The Chromaffin Granule and Synaptic Vesicle Amine Transporters Differ in Substrate Recognition and Sensitivity to Inhibitors*

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Classical studies using bovine chromaffin granules have defined the physiologic and pharmacologic properties of the vesicular amine transporter that packages monoamine transmitters into intracellular vesicles for subsequent regulated release. The recent isolation of two distinct but closely related cDNA clones encoding vesicular amine transport suggests that the activity expressed in the brain (synaptic vesicle amine transporter or SVAT) may differ significantly from the previously described adrenal gland activity (chromaffin granule amine transporter or CGAT). A direct comparison of the two transporters now shows that SVAT has a higher affinity than CGAT for monoamine substrates, in particular for histamine. In addition, SVAT shows approximately 10-fold greater sensitivity to tetrabenazine than CGAT. [3H]Dihydrotetabenazine shows no detectable binding to CGAT but does bind to SVAT, accounting for the differential sensitivity. Furthermore, methamphetamine preferentially inhibits transport by SVAT relative to CGAT, apparently by competing at the site of amine recognition rather than by disrupting the vesicular pH gradient. These previously unsuspected differences in the storage of monoamine transmitter in the central nervous system and the adrenal gland may help to account for several classic pharmacological observations.

The regulated release of neurotransmitter depends on its storage within vesicles that fuse with the plasma membrane in response to neural activity. In contrast to neural peptides that sort into a regulated secretory pathway as precursor proteins (DeCamilli and Jahn, 1990; Trimble et al., 1991; Kelly, 1991), the storage of classical neurotransmitters involves transport from the cytoplasm, where synthesis occurs and where transmitter accumulates after removal from the synapse. Previous work has defined four classes of vesicular neurotransmitter transport activity: one for monoamines, another for acetylcholine, a third for glutamate and a fourth for γ-aminobutyric acid and glycine (Sudhof and Jahn, 1991; Edwards, 1992).

Pharmacological observations have helped to define the physiologic role of vesicular monoamine transport. The Rauwolfia alkaloid reserpine inhibits vesicular amine transport, depleting monoamine stores and reducing blood pressure. Reserpine also produces a syndrome resembling depression, suggesting that monoamines may have a role in affective disorders (Friez, 1954). The synthetic inhibitor tetrabenazine has similar but more transient effects than reserpine and depletes predominantly central monoamine stores (Carlsson, 1963). In contrast, psychostimulants such as amphetamines interfere with transmitter storage by releasing large amounts of monoamine into the synapse (Di Chiara and Imperato, 1988; Carboni et al., 1989).

Bovine chromaffin granules express an easily detectable monoamine transport activity and have provided a model system to study vesicular transport (Johnson, 1988). Competition studies show that a single transport activity expressed in these granules recognizes each of the amine transmitters with high affinity. This relative lack of substrate specificity differs from the plasma membrane transporters for norepinephrine, dopamine, and serotonin, each of which has a clear substrate preference (Amara and Kuhar, 1993). In further contrast to plasma membrane transport, which uses the sodium gradient across the plasma membrane to drive cotransport of the neurotransmitter, amine transport into chromaffin granules depends on a pH gradient generated by a vacuolar H⁺-ATPase (Kanner and Schuldiner, 1987; Johnson, 1988) and involves the exchange of two luminal protons for a cytoplasmic amine (Knott et al., 1981). Reserpine and tetrabenazine inhibit amine transport into bovine chromaffin granules, apparently by interacting at two distinct sites (Scherman and Henry, 1984).

Although vesicular amine transport has been studied most extensively in bovine chromaffin granules, a wide range of cell types outside the adrenal medulla express a similar activity. These include mast cells, basophils, and platelets (Henry and Scherman, 1989), as well as monoamine cell populations in the brain (Desnos et al., 1990). Reserpine and tetrabenazine deplete amines in these cell types, but poor access and reduced availability has limited the study of their vesicular amine transport activity.

