The Neurotoxin 1-Methyl-4-phenylpyridinium Is a Substrate for the Canalicular Organic Cation/H⁺ Exchanger

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ABSTRACT
Hepatic organic cation transport consists, in part, of carrier-mediated sinusoidal uptake stimulated by an inside-negative membrane potential and canalicular excretion driven by electroneutral organic cation/H⁺ exchange. Intracellular organic cation transport involves sequestration into acidified organelles, also mediated by organic cation/H⁺ exchange. A sinusoidal organic cation transporter has been cloned; however, canalicular organic cation transport has not been characterized at the molecular level. On the assumption that hepatic organic cation/H⁺ exchange resembles monoamine transport in synaptic vesicles, we examined, using canalicular rat liver plasma membrane vesicles, the transport of 1-methyl-4-phenylpyridinium (MPP⁺), a neurotoxin taken up by a synaptic vesicular monoamine transporter that has been cloned. Under voltage-clamped conditions, an outwardly directed H⁺ gradient stimulated [³H]MPP⁺ uptake, compared with uptake under pH-equilibrated conditions, consistent with electroneutral MPP⁺/H⁺ exchange. Substrates for canalicular organic cation/H⁺ exchange cis-inhibited pH-dependent MPP⁺ uptake. Equilibrium exchange of [¹⁴C]tetraethylammonium was inhibited by MPP⁺ in a concentration-dependent manner, consistent with a direct interaction of MPP⁺ with the organic cation carrier. Carrier-mediated MPP⁺ uptake exhibited saturability, with kinetic parameters similar to those described for canalicular tetraethylammonium/H⁺ exchange. Canalicular [³H]MPP⁺ uptake was ATP-independent and, thus, distinct from P-glycoprotein-mediated efflux. The finding that MPP⁺ is a substrate for canalicular organic cation/H⁺ exchange is applicable to studies, using degenerate oligonucleotides complementary to sequences conserved in neurotransmitter transporters, aimed at cloning this transporter.

The liver plays an essential role in the uptake and elimination of a wide range of endobiotics and xenobiotics, including secondary, tertiary or quaternary amines bearing a net positive charge on one or more nitrogen groups at physiological pH. Previous studies, using isolated perfused liver (Meijer et al., 1970) and isolated hepatocytes (Eaton and Klaassen, 1978; Mol et al., 1988), established that the hepatobiliary transport of organic cations is a carrier-mediated transport process, dependent on physicochemical features such as lipophilicity and molecular weight. Recent studies in isolated liver plasma membrane vesicles have begun to characterize both the driving force for carrier-mediated hepatic organic cation uptake and the mechanism for canalicular organic cation excretion. At least three sinusoidal organic cation transport processes have been identified. Sinusoidal uptake of the endogenous organic cation N¹-methyl-2-pyrrolidone was found to be mediated by an electroneutral organic cation/H⁺ antiport (Moseley et al., 1990). In addition, a thiamine/H⁺ antiport is present on the sinusoidal membrane that is, on the basis of substrate specificity studies, distinct from N¹-methyl-2-pyrrolidone/H⁺ exchange (Moseley et al., 1992b). In contrast, transport of the exogenous organic cation TEA across the sinusoidal membrane was found to be a carrier-mediated process stimulated by an inside-negative membrane potential, whereas canalicular transport was driven by an organic cation/H⁺ antiport (Moseley et al., 1992a). Canalicular organic cation/H⁺ exchange may reflect, in part, the exocytic insertion of this transporter from an acidified intracellular compartment to this membrane domain. ATP-dependent organic cation uptake has been demonstrated in both purified rat liver multivesicular bodies, i.e., prelysosomal endocytic vesicles that are acidified by an electrogenic H⁺-ATPase (Van Dyke et al., 1992), and lysosomes (Moseley and Van Dyke, 1995). Intracellular sequestration of organic cations into acidified compartments in this manner may reduce the free cytoplasmic concentration

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ABBREVIATIONS: biLPM, basolateral liver plasma membrane; cLPM, canalicular liver plasma membrane; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MPP⁺, 1-methyl-4-phenylpyridinium; TBUA, tributylmethylammonium; TEA, tetraethylammonium; VMAT, vesicular monoamine transporter.
of organic cations to values at or below their Nernst potentials and may account for the absence of an active transport process for certain organic cations at the sinusoidal membrane domain.

