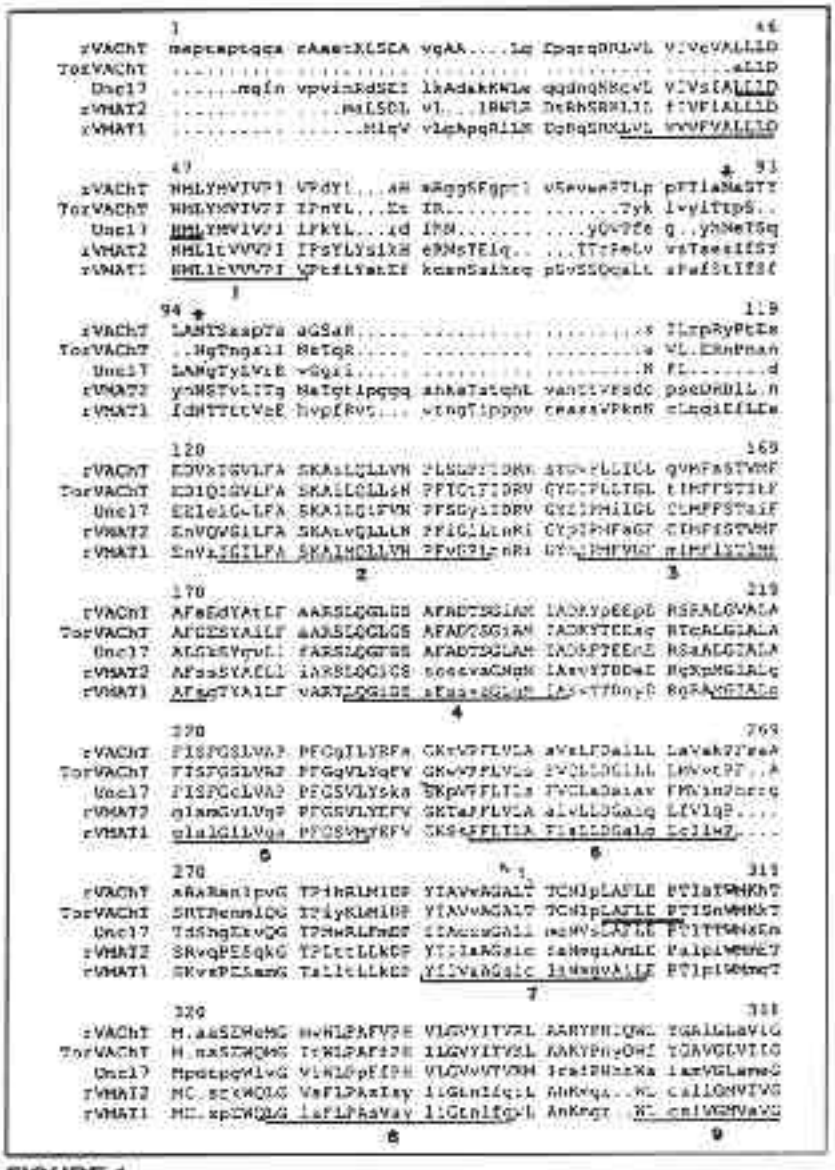

FIGURE 1.