To understand the mechanism and regulation of vesicular amine transport, we have previously used selection in the neurotoxin MPP⁺ to isolate a cDNA clone encoding a chromaffin granule amine transporter. MPP⁺ is the active metabolite of MPTP, a toxin that injures dopaminergic cells in the substantia nigra to produce a model of Parkinson's disease. Vulnerable cells accumulate MPP⁺ by uptake through a plasma membrane monoamine transporter (Javitch et al., 1985; Snyder et al., 1986). Inside the cell, MPP⁺ enters mitochondria and inhibits oxidative phosphorylation (Ramsay and Singer, 1986; Krueger et al., 1990). The cloned vesicular transporter protects against MPP⁺ by sequestering the toxin away from its primary site of action in the mitochondria (Liu et al., 1992a). The cDNA conferring resistance to MPP⁺ encodes a vesicular transport activity that recognizes all of the amine transmitters, depends on a proton gradient, and shows inhibition by reserpine and to a lesser extent tetrabenazine. The sequence of the cDNA predicts a protein with 12 transmembrane domains and remote homology to several bacterial antibiotic resistance proteins. Northern blot analysis to determine the distribution of the cloned sequence shows expression in the adrenal but not in the central nervous system.
nervous system, suggesting the existence of additional vesicular transporters. Indeed, screening of a brainstem cDNA library under reduced stringency resulted in the isolation of a highly related sequence expressed in the brain and not the adrenal (Liu et al., 1992b; Erickson et al., 1992). The high degree of sequence similarity between the chromaffin granule amine transporter (CGAT) and the brainstem cDNA identified by hybridization under reduced stringency suggests that the brainstem cDNA encodes a vesicular neurotransmitter transporter. Furthermore, the expression of this sequence in brainstem monoamine populations suggests a role in the vesicular transport of monoamines (Liu et al., 1992b). The isolation of these two distinct but closely related cDNAs suggests that monoamine storage in the brain may differ from storage in the adrenal gland.

EXPERIMENTAL PROCEDURES

Transient Expression—The CGAT and SVAT cDNAs cloned into the expression vector CDMS were transfected into COS cells by electroporation. Briefly, COS cells grown in Dulbecco's modified Eagle's medium and 10% fetal calf serum (FCS) were detached from the plate, homogenized at 0.01-mm clearance in cold 0.32 M sucrose, 10 mM HEPES-KOH, (pH 7.4) (SH buffer) containing 5 mM Mg-EGTA, 2 mg/ml leupeptin, and 0.2 mM diisopropyl fluorophosphate. After removal of the cell debris by centrifugation at 3500 g for 5 min, the mixture was transferred to a cold cuvette (0.4-cm gap) (Bio-Rad), electrotommmorphased (0.22 kV, 960 microfarads), returned to ice for 10 min, and then plated in Dulbecco's modified Eagle's medium with 10% FCS. Transport activity was measured 2–4 days after electroporation.

Stable Expression—CHO cells co-transfected with 10 μg of SVAT:CDMS and 1 μg of RSV-neo by the calcium phosphate method (Graham and van der Eb, 1973) were selected for 1 week in 400 ng/ml excess G418 (Life Technologies, Inc.) and then in 1 μg MPP+ for an additional 3 weeks. Before the measurement of transport activity, the transformants were maintained in the absence of MPP+ for at least 2 weeks.

Transport Assay—COS or CHO transfectedants were harvested and homogenized at 0.01-mm clearance in cold 0.32 M sucrose, 10 mM HEPES-KOH, (pH 7.4) (SH buffer) containing 5 mM Mg-EGTA, 2 mg/ml leupeptin, and 0.2 mM diisopropyl fluorophosphate. After removal of the cell debris by centrifugation at 3500 g for 5 min, 10 μl of the supernatant (100 μg of protein) was added to 200 μl of SH buffer containing 4 mM KCl, 2 mM MgSO4, 2.5 mM ATP, and 10–20 nM [3H]serotonin or 44 nM [3H]dopamine (Du Pont NEN) with inhibitors and competitors as indicated and incubated at 25 °C for 2 min, unless indicated otherwise.