Using functional expression cloning in Xenopus oocytes, an organic cation transporter (OCT1) has been identified (Grundemann et al., 1994). This cDNA codes for a 62-kDa protein that is not homologous to any other known protein and that has the functional characteristics of basolateral organic cation uptake in kidney and liver (Grundemann et al., 1994). Organic cation uptake mediated by OCT1 is not affected by pH gradients and exhibits a $K_m > 10$ times lower than the apparent $K_m$ of renal brush-border organic cation/H+ exchange (Grundemann et al., 1994). Therefore, organic cation/H+ exchange, localized to the apical membrane where it can govern organic cationic drug excretion, has not been characterized at the molecular level in any epithelium, despite considerable effort, suggesting the need for alternative strategies.

The process of intraneuronal sequestration of neurotransmitters is mediated by the vesicular neurotransmitter transporters, among which the VMATs have been the best studied (Schuldiner et al., 1995). The VMATs use a H+ electrochemical gradient generated by a vacuolar-type H+-ATPase to couple efflux of two H+ ions with neurotransmitter uptake into synaptic vesicles. Several human, bovine and rat VMATs have been cloned (Erickson et al., 1992; Liu et al., 1992). Shared features of canalicular and lysosomal organic cation/H+ exchange (Moseley and Van Dyke, 1995; Moseley et al., 1992a) have led us to speculate that hepatic organic cation transport resembles monoamine transport in synaptic vesicles. Therefore, in this study we have examined, using rat cLPM vesicles, the transport of MPP+ , a neurotoxin that causes parkinsonism-like symptoms and that is taken up in exchange with H+ (Moriyama et al., 1993) by VMATs. By demonstrating that MPP+ , a substrate for VMATs, is also a substrate for canalicular organic cation/H+ exchange, we have taken the initial step in a strategy that may lead to the cloning of the hepatic organic cation/H+ exchanger.

**Methods**

**Materials.** N-[methyl-3H]MPP+ (80 Ci/mmol), [3H]TEA bromide (3.0 mCi/mmole) and [6-3H]taurocholic acid (2.1 Ci/mmole) were purchased from DuPont-New England Nuclear (Boston, MA). Meiperphenidol [Darstine, (1,3-hydroxy-5-methyl-4-phenylhexyl)-1-methylpyridinium bromide)] was a gift from Merck, Sharp & Dohme. TBuMA was purchased from Fluka Chemical (Ronkonkoma, NY). All other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Valinomycin and carbonyl cyanide p-trifluoromethoxyphenylhydrazone were stored in absolute ethanol (Aaper Alcohol and Chemical, Shelbyville, KY). When used, ethanol was also added to controls; the total concentrations of ethanol in membrane vesicle suspensions were identical and did not exceed 0.25% (v/v). All water used in preparing media was deionized, and all solutions were filtered through 0.22-μm Millipore filters before use.

**Preparation of rat liver plasma membrane vesicles.** The method for isolation of bLPM and cLPM vesicles, as well as their biochemical and morphological characterization, has been described in detail elsewhere (Meier et al., 1984b; Moseley et al., 1986). These studies have demonstrated minor contamination with intracellular organelles and nearly complete separation of cLPM from bLPM vesicles, as reflected by the virtual absence of Na+,K+-ATPase activity, glucagon-stimulatable adenylate cyclase activity and intact secretory components in cLPM vesicles (Meier et al., 1984b). Freeze-fracture analysis has revealed that ~80% of cLPM and ~73% of bLPM vesicles exhibit right-side-out configuration (Meier et al., 1984a; Moseley et al., 1986), in which the extravesicular membrane face corresponds to the bile luminal or sinusoidal surface, respectively, in vivo. Immediately after isolation, membranes were suspended in the desired incubation medium (exact composition is designated in the figure and table legends), at a protein concentration of 5 to 10 mg/ml, and stored at ~70°C before transport studies. Protein concentration was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard. Interference of various buffer solutions with the protein assay was accounted for by determining separate standard curves for each buffer system.

**Lysosome preparation.** Lysosomes were purified from fasting male rat livers as previously described (Moseley and Van Dyke, 1995; Van Dyke, 1993; Yamada et al., 1984), 18 hr after i.v. injection of 60 mg of fluoroescin isothiocyanate-dextran dissolved in 0.9% saline. Lysosomes obtained in this manner appear to be free of contamination by other organelles when assessed by electron-microscopic morphological or marker enzyme analysis (Van Dyke, 1993; Yamada et al., 1984). Lysosomes were resuspended in ice-cold buffer containing 140 mM potassium glutonate, 70 mM sucrose and 30 mM Bis-Tris, pH 7.0. Lysosomes were kept at 4°C for 1 to 2 hr before use, to allow equilibration of proton gradients, and were used fresh within 4 hr of preparation. Protein concentrations were measured by the method of Lowry et al. (1951).