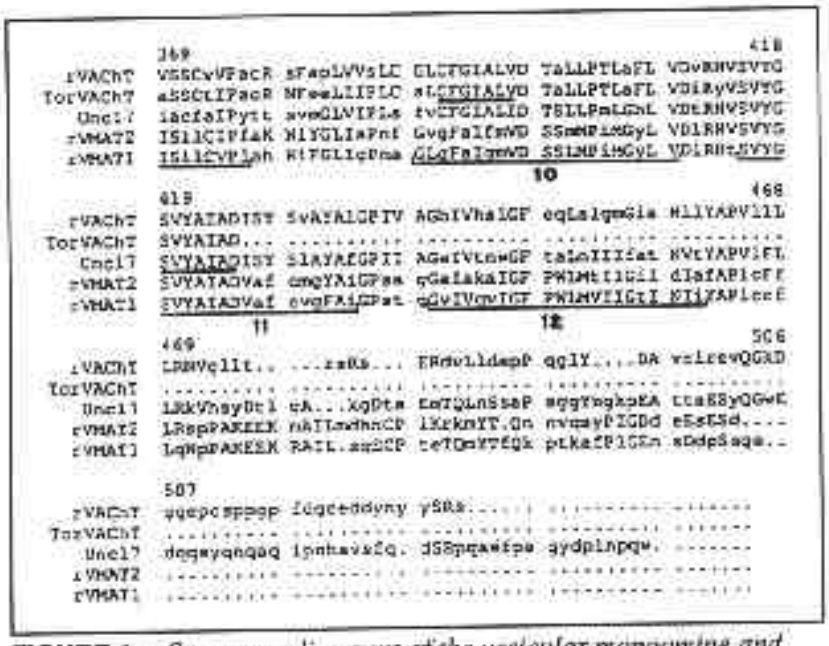

FIGURE 1. *Sequence alignment of the vesicular monoamine and putative vesicular acetylcholine transporters. rVMAT-1 and rVMAT-2 indicate the rat chromaffin granule amine transporter and rat synaptic vesicle amine transporter, respectively. Unc17, TorVAChT and rVAChT denote the putative vesicular acetylcholine transporters from* C. elegans, T. californica, *and rat. Upper case letters denote the consensus and lower case the divergent residues. The underlined sequences in unc17 indicate the regions used to design degenerate oligonucleotide primers for PCR amplification of TorVAChT. The underlined residues in TorVAChT indicate the regions used to design degenerate oligonucleotide primers for PCR amplification of rVAChT. Brackets mark the predicted transmembrane domains, asterisks the potential sites for N-linked glycosylation in rVAChT. The twelfth transmembrane domain and the C-terminus contain a leucine-rich region. The numbers refer to amino acid residues of rVAChT.*

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TWO DISTINCT GENES ENCODE VESICULAR AMINE TRANSPORT

Classical studies have shown that monoamine cell populations in the central nervous system (CNS) express vesicular amine transport activity that is inhibited by reserpine and tetrabenazine. However, Northern blot analysis of polyA+ ribonucleic acid (RNA) from different tissues including the brain showed expression of sequences encoding the chromaffin granule amine transporter (CGAT or VMAT-1) in only the adrenal gland. Although low levels of expression or expression by a small proportion of cells could account for the failure to detect a signal, the authors pursued the alternative possibility that the brain expresses a distinct vesicular amine transporter. Screening a brainstem cDNA library with the CGAT cDNA as probe under moderately reduced stringency revealed a second distinct but closely related transporter, originally termed the synaptic vesicle amine transporter (SVAT or VMAT-2) (Liu et al. 1992b) (figure 2). Northern analysis showed expression of this sequence in the brainstem but not the adrenal gland. In situ hybridization also showed expression by the expected dopaminergic cell groups in the substantia nigra and ventral tegmental area, the noradrenergic neurons of the locus coeruleus, and serotonergic neurons of the dorsal raphe, consistent with the previously demonstrated recognition of multiple monoamine transmitters with similar affinity by the transporter from bovine chromaffin granules. Thus, chromaffin cells and central neurons express distinct but highly related vesicular monoamine transporters.

Surprisingly, an amine transporter purified from bovine chromaffin granules corresponds more closely to the central rat transporter than to the adrenal transporter (Howell et al. 1994). Purification of proteins labeled by 3H-reserpine had in fact yielded two proteins that differ in isoelectric point (Stern-Bach et al. 1990), with the sequence of one corresponding to VMAT-2 (Howell et al. 1994). Additional study has further shown that the bovine and rat adrenal glands contain both transporters, although the rat adrenal contains an overwhelming preponderance of the expected VMAT-1 transporter (Peter, et al., in press). Further, the purified bovine transporter showed high sensitivity to inhibition by tetrabenazine (Howell et al. 1994), whereas rat VMAT-1 did not (Liu et al. 1992b). To under-stand the basis for these differences in pharmacology as well as to identify other differences that may play a role in synaptic transmission and possibly protect against neural degeneration, the authors have characterized the functional properties of cloned VMAT-1 and VMAT-2 (Peter et al. 1994).