The reaction was terminated by dilution into cold SH buffer followed by filtration through 0.2 μm Supor 200 membranes (Gelman). Radioactivity bound to the filter was then measured by scintillation counting in 2.5 ml of CytoScint (ICN). Nonspecific binding was assessed in the presence of 100 μM cold tetrabenazine. Transfected cells expressing the transporters were harvested and transfected with the CDM8 vector alone, whereas CHO transfectedants expressing the transporters accumulated 5–10 times more [3H]serotonin than wild-type CHO cells.

Reserpine Binding—Reserpine binding was measured as described by Rudnick et al. (1990). Membranes were prepared as described above for the transport assay. Aliquots of 10 μl (~100 μg of protein) were added to 1 ml of cold SH buffer containing 4 mM KCl, 4 mM MgSO4, 5 mM ATP, and 2 nM [3H]reserpine. After incubation at 30 °C for 5 min in the case of competition studies, free reserpine was separated from reserpine bound to cell membranes by centrifugation for 2 min using a prepacked 3-ml column of Sephadex LH-20 (Pharmacia LKB Biotechnology Inc.). For the measurement of transporter number, the incubation was continued for 30 min. Radioactivity in the excluded volume was measured by scintillation counting in 5 ml of Ecolume (ICN). Nonspecific binding was assessed in the presence of 2 μM cold reserpine. The measurements were performed in duplicate and repeated two to three times, with the standard error usually less than 10% of the mean. Stable CHO transfectants expressing the transporters bound 5–10 times more [3H]reserpine than wild-type CHO cells.

Tetrabenazine Binding—Wild-type cells and stable transfectedants expressing CGAT and SVAT were homogenized at 0.01-mm clearance in cold 0.15 M NaCl, 0.01 mM K-HEPES, pH 7.4 (HBS). After centrifugation at low speed to remove cell debris, the supernatant was collected by centrifugation, resuspended in 1.2 ml of SH buffer containing 125 μM tetrabenazine, followed by filtration through 0.2 μm Supor 200 membranes (Gelman). The radioactivity bound to the membranes was then determined by scintillation counting in 2.5 ml of CytoScint (ICN). Nonspecific binding was assessed in the presence of 100 μM cold tetrabenazine. Transfected cells expressing the transporters were harvested and transfected with the CDM8 vector alone, whereas CHO transfectedants expressing the transporters accumulated 5–10 times more [3H]serotonin than wild-type CHO cells.

Measurement of ΔpH—Wild-type CHO cells and stable CHO transfectedants expressing CGAT and SVAT were homogenized in SH buffer and a low speed supernatant prepared as described above. Ten μl (~100 μg of protein) of the supernatant was diluted in 4 ml of cold 0.2 M sucrose and 40 mM HEPES-KOH (pH 8.0), containing 2 nM [3H]dihydrotetrabenazine. After incubation at 30 °C for 30 min, 100-μl aliquots were diluted in 4 ml of cold 0.2 M sucrose and 40 mM HEPES-KOH (pH 8.0) containing 125 μM tetrabenazine, followed by filtration through 0.2 μm Supor 200 membranes (Gelman). The radioactivity bound to the membranes was then determined by scintillation counting in 2.5 ml of CytoScint (ICN). Nonspecific binding was assessed in the presence of 100 μM cold tetrabenazine. Transfected cells expressing the transporters were harvested and transfected with the CDM8 vector alone, whereas CHO transfectedants expressing the transporters accumulated 5–10 times more [3H]serotonin than wild-type CHO cells.

To determine whether the cDNA obtained from the brainstem library has transport activity in a quantitative transport assay using membrane vesicles, we transfected COS cells with the cDNA subcloned into an appropriate expression vector, homogenized the cells, and removed the nuclei and debris by centrifugation at low speed. Membrane vesicles prepared from transfected COS cells accumulate increasing amounts of [3H]dopamine with time and the proton ionophore CCCP abolishes transport, as expected for an activity that depends on a pH gradient (Fig. 1). Membranes from cells transfected with the CDMS vector alone accumulate substantially less dopamine.