**Transport measurements.** Frozen membrane vesicle suspensions were rapidly thawed by immersion in a 37°C water bath, diluted to the desired protein concentration (3–5 mg/ml) and vesiculated by aspiration 10 times through a 25-gauge needle. Transmembrane transport of [3H]MPP+ (0.25 μCi/sample), [3H]TEA (0.1 μCi/sample) and [3H]taurocholate (0.25 μCi/sample) was measured by a rapid Millipore filtration technique. Uptake into 20 μl of membrane vesicle suspension was initiated at 25°C by addition of 80 μl of reaction medium containing radiolabeled substrate. The exact composition of the reaction media is given in the figure and table legends for the individual experiments. After incubation for the designated time intervals, transport was terminated by the addition of 3 ml of ice-cold stop solution, consisting of 204 mM sucrose, 150 mM potassium glutonate, 10 mM HEPEs/Tris, pH 7.5, 5 mM magnesium glutonate, 0.2 mM calcium glutonate and 1 mM TBuMA (for uptake studies involving pH gradients); 175 mM sucrose, 150 mM potassium glutonate, 10 mM HEPEs/Tris, pH 7.5, 5 mM magnesium glutonate, 0.2 mM calcium glutonate and 1 mM TBuMA (for electrogenicity studies); 250 mM sucrose, 10 mM Tris, pH 7.4, 0.2 mM CaCl2, 10 MgCl2 and 1 mM TBuMA (for ATP-dependency studies). For studies of the lysosomal uptake of [3H]MPP+, uptake buffer contained 140 mM potassium glutonate, 70 mM sucrose, 30 mM Tris, pH 7.0, and an ATP-regenerating system (9 mM phosphoenolpyruvate, 2.5 mM NADH, 16 U/ml pyruvate kinase and 19 U/ml lactate dehydrogenase), and uptake was initiated by addition of lysosomes to buffer containing [3H]MPP+ and either 5 mM magnesium glutonate or 5 mM MgATP. Aliquots of the incubation mixture were removed at designated times, and uptake was terminated by the addition of 3 ml of ice-cold stop solution, consisting of 140 mM potassium glutonate, 70 mM sucrose and 30 mM Tris, pH 7.0. Membrane vesicle- and lysosome-associated ligands were separated from free ligand by filtration under vacuum through a 0.45-μm Millipore filter (type HAWP; Millipore Corp., Bedford, MA) that had been presoaked in stop solution. The filter was washed twice with 3 ml of stop solution, dissolved in Redisolv HP (Beckman Instruments, Palo Alto, CA) and counted in a Beckman LS 1801 liquid scintillation counter. Nonspecific binding of isotope to filter and membrane vesicles (determined in each experiment by addition, at 0–4°C, of incubation medium and stop solution to 20 μl of membrane suspension) was subtracted from all vesicle uptake determinations. Nonspecific binding of isotope to filter and lysosomes (determined in duplicate at time 0) was subtracted from all lysosome uptake determinations. Unless otherwise
indicated, all incubations were performed in triplicate, and all observations were confirmed with three or more separate preparations.

Data analysis. All values are expressed as mean ± S.E. The data were compared by Student’s t test; differences were considered to be statistically significant at P < .05.

Results

The effect of an outwardly directed H+ gradient on \[^{3}H\]MPP\(^{+}\) uptake was examined first in cLPM vesicles (fig. 1). Uptake of 1 μM MPP\(^{+}\) in the presence of an outwardly directed H\(^{+}\) gradient (pH\textsubscript{in} 5.9/pH\textsubscript{out} 7.9), under voltage-clamped conditions (K\textsuperscript{+} \textsubscript{in} = K\textsuperscript{+} \textsubscript{out} in the presence of the K\textsuperscript{+} ionophore valinomycin at 5 μg/mg protein), was significantly greater than uptake under pH-equilibrated (pH\textsubscript{in} 7.9/pH\textsubscript{out} 7.9) conditions. Intravesicular volume, as reflected by equilibrium uptake values, was similar in the presence and absence of a pH gradient. As an additional control to exclude an effect of pH per se on MPP\(^{+}\) uptake, uptake of 1 μM MPP\(^{+}\) was measured, in separate experiments, under pH\textsubscript{in} 5.9/pH\textsubscript{out} 5.9 conditions. As also shown in figure 1, MPP\(^{+}\) uptake in the presence of an outwardly directed H\(^{+}\) gradient (pH\textsubscript{in} 5.9/pH\textsubscript{out} 7.9) was significantly greater than uptake under these pH-equilibrated conditions.