Using expression in a heterologous system, it has been determined that VMAT-1 and VMAT-2 differ in their physiological properties as well as in their pharmacology (Peter et al. 1994). Both VMAT-1 and VMAT-2 protect against MPP+ toxicity after expression in CHO cells, and these stably transformed cell lines have been used to determine their functional characteristics. VMAT-2 has an approximately twofold to threefold higher affinity for most monoamine substrates than VMAT-1 (table 1). Both transporters have the highest affinity for serotonin, followed by dopamine, then norepinephrine and epinephrine. However, the transporters differ dramatically in their affinity for histamine, with low micromolar concentrations inhibiting transport of 3H-serotonin by VMAT-2 but two orders of magnitude more required to inhibit VMAT-1.

To further compare the physiologic and pharmacologic properties of VMAT-1 and VMAT-2, the authors have investigated the interaction of the transporters with the antihypertensive drug reserpine. Using membranes prepared from stably transfected CHO cell lines, it has been found that reserpine inhibits transport of amines by both VMAT-1 and VMAT-2 with high potency and binds to VMAT-1 with two distinct affinities (Peter et al. 1994; Schuldiner et al. 1993a). Previous work had shown that monoamines inhibit reserpine binding with an affinity similar to their apparent affinity as substrates for transport, indicating that reserpine binds at or near the site of substrate recognition. In addition, the imposition of ΔpH accelerates the rate of reserpine binding, suggesting that reserpine can detect conformational changes in the protein that occur during the transport cycle (Rudnick et al. 1990; Scherman and Henry 1984; Weaver and Deupree 1982). Both of these observations have been confirmed for VMAT-1 expressed in CHO cells (Schuldiner et al. 1993a). Binding to reserpine also enables quantitation of the transporter and hence calculation of the turnover number for VMAT-1 (~10/min) and VMAT-2 400/min at saturating substrate concentrations and 29°C (Peter et al. 1994). Thus, the transporter expressed by dopaminergic cells susceptible to MPTP (VMAT-2) has a higher apparent affinity for substrates and a faster turnover than the transporter expressed by more resistant cells in the adrenal medulla (VMAT-1) and so cannot account for the differential vulnerability seen in MPTP toxicity and PD. Rather, the results suggest that vesicular amine transport acts to protect against endogenous or exogenous toxins in both cell types but does not suffice to protect neurons in the substantia nigra.

In contrast to the apparent similarity of their interaction with reserpine, VMAT-1 and VMAT-2 differ in their interaction with another inhibitor of transport, tetrabenazine. As noted above, high concentrations of tetra-

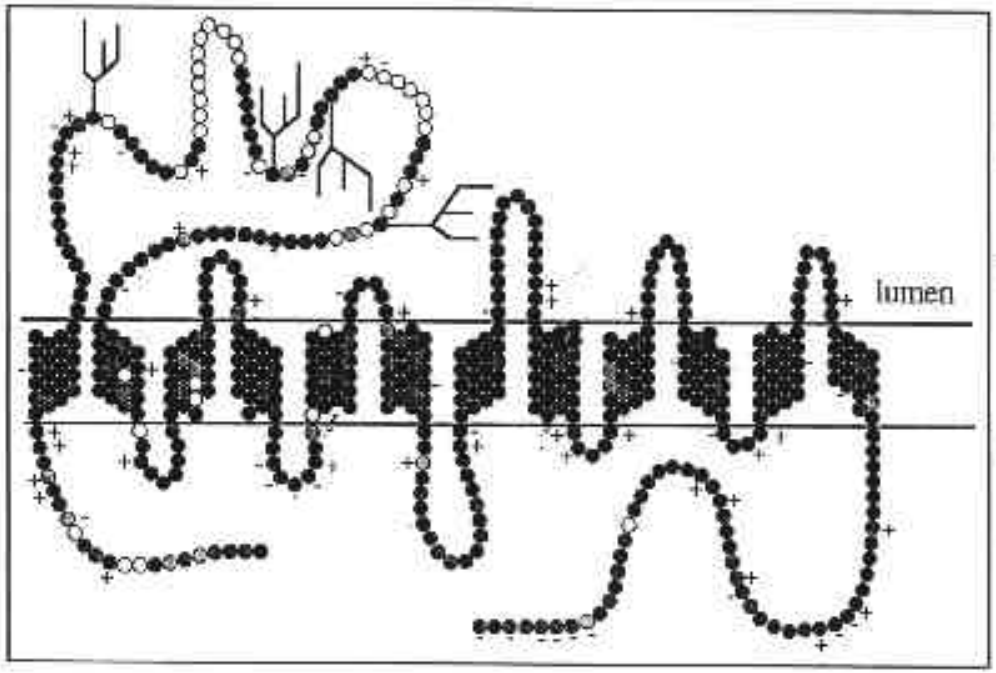

FIGURE 2.