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1 The abbreviations used are: CGAT, chromaffin granule amine transporter; SVAT, synaptic vesicle amine transporter; FCS, fetal calf serum; CHO, Chinese hamster ovary; CCCP, carbonyl cyanide-m-chlorophenylhydrazone.
Thus, the rat brainstem cDNA encodes a brain SVAT related to CGAT in function as well as predicted structure.

**Substrate Preference**—The existence of two distinct sequences encoding CGAT and SVAT suggests that these vesicular transporters may differ in specific functional characteristics. To compare CGAT and SVAT directly, we prepared membranes from COS cells transiently transfected with each of the two cDNAs. We first compared the kinetics of serotonin transport encoded by the two sequences (Fig. 2). Using incubation for 2 min as a measure of the maximal initial rate of uptake in this heterologous system, CGAT has a lower apparent affinity for the transport of serotonin ($K_m \sim 0.3 \mu M$) than SVAT ($K_m \sim 0.18 \mu M$).

To measure the substrate affinities more accurately, we transfected the SVAT cDNA into CHO cells. Selection in MPP+ resulted in the isolation of stable CHO transformants expressing easily detectable vesicular amine transport activity. The measurement of serotonin transport in the presence of other compounds as inhibitors demonstrated the competitive nature of the interaction (data not shown). Table I shows that both CGAT and SVAT have the highest apparent affinity for serotonin. The affinities ($K_i$) for dopamine, epinephrine, and norepinephrine appear essentially indistinguishable in the case of both transporters. In addition, neither transporter shows inhibition by 3,4-dihydroxyphenylalanine (data not shown). Since mast cells store histamine for regulated release, we also examined the affinity of the two vesicular amine transporters for histamine. Interestingly, CGAT has an extremely low affinity for histamine ($K_i \sim 436 \mu M$), whereas SVAT has an affinity in the range of the other amine substrates ($K_i \sim 3 \mu M$) (Table I).

**Differential Tetrabenazine Sensitivity**—Classical pharmacologic studies show that two major inhibitors of vesicular amine transport, reserpine and tetrabenazine, deplete monoamines in vivo. In contrast to reserpine, tetrabenazine depletes predominantly central rather than peripheral amine stores (Carlsson, 1965). Both drugs inhibit amine transport into bovine chromaffin granules, but reduced access and amounts of tissue have limited the study of drug sensitivity of transporters from other species or from the central nervous system. The availability of cDNA clones encoding both rat CGAT and SVAT now allows us to compare their sensitivity to reserpine and to determine if the differential tetrabenazine sensitivity derives from differences in the transporter or from differences in some other aspect of amine storage such as the cellular environment.

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**Table I**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>CGAT $\mu M$</th>
<th>SVAT $\mu M$</th>
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<tbody>
<tr>
<td>Serotonin</td>
<td>0.85 ± 0.23</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1.56 ± 0.35</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.86 ± 0.11</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2.5 ± 0.4</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td>Histamine</td>
<td>436 ± 36</td>
<td>3.06 ± 1.0</td>
</tr>
<tr>
<td>MPP+</td>
<td>2.8 ± 0.6</td>
<td>1.6 ± 0.45</td>
</tr>
</tbody>
</table>

Reserpine inhibits the transport of $[3H]$serotonin by membranes prepared from COS cells transiently transfected with CGAT (Fig. 3A) with a potency ($IC_{50} \sim 100 \mu M$) in the same range as previously found in CGAT stable transfectants (Liu et al., 1992b). In the same transient expression system, SVAT shows a similar sensitivity to reserpine ($IC_{50} \sim 80 \mu M$). Thus, inhibition of both CGAT and SVAT by reserpine presumably accounts for the depletion of both peripheral and central amine stores by this drug in *vivo*.