As additional evidence for the presence of carrier-mediated MPP\(^{+}\) transport in cLPM vesicles, we next examined whether the presence of unlabeled intravesicular organic cationic substrate was capable of stimulating the uptake of extravesicular \[^{3}H\]MPP\(^{+}\) (trans-stimulation). As shown in table 1, in the absence of a pH gradient, the initial rates of 1 mM \[^{3}H\]MPP\(^{+}\) uptake were significantly greater in cLPM vesicles preloaded with 5 mM TEA. Equilibrium uptake values (60 min) were similar in the presence or absence of intravesicular TEA.

For cis-inhibition studies, the effects of several organic cations on pH-dependent MPP\(^{+}\) uptake in cLPM vesicles were examined (table 2). Substrates for canalicular organic cation/H\(^{+}\) exchange, including TEA (Moseley et al., 1992a), TBuMA (Moseley et al., 1996), darstine, primaquin and de-cyinum (Moseley and Van Dyke, 1995), significantly inhibited pH-dependent MPP\(^{+}\) uptake. N-Methylnicotinamide, an endogenous organic cation that is not a substrate for canalicular organic cation/H\(^{+}\) exchange, had no effect on pH-dependent MPP\(^{+}\) uptake. These findings are consistent with MPP\(^{+}\) being a substrate for canalicular organic cation/H\(^{+}\) exchange. Because it is possible that these organic cations exert cis-inhibitory effects on pH-dependent MPP\(^{+}\) uptake by dissipation the H\(^{+}\) gradient, rather than by directly interacting with the organic cation carrier, the effect of MPP\(^{+}\) on TEA uptake under equilibrium exchange conditions was also examined. As shown in figure 2, TEA uptake measured under equilibrium conditions ([TEA]\textsubscript{in} = [TEA]\textsubscript{out} = 1 mM and pH\textsubscript{in} = pH\textsubscript{out} = 7.4) was inhibited by MPP\(^{+}\) in a concentration-dependent manner. Inhibition of TEA uptake by MPP\(^{+}\) in the absence of a H\(^{+}\) gradient is consistent with a direct interaction of MPP\(^{+}\) with the canalicular organic cation/H\(^{+}\) exchanger.

Canalicular membrane vesicles exhibit ATP-dependent transport of daunomycin and other cytotoxic drugs, mediated by P-glycoprotein (Kamimoto et al., 1989). In other epithelia, P-glycoprotein-mediated multidrug resistance is characterized by a broad range of structurally heterogeneous substrates, including hydrophobic organic cations of low molecular weight (Nogae et al., 1989; Pearce et al., 1989). Recently, ATP-dependent transport of certain bulky aromatic organic cations across the canalicular membrane was described (Muller et al., 1994). However, canalicular transport of TEA and TBUVA was found to be ATP-independent (Moseley and Van Dyke, 1995; Moseley et al., 1996). Therefore, in the absence of a pH gradient, the effect of ATP on the time course of MPP\(^{+}\) uptake was examined. As shown in table 3, there was no significant difference in MPP\(^{+}\) uptake in the presence of ATP and an ATP-regenerating system vs. MPP\(^{+}\) uptake in the absence of ATP. As a control, the effect of ATP on the time course of \[^{3}H\]taurocholate uptake was also examined. As previously shown (Nishida et al., 1991), taurocholate uptake was significantly enhanced in the presence of ATP. These results suggest that the canalicular transport of MPP\(^{+}\) is also ATP-independent and distinct from P-glycoprotein-mediated drug efflux.

The kinetic features of pH-dependent MPP\(^{+}\) transport were also determined. In cLPM vesicles, the concentration dependence of the initial uptake rates was studied over a MPP\(^{+}\) concentration range of 0.001 to 1 mM. Carrier-mediated uptake was determined by subtracting a diffusional component of uptake (\[^{3}H\]MPP\(^{+}\) uptake under pH-equilibrated conditions) from total uptake. As shown in figure 3, carrier-mediated uptake exhibited saturability with increas-
ing concentrations of MPP⁺. The data in figure 3 (inset) are presented as an Eadie-Hofstee plot (initial velocity/substrate concentration vs. initial velocity), demonstrating a single transport system with an apparent $K_m$ of 0.3 mM and an apparent $V_{max}$ of 0.4 nmol/mg protein/15 sec.