Predicted structures of VMAT-1 and VMAT-2. The lumen of the vesicle is above, the cytoplasm below. Basic and acidic amino acid residues are indicated by a plus and minus, respectively. Branched structures in the first lumenal loop indicate potential N-linked carbohydrates. The residues in black are identical between the two vesicular amine transporters, the white residues divergent. The lightly stippled residues are conservative differences between VMAT 1 and VMAT 2. The darkly stippled residues are residues of VMAT-2 that diverge from VMAT-1 but show conservation to human VMAT-2.

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TABLE 1. Apparent affinity of VMAT-1 and VMAT-2 for monoamine substrates and MPP+. Standard transport assay (Liu et al. 1992b) was performed at 29oC for 2 minutes using 3H-serotonin. The values indicate the Km for serotonin and the Kis for other compounds.

Substrate	VMAT-1	VMAT-2
	μM	μM
Serotonin	0.85 ± 0.23	0.19 ± 0.04
Dopamine	1.56 ± 0.35	0.32 ± 0.04
Epinephrine	1.86 ± 0.11	0.47 ± 0.05
Norepinephrine	2.5 ± 0.4	0.33 ± 0.06
Histamine	436 ± 36	3.06 ± 1.0
MPP+	2.8 ± 0.6	1.6 ± 0.45

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benazine are required to inhibit transport by VMAT-1, whereas the purified bovine chromaffin granule transporter shows sensitivity to low nanomolar amounts. Consistent with the sequence similarity to the purified bovine transporter, VMAT-2 also shows sensitivity to nanomolar concentrations of tetrabenazine (figure 3) (Peter et al. 1994). The differential inhibition of VMAT-1 and VMAT-2 by tetrabenazine, although not anticipated, does account for several classic pharmacological observations. In contrast to reserpine, which depletes both peripheral and central monoamine stores, tetrabenazine depletes predominantly central stores and so causes less hypotension (Carlsson 1965). Differences in the turnover of monoamine stores in the adrenal gland and the brain had been invoked to explain this differential effect (Scherman and Boschi 1988). However, differential inhibition of VMAT-1 and VMAT-2 by tetrabenazine now seems far more likely to account for the observations.

In further contrast to reserpine, classical studies have shown that only large amounts of monoamine transmitters displace 3H-dihydrotetrabenazine from the bovine transporter (Scherman and Henry 1984) and ΔpH does not

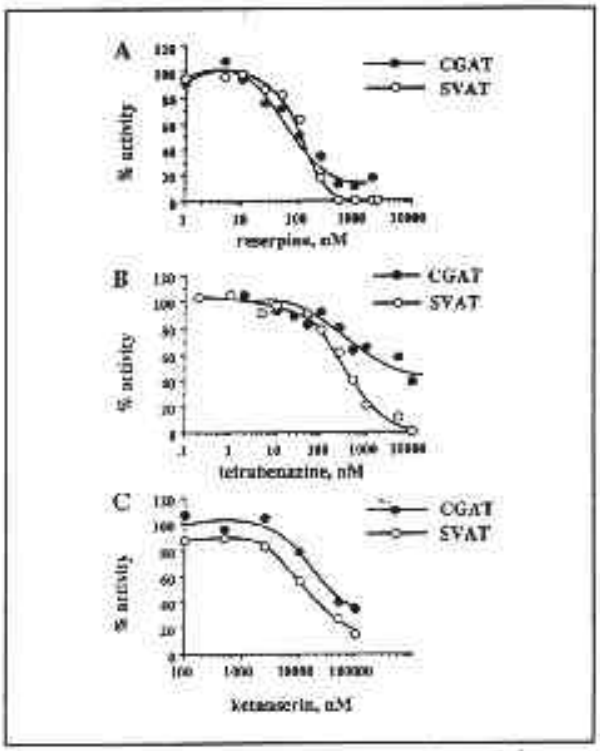

FIGURE 3. *Differential inhibition of VMAT-1 (CGAT) and VMAT-2 (SVAT) by tetrabenazine but not reserpine or ketanserin. Dose-response analysis of ^{3}H-serotonin transport by membranes from COS cells transiently transfected with the two cDNAs. Reserpine inhibits the two vesicular amine transporters with similar potency (A), but tetrabenazine shows substantially higher potency as an inhibitor of VMAT-2 than VMAT-1 (B). Ketanserin is thought to interact with the same site as tetrabenazine but inhibits VMAT-1 and VMAT-2 with similar potency (C).*