Classical studies using bovine chromaffin granules show that tetrabenazine inhibits vesicular amine transport in the nanomolar range (Pletscher, 1977). Previous work using the rat CGAT cDNA indicated considerably reduced sensitivity to tetrabenazine, with the inhibition of transport in the micromolar range (Liu et al., 1992b). This reduced sensitivity may derive from the non-neural environment of CHO fibroblasts in which we have expressed CGAT. To address this possibility, we measured the drug sensitivity of vesicular amine transport in membranes prepared from neuroendocrine rat PC12 cells, the cell line from which the CGAT cDNA originally derives, and found similarly reduced sensitivity to tetrabenazine (data not shown). Thus, heterologous expression in a non-neural cell line does not alter the sensitivity of rat CGAT to tetrabenazine and transient expression of CGAT in COS cells shows a similarly reduced sensitivity to this drug ($IC_{50} \sim 3 \mu M$) (Fig. 3B), with only extremely high concentrations ($50 \mu M$) inhibiting transport completely. Interestingly, SVAT expressed in the same transient system has considerably greater sensitivity to tetrabenazine ($IC_{50} \sim 300 \mu M$), further indicating that the non-neural environment does not account for the reduced sensitivity of CGAT. The differential sensitivity of CGAT and SVAT to this drug also appears to account for its relatively selective depletion of central monoamine stores in some mammals (Carlsson, 1965).

Although both reserpine and tetrabenazine inhibit the transport of amines into vesicles, binding studies indicate that these two inhibitors interact with the bovine chromaffin granule transporter at distinct sites (Darchen et al., 1989). Nonetheless, tetrabenazine can prevent reserpine binding to the transporter, indicating that these two sites interact. Previous studies in stable CHO transfectants also show that tetrabenazine can prevent reserpine binding to CGAT (Schuldiner et al., 1993). To determine whether the differential sensitivity of the two transporters to tetrabenazine extends to the ability of tetrabenazine to prevent reserpine binding to CGAT and SVAT, we have directly compared reserpine binding in the stable CHO transfectants. Tetrabenazine inhibits reserpine binding in membranes expressing SVAT with a greater affinity ($IC_{50} \sim 320 \mu M$) than in membranes expressing CGAT ($IC_{50} \sim 6 \mu M$) (Fig. 4), suggesting...
that the differential potency of tetrabenazine as an inhibitor of transport reflects differences in its ability to interfere with the site of amine recognition.

The differences in the ability of tetrabenazine to displace reserpine could in turn reflect either differences in the mechanism of drug action or in its binding to the transporters. To distinguish between these possibilities, we have also determined the ability of \( ^{3} \text{H} \)-dihydrotetrabenazine to bind to membranes from transfected cells. Dihydrotetrabenazine binds to membranes from stable transformants expressing SVAT. The binding to SVAT saturates in the very low nanomolar range (data not shown). However, dihydrotetrabenazine shows no detectable binding to membranes from stable transformants expressing CGAT. Table II shows equilibrium binding to membranes expressing CGAT and SVAT normalized for transport activity. Thus, differences in tetrabenazine binding account for its increased potency as an inhibitor of transport by SVAT compared to CGAT.

Ketanserin, a serotonin receptor ligand, inhibits vesicular amine transport and is a competitive inhibitor of tetrabenazine binding to the vesicular amine transporter, suggesting that these compounds interact with a similar site on the protein (Darchen et al., 1988). To determine whether the differential sensitivity of CGAT and SVAT to tetrabenazine reflects a general feature of this site or a feature specific to the binding of tetrabenazine, we have determined the ability of ketanserin to inhibit transport of \( ^{3} \text{H} \)-serotonin by CGAT and SVAT. In contrast to tetrabenazine, which shows a 10-fold difference in potency between the two amine transporters, ketanserin inhibits transport with roughly similar potency for both CGAT (IC\(_{50} \sim 12 \, \mu \text{M}) and SVAT (IC\(_{50} \sim 10 \, \mu \text{M}) (\text{Fig. 3C}).