Hepatic lysosomes sequester certain organic cationic drugs, most likely via an organic cation/H⁺ antiport driven by H⁺-ATPase, with characteristics of canaliculic organic cation/H⁺ exchange (Moseley and Van Dyke, 1995). These observations led us to speculate that this intracellular compartment may undergo exocytosis at the canaliculic membrane domain, resulting in the insertion of an organic cation/H⁺ exchanger that would favor net organic cation excretion into bile. As shown in figure 4, the uptake of 10 μM [³H]MPP⁺ by rat liver lysosomes was also significantly increased by the presence of ATP in the incubation buffer.

Sinusoidal uptake of TEA (Moseley et al., 1992a) and TBuMA (Moseley et al., 1996) is stimulated by an inside-negative membrane potential. The effect of membrane potential on MPP⁺ transport in bILPM vesicles was, therefore, also examined. As demonstrated in figure 5, MPP⁺ uptake in bILPM vesicles was significantly greater in the presence than in the absence of a valinomycin-induced intravesicular negative K⁺ diffusion potential.

**Discussion**

The present study demonstrates that hepatic MPP⁺ transport resembles that previously described for carrier-mediated TEA and TBuMA transport (Moseley et al., 1992a, 1996). In cILPM vesicles, transport is mediated by coupled exchange of MPP⁺ for H⁺, whereas MPP⁺ uptake in bILPM vesicles is mediated by an electrically conductive pathway. The substrate specificity and kinetic features of MPP⁺/H⁺ exchange in cILPM vesicles are similar to those described for canaliculic organic cation/H⁺ exchange (Moseley and Van Dyke, 1995; Moseley et al., 1992a, 1996). MPP⁺ inhibits TEA uptake in a concentration-dependent manner in cILPM vesicles in the absence of a H⁺ gradient, consistent with a direct interaction of MPP⁺ with the canaliculic organic cation/H⁺ exchanger. Furthermore, rat liver lysosomes sequester
MPP\(^+\) in the presence of ATP, similar to processes described for TEA (Moseley and Van Dyke, 1995) and TBU MA (Van Dyke et al., 1992), which most likely involves an organic cation/H\(^+\) antiport with characteristics of canalicular organic cation/H\(^+\) exchange. Although the hepatic transport of MPP\(^+\) has not been characterized to the same extent as that of other organic cations (Moseley and Van Dyke, 1995; Moseley et al., 1992a, 1996), these results establish MPP\(^+\) as a substrate for hepatic organic cation transport processes and, in particular, canalicular organic cation/H\(^+\) exchange.

MPP\(^+\) and its analog 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine are also substrates for the organic cation/H\(^+\) exchanger in renal brush-border membrane vesicles (Lazaruk and Wright, 1990; Sokol et al., 1987). The finding that MPP\(^+\) is a substrate for the hepatic transporter serves to reinforce the similarities in organic cation transport by these two epithelia. Studies in isolated renal brush-border and basolateral membrane vesicles have demonstrated that carrier-mediated organic cation transport mediated across the basolateral membrane is driven by an inside-negative potential difference, whereas apical transport is mediated by electroneutral organic cation/H\(^+\) exchange (Pritchard and Miller, 1993; Ulrich, 1994). Intracellular transport of organic cations in the kidney involves sequestration into acidified compartments mediated by organic cation/H\(^+\) exchange (Pritchard et al., 1994), a process that is recapitulated in the liver (Moseley and Van Dyke, 1995; Van Dyke et al., 1992).