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influence binding, indicating that tetrabenazine does not bind at the site of substrate recognition and recognition of the drug does not change during the transport cycle. However, tetrabenazine can prevent reserpine binding, suggesting an allosteric interaction between the two sites (Darchen et al. 1989). In the case of VMAT-1 and VMAT-2, tetrabenazine also inhibits 3H-reserpine binding but with distinct potencies that correspond to the differential sensitivity of transport to tetrabenazine (Peter et al. 1994). Thus, the difference in tetrabenazine sensitivity between VMAT-1 and VMAT-2 does not reflect a difference in the interaction between these two sites. Rather, additional study with 3H-dihydrotetrabenazine has shown binding to VMAT-2 but not VMAT-1, indicating a simple difference in drug recognition (Peter et al. 1994). The vesicular amine transporters also differ in their interaction with psychostimulants.

VESICULAR AMINE TRANSPORT AND PSYCHOSTIMULANTS

The authors have found that the two vesicular amine transporters differ in their interaction with amphetamines. Methamphetamine inhibits the transport of 3H-serotonin by VMAT-2 much more potently than transport by VMAT-1 (figure 4) (Peter et al. 1994). Although amphetamines can inhibit transport by the dissipation of ΔpH (Sulzer and Rayport 1990), the differential inhibition of VMAT-1 and VMAT-2 and the stereospecificity of the inhibition make a direct interaction far more likely. Indeed, meth-amphetamine inhibits reserpine binding to VMAT-2 with greater potency than to VMAT-1, indicating interaction with the site of substrate recog-nition (Peter et al. 1994). Nonetheless, the significance of this interaction for the mechanism by which psychostimulants induce monoamine efflux remains uncertain. In particular, the lipophilic nature of amphetamines may not require specific transport into vesicles to dissipate ΔpH. Alternatively, recognition by the transporter may promote efflux through an exchange mechanism. However, dissection of the role that the transporter plays in efflux induced by dissipation of ΔpH and efflux induced by amphetamines requires the development of an appropriate heterologous expression system.

Since amphetamines act by inducing the release of stored monoamines, the site of storage also appears critical to their action as toxins. Indeed, amphetamines produce free radical injury localized to the sites where vesicles accumulate (Cubells et al. 1994). The site of monoamine storage appears to differ from other classical transmitters. Small (40 nm), clear synaptic vesicles contain such classical transmitters as acetylcholine,

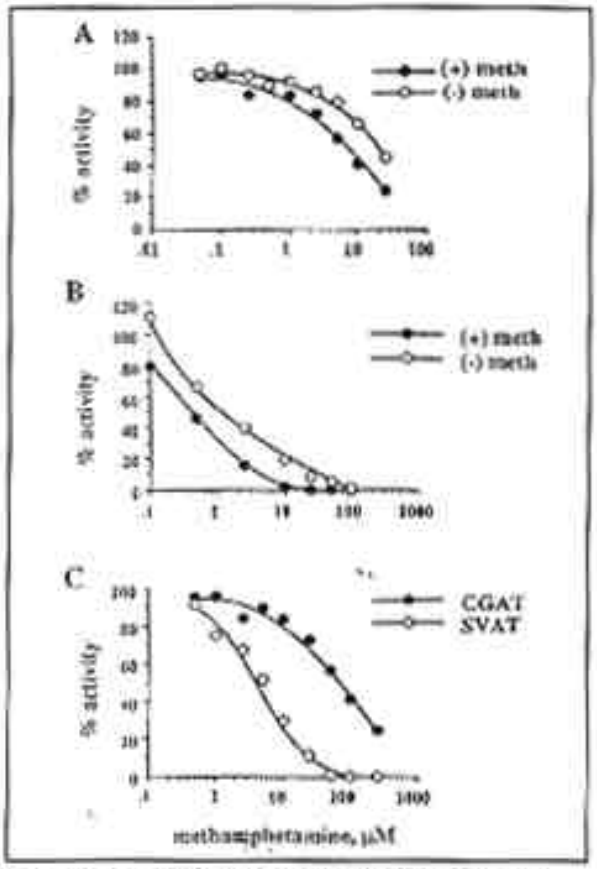

FIGURE 4. *Methamphetamine inhibits VMAT-1 (CGAT) and VMAT-2 (SVAT). Using membranes from stable CHO transformants expressing the two vesicular amine transporters, methamphetamine inhibits serotonin transport by VMAT-1 (A) and VMAT-2 (B) with the anticipated stereospecificity. However, the two transporters differ substantially in their sensitivity to the psychostimulant (C).*