Affinity for MPP\(^+\) and Intrinsic Transport Rate—The ability of CGAT to protect against MPP\(^+\) toxicity suggests that the transporter recognizes this toxin, as well as endogenous monoamine substrates. The relative susceptibility of dopaminergic neurons in the substantia nigra to MPTP compared with resistant adrenomedullary cells raises the possibility that SVAT may recognize MPP\(^+\) differently than CGAT. The ability to select stable SVAT transporters in MPP\(^+\), however, indicates that rat SVAT can also protect against the toxin. Unfortunately, \( ^{3} \text{H} \)-MPP\(^+\) does not show time-dependent saturable uptake in these membrane preparations, possibly due to its rapid equilibration into multiple organelles as a lipophilic cation (Ramsay and Singer, 1986). Substantial endogenous MPP\(^+\) binding or influx complicates the analysis of \( ^{3} \text{H} \)-MPP\(^+\) transport into membranes from transfected cells. In order to compare MPP\(^+\) recognition, we have determined the ability of MPP\(^+\) to inhibit the transport of \( ^{3} \text{H} \)-serotonin by CGAT and SVAT. Using this assay, MPP\(^+\) inhibits the two transporters with similar potency (Table I), at an apparent affinity near the lower end of the range for endogenous monoamine substrates.

To characterize further the interaction between MPP\(^+\) and the transporters, we have also measured the ability of MPP\(^+\) to prevent reserpine binding to both CGAT and SVAT (Table III).
MPP⁺ inhibits reserpine binding to both transporters with an affinity only slightly less than its affinity as an inhibitor of transport and the affinity for SVAT appears slightly (2-fold) higher than for CGAT. Since reserpine appears to bind at the site of amine recognition, these results together with the ability of the transporter to protect against MPP⁺ toxicity (presumably by transporting the toxin into vesicles) support the hypotheses that MPP⁺ interacts with the transporter at a site similar to that for normal substrates and that the affinity of SVAT may even exceed that of CGAT.

Although CGAT and SVAT recognize MPP⁺ with similar affinity, the differential sensitivity of adrenal chromaffin cells and central monoamine populations may derive from differences in the intrinsic rate of monoamine transport. To measure the intrinsic transport rate, the observed transport activity can be related to the number of transporters expressed. Since potential differences in translation complicate the use of mRNA to quantitate transporter number and possible differences in affinity complicate the use of antibody probes, we used reserpine binding to determine transporter number (Schuldiner et al., 1993). In particular, we have used reserpine binding to normalize the serotonin transport activity expressed in stable transformants. With an amount of membranes in the linear range for measurement of both transport activity and reserpine binding, the results show that CGAT and SVAT have intrinsic transport rates of approximately 10/min and 40/min, respectively. Thus, CGAT and SVAT cDNAs from the rat encode transporters recognizing MPP⁺ with similar affinity and with similar rates of serotonin transport. Indeed, SVAT has both a slightly higher affinity and a slightly higher turnover number, suggesting that although we have not directly examined the transport of MPP⁺, differences in these parameters do not appear to account for the differential sensitivity of adrenal and central monoaminergic populations to MPTP, at least in the rat.

**Methamphetamine Sensitivity**—Psychostimulants such as the amphetamines act by inducing the massive release of monoamines into central synapses (Di Chiara and Imperato, 1988). The mechanism by which amphetamine and its derivatives induce the release of amines is not well understood. These drugs may enter monoamine cells through the plasma membrane amine transporters (Rudnick and Wall, 1992a, 1992b, 1992c) and act as weak bases to disrupt intracellular pH gradients, inducing the release of stored monoamines, possibly through diffusion across the vesicular membrane (Sulzer and Rayport, 1980). In the case of 3,4-methylenedioxymethamphetamine (MDMA), exchange across a plasma membrane serotonin transporter then permits efflux of the monoamine into the synapse (Rudnick and Wall, 1992a). It is not clear, however, whether amphetamines interact directly with the vesicular transporter. The transporter may recognize the amphetamines as substrates and drive their accumulation in vesicles, increasing luminal pH and leading to efflux of vesicular contents. Alternatively, extravesicular amphetamine may penetrate the vesicles nonspecifically and induce efflux specifically through the vesicular transporter. Thus, amphetamines may interact either directly or indirectly with the vesicular transporter.