MPP\(^+\) accumulates within isolated rat hepatocytes, resulting in cell death (Singh et al., 1988). Intracellular calcium release from mitochondria plays a role in MPP\(^+\)-induced hepatotoxicity (Kass et al., 1988), and MPP\(^+\) uptake, driven by the membrane potential, has been reported in liver mitochondria (Ramsay and Singer, 1986). The mechanisms, however, for the hepatic uptake of MPP\(^+\) have not been previously defined in a membrane vesicle model. Using functional expression cloning in Xenopus laevis oocytes, a rat renal organic cation transporter (OCT1) was recently identified, with features, including potential-dependent but pH-independent uptake of TEA, characteristic of basolateral organic cation uptake in the proximal tubule (Grundemann et al., 1994). MPP\(^+\) effectively inhibited TEA uptake in OCT1

### TABLE 3

**Effect of ATP on MPP\(^+\) and taurocholate uptake in cLPM vesicles**

Membrane vesicles were incubated with 250 mM sucrose, 10 mM HEPES/Tris, pH 7.4, 0.2 mM CaCl\(_2\). The time course of uptake of 1 \(\mu\)M [\(^3\)H]MPP\(^+\) and 1 \(\mu\)M [\(^3\)H]taurocholate was measured at 37°C in medium containing 250 mM sucrose, 10 mM HEPES/Tris, pH 7.4, 0.2 mM CaCl\(_2\), 10 MgCl\(_2\) and an ATP-regenerating system (3 mM creatine and 3.6 \(\mu\)g creatine phosphokinase), without (∓ATP) or with (∽ATP) 1.2 mM ATP. Data are means ± S.E. of quadruplicate analyses of three separate membrane vesicle preparations.

<table>
<thead>
<tr>
<th>Condition</th>
<th>MPP(^+) Uptake</th>
<th>Taurocholate Uptake</th>
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<tbody>
<tr>
<td></td>
<td>30 sec</td>
<td>60 sec</td>
</tr>
<tr>
<td>−ATP</td>
<td>6.6 ± 0.8</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td>+ATP</td>
<td>4.2 ± 1.0</td>
<td>4.3 ± 0.6*</td>
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* \(P < .05\).

** \(P < .005\).
cRNA-injected oocytes (Grundemann et al., 1994). Northern blot analysis and in situ hybridization suggest that this transporter is also expressed in liver and intestine (Grundemann et al., 1994). If so, it is distinctly possible that OCT1 mediates sinusoidal MPP⁺ uptake, as described in this study. The recent description of the hepatic uptake of MPP⁺ by cultured rat hepatocytes and human embryonic kidney cells heterologously expressing OCT1 (Martel et al., 1996) is consistent with our findings in bLPM vesicles. However, in all studies to date, pH-dependent organic cation transport has not been functionally expressed in the Xenopus oocyte model (Grundemann et al., 1994; Hori et al., 1992), and alternative strategies may be required to clone organic cation/H⁺ exchangers.

Synaptic transmission involves the regulated release of neurotransmitters stored in subcellular organelles into the synaptic cleft, followed by reuptake of neurotransmitters back to the presynaptic terminal or into glial elements. The process of intraneuronal sequestration is mediated by the vesicular neurotransmitter transporters, among which the VMATs have been the best studied (Schuldiner et al., 1995). Whereas neurotransmitter reuptake, except in the case of acetylcholine, is a Na⁺-dependent process, the VMATs use an H⁺ electrochemical gradient generated by a vacuolar-type H⁺-ATPase to couple efflux of two H⁺ ions with neurotransmitter uptake. Several human, bovine and rat VMATs have been cloned. VMAT1, expressed only in adrenal medulla, confers resistance to MPP⁺ when transfected into Chinese hamster ovary cells (Liu et al., 1992). A transporter similar to but distinct from VMAT1 has been identified in brain (VMAT2) (Erickson et al., 1992; Liu et al., 1992). The predicted sequences of the VMATs show significant homology with a growing class of drug-resistant proteins from prokaryotes and eukaryotes that, on the basis of a dependence of an H⁺ electrochemical gradient for transport activity, have been termed toxin-extruding antiporters, or TEXANs (Schuldiner, 1994). Thus far, only the VMATs have been identified as mammalian members of the TEXAN family (Schuldiner, 1994). However, it is possible that the mammalian TEXANs play more of a role in the whole organism, other than neurotransmitter transport. Our finding that VMATs and canalicular organic cation/H⁺ exchange share MPP⁺ as a substrate establishes the validity of a strategy, using degenerate oligonucleotides complementary to sequences conserved in these neurotransmitter transporters, aimed at cloning canalicular organic cation/H⁺ exchange.

In conclusion, the neurotoxin MPP⁺ is a substrate for hepatic organic cation transport processes and, in particular, canalicular organic cation/H⁺ exchange, as previously described for TEA and TBuMA. By taking advantage of our understanding of the molecular mechanisms of monoamine transport in synaptic vesicles, which shares features with canalicular organic cation/H⁺ exchange, these findings provide a strategy that may lead to the cloning of this transporter.

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References

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