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GABA, glycine, and glutamate; they mediate typical fast synaptic trans-mission and cluster over the nerve terminal (De Camilli and Jahn 1990; Sudhof and Jahn 1991; Trimble et al. 1991). However, monoamines appear to be stored with neural peptides in larger, dense core vesicles in at least some tissues such as the adrenal gland (and PC12 cells). In contrast to synaptic vesicles, dense core vesicles (or secretory granules in endocrine cells) mediate the relatively slow release of neuromodulators and occur in the cell body and dendrites as well as the nerve terminal. Thus, the site of monoamine storage has profound consequences for its role in signaling. Interestingly, smaller, occasionally dense cored vesicles that cluster over the synapse appear to store monoamines in the CNS.

Since localization of the vesicular amine transporters determines the site of monoamine storage, the authors have examined the distribution of endoge-nous VMAT-1 in the neuroendocrine PC12 cell line using a polyclonal antibody generated against a peptide derived from the C-terminus of the protein. By both immunofluorescence and density gradient centrifugation through several different media, VMAT-1 sorts to dense core vesicles (figure 5), accounting for the pattern of monoamine storage (Liu et al. 1994). Only small amounts of immunoreactive material appear in lighter synaptic-like microvesicles. Thus, in contrast to the numerous peptides that sort to the regulated secretory pathway, VMAT-1 is the first integral mem-brane protein identified that is preferentially expressed on dense core vesicles rather than synaptic vesicles. Since neural peptides apparently sort to this pathway by aggregation (Burgess and Kelly 1987; Chanat and Huttner 1991), the availability of a membrane protein may enable identi-fication of a specific sorting signal. The identification of this signal may then help to explain how aggregated lumenal proteins such as neural peptides sort to the pathway. In addition, VMAT-1 contains signals for endocytosis, accounting for the detection of transport activity using membranes from CHO cells (Liu et al. 1992b). However, the storage of central monoamines in smaller vesicles requires explanation. It may derive from the expression of a transporter (VMAT-2) with distinct sorting sequences or from expression within a different cell type. As a result of sorting to different types of vesicle, monoamines may play distinct roles in synaptic transmission and influence the activity of amphetamines.

CONCLUSIONS

The authors have used selection in the neurotoxin MPP+ to isolate a cDNA clone encoding vesicular amine transport. The protein protects