To assess the potential for a specific interaction, we have determined the effect of one amphetamine derivative, methamphetamine, on transport encoded by the rat CGAT and SVAT cDNAs. Inhibition by methamphetamine reproduces the stereospecificity observed in previous studies using a variety of related amphetamines (Innes and Nickerson, 1976; Rudnick and Wall, 1992a), with the (+) stereoisomer more potent than the (-) isomer (Fig. 5, A and B). Interestingly, the transport of [³H]serotonin by SVAT exhibits a much greater sensitivity to (+)-methamphetamine (IC₅₀ ≈ 0.4 µM) than CGAT (IC₅₀ ≈ 7 µM) (Fig. 5C). Thus, the brain transporter appears to show a higher affinity for methamphetamine than the adrenal transporter. However, it is possible that methamphetamine may interfere with transport by disrupting the vesicular pH gradient. While this effect cannot account for the increased potency as an inhibitor of SVAT, it may contribute to the inhibition of CGAT.

To determine the effect of methamphetamine on ΔpH, we have used the pH-sensitive fluorescent dye acridine orange. Using this assay, methamphetamine dissipates the pH gradi-
ent in wild-type CHO cells but only at relatively high concentrations, 2 orders of magnitude higher than the concentrations that inhibit amine transport (data not shown). This suggests that the dissipation of $\Delta$H does not contribute significantly to the inhibition of amine transport by methamphetamine. Furthermore, methamphetamine may inhibit amine transport by interacting with the protein at the site of amine recognition. To address this possibility, we used reserpine binding as a measure of interaction at this site. Methamphetamine competes at high affinity with reserpine for binding (Table III), strongly supporting an interaction at the site of substrate recognition. In addition, the affinity for displacement of reserpine corresponds well with the potency as a transport inhibitor, with the affinity for SVAT significantly greater than that for CGAT.

**DISCUSSION**

The results described here show that a cDNA (SVAT) isolated from a rat brainstem library encodes vesicular amine transport in an assay using membranes prepared from both transiently and stably transfected fibroblasts. The use of this assay has also enabled a direct comparison of the central and adrenal vesicular transport activities and indicates significant differences in substrate affinity, drug binding, and drug sensitivity. In terms of substrate preference, both transporters recognize serotonin with higher affinity than dopamine, epinephrine, or norepinephrine. However, SVAT has a generally higher affinity than CGAT for all substrates. This may derive from the more stringent requirements for packaging of monoamines in the central nervous system as compared to the adrenal gland. The higher affinity of SVAT would help to lower cytoplasmic concentrations of potentially toxic transmitters such as dopamine in central monoamine populations to a greater extent than CGAT, which principally transports less toxic transmitters such as epinephrine.

CGAT and SVAT differ dramatically in their affinity for histamine. Histamine competes with $[3H]$serotonin for transport by SVAT with an affinity similar to that for the catecholamine and indoleamine substrates, whereas it inhibits transport by CGAT at only extremely high concentrations. This marked difference in substrate recognition between the two vesicular amine transporters strongly suggests a specialized role for SVAT in histamine transport in vivo. Indeed, immunostaining indicates the expression of SVAT but not CGAT by mast cells. Thus, differences in the pattern of expression correlate with differences in substrate recognition by the two transporters. The absence of hydroxyl groups in histamine further suggests that SVAT does not require this substituent for substrate recognition. CGAT may require hydroxyl groups for recognition, possibly accounting for additional differences between the two transporters.

Reserpine inhibits the transport of $[3H]$serotonin by both CGAT and SVAT with a potency in the nanomolar range. This result in vitro correlates with the known pharmacology in vivo, where reserpine depletes both peripheral and central monoamine stores. Previous studies have also shown that reserpine binds essentially irreversibly to the transporter (Rudnick et al., 1990), presumably accounting for the long duration of its effects in vivo. In contrast, tetrabenazine dissociates more rapidly from the transporter and affects primarily central stores in vivo (Carlsson, 1965). Since bovine chromaffin granules show great sensitivity to tetrabenazine (Johnson, 1988), the observed differences in sensitivity to tetrabenazine between adrenal and central amine stores were attributed to differences in the amount of time required to fill the two stores of monoamine (Scherman and Boschi, 1988). Chromaffin granules contain much larger amounts of monoamine than related vesicles in the brain and so take longer to fill. The short-acting tetrabenazine would presumably not deplete them as effectively as the longer-acting reserpine. However, we now show that rat SVAT has a much greater sensitivity to tetrabenazine than rat CGAT, in terms of both the inhibition of transport and the inhibition of reserpine binding. Thus, preferential inhibition of SVAT presumably accounts for the predominant depletion of central monoamine stores by tetrabenazine in vivo. In addition, the reduced sensitivity of CGAT to tetrabenazine does not constitute an artifact of the heterologous expression system. Rather, the rat chromaffin granule transporter exhibits a much lower sensitivity to tetrabenazine than the previously characterized bovine transporter. Thus, in terms of tetrabenazine sensitivity, the bovine chromaffin granule transporter resembles rat SVAT rather than CGAT. Interestingly, the sequence of a bovine cDNA predicts a protein more closely related to rat SVAT than rat CGAT.

The differential binding of CGAT and SVAT to dihydrotetrabenazine coincides with previous studies of tetrabenazine binding in different tissues to make several predictions about the distribution of these two transport activities. As expected, tetrabenazine binds with high affinity to membranes prepared from the brain (Scherman, 1986), which has been shown to express SVAT mRNA (Liu et al., 1992b). In addition, tetrabenazine binds with high affinity to sympathetic neurons (Desnos et al., 1990), suggesting that these cells also express SVAT. Indeed, preliminary observations do suggest that post-ganglionic sympathetic neurons, unlike the embryologically related adrenal gland, express SVAT rather than CGAT.

In terms of the mechanism of tetrabenazine action, we have found that the differential potency as an inhibitor of transport by CGAT and SVAT simply reflects differences in the drug’s ability to bind to the two proteins. However, we also show that ketanserin, which interacts with the transporter at the same site as tetrabenazine, does not inhibit CGAT and SVAT differentially, indicating specific differences in the interaction with tetrabenazine rather than in the site as a whole.

Drug binding studies also enable the experimental determination of transporter number and hence turnover rate. Using reserpine to assess the number of transporters in stable transfectants, we estimate a turnover number of 10–40/min. Tetrabenazine binding yields a similar turnover number for SVAT indicating that the two drugs bind with approximately the same stoichiometry. (Tetrabenazine cannot be used to measure the turnover number for CGAT since the drug shows no detectable binding to this protein.) The turnover number falls short of the 2½ previously reported for bovine chromaffin granules (Scherman and Boschi, 1988) but coincides well with the values obtained using the purified bovine transporter (Stern-Bach et al., 1990) and brain regions (10–35/min) (Scherman, 1986). In addition, CGAT and SVAT show similar turnover numbers, making it unlikely that differences in the rate of transport account for the selective vulnerability of central monoamine populations in both MPTP toxicity and idiopathic Parkinson’s disease.

Using the rat CGAT and SVAT cDNA clones, we have found that methamphetamine interacts specifically with both CGAT and SVAT. Although work with other amphetamines has suggested inhibition of transport by interference with $\Delta$H, the similar potency of methamphetamine as an inhibitor of trans-
port and as an inhibitor of reserpine binding shown here suggests that the stimulant interacts directly with the transporter. Furthermore, methamphetamine inhibits vesicular amine transport by both CGAT and SVAT with the expected stereospecificity but has an approximately 15-fold greater potency for SVAT than CGAT. Interestingly, in vivo studies suggest that small doses of methamphetamine have a predominantly central rather than peripheral effect (Innes and Nickerson, 1975). The differential affinity of methamphetamine for SVAT and CGAT may account for these selective effects and suggests that vesicular amine transport may play a crucial role in the action of amphetamines.

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Vesicular Amine Transport: CGAT Versus SVAT