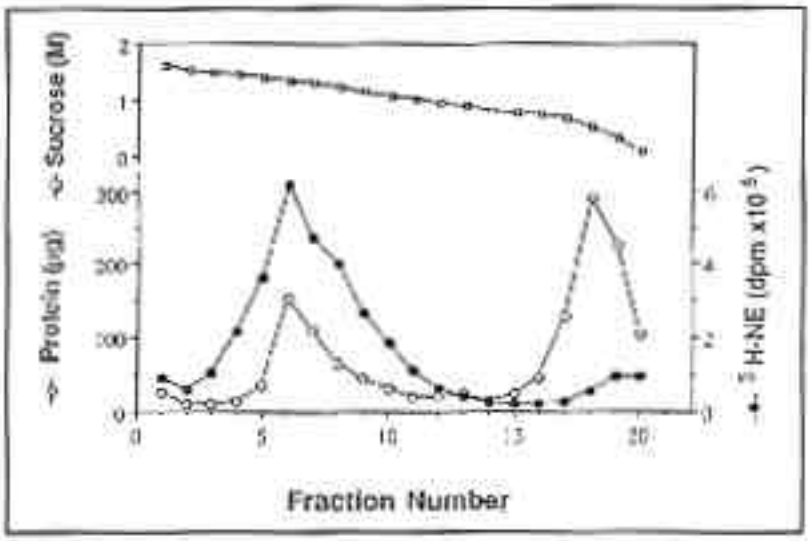

FIGURE 5a. *Distribution of VMAT-1 by equilibrium sedimentation of PC12 membranes through sucrose. The cells were loaded overnight with ^{3}H-norepinephrine, the nuclear debris pelleted, and the supernatant separated by sedimentation through a continuous 0.6 to 1.6 M sucrose gradient. Fractions were collected from the bottom and the accumulation of radioactivity indicates large dense core vesicles centered at fraction 6 (A). Western blot analysis shows that VMAT-1 also centers around fraction 6 together with secretogranin II, away from the peak for synaptic-like microvesicles marked by synaptophysin (B). VMAT-1 occurs in two forms of different molecular weight.*

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against MPP+ by sequestering it in vesicles, away from its primary site of action in mitochondria. Interestingly, the sequence of the cDNA predicts a protein with 12 transmembrane domains but no strong relationship to previously reported sequences other than several bacterial antibiotic-resistant proteins, suggesting evolution from ancient detoxification systems. Molecular cloning has further demonstrated that two distinct

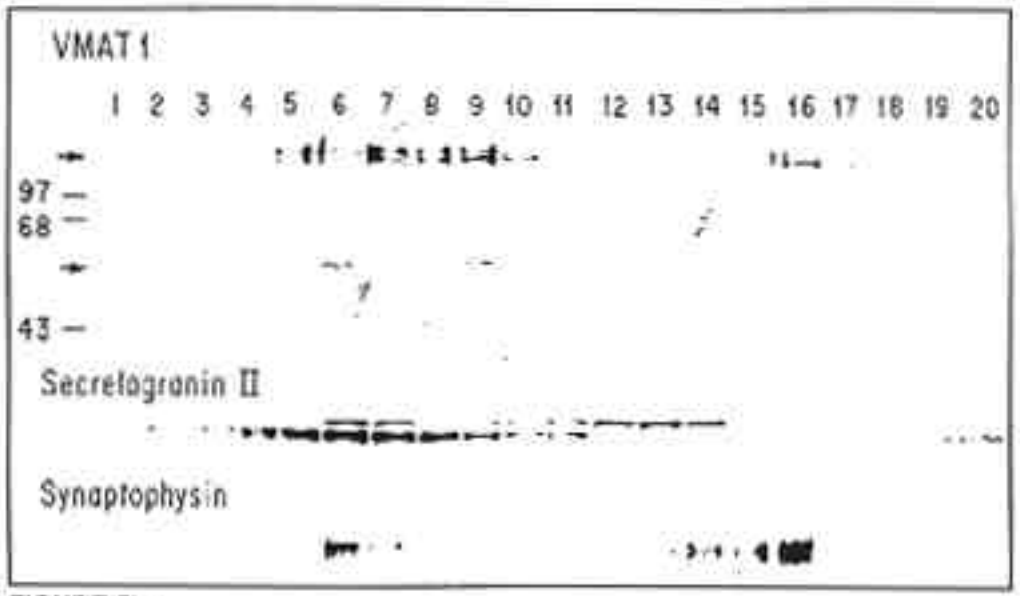
VMAT 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
97
68
43
Secretogranin II
Synaptophysin

FIGURE 5b.

proteins mediate vesicular amine transport in the adrenal gland and central nervous system (VMAT-1 and VMAT-2, respectively). Using membrane vesicles from cells transfected with the two cDNAs, it has been found that they differ in physiological and pharmacological properties, including the interaction with amphetamines. In conjunction with the development of appropriate expression systems and efflux assays, the availability of the cDNA clones will enable scientists to dissect the mechanism of amphetamine action. In particular, does amphetamine-induced efflux involve reversal of the vesicular transporter? Do amphetamines simply dissipate ΔpH or do they interact directly with the transport protein? The storage of monoamines within dense core vesicles and synaptic vesicles will also influence the site of monoamine release and hence the psychostimulant and neurotoxic action of amphetamines. It has been found that VMAT-1 sorts preferentially to dense core vesicles in PC12 cells. To understand the psychostimulant and neurotoxic actions of amphetamines, researchers must now examine the subcellular localization of VMAT-2 in the CNS. In particular, how does the intracellular trafficking differ from VMAT-1 in the adrenal gland and PC12 cells? What is the mechanism of sorting?

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