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Effect of P-glycoprotein modulators on the human extraneuronal monoamine transporter

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Abstract

The aim of this work was to investigate the effect of P-glycoprotein modulators on human extraneuronal monoamine transporter (EMT)-mediated transport. The experiments were performed using a cell line from human embryonic kidney (HEK293 cells) stably transfected with pcDNA3hEMT (293_{hEMT}), or with pcDNA3 alone ($293_{control}$). Of the P-glycoprotein modulators tested, rhodamine123, verapamil and daunomycin concentration-dependently inhibited EMT-mediated uptake of [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP^+). The corresponding IC_{50}'s were found to be 3.6, 37 and 130 μM, respectively. By contrast, vinblastine, digitoxin and cyclosporine A were devoid of effect. The endogenous organic cation tyramine, but not choline, inhibited EMT-mediated transport (IC_{50} of 468 μM). Moreover, L-arginine and L-histidine (up to 1 mM) did not affect [^{3}H]MPP^+ uptake. Finally, MPP^+ and tyramine *trans*-stimulated [^{3}H]MPP^+ uptake, but rhodamine123 had no effect, and verapamil and daunomycin *trans*-inhibited [^{3}H]MPP^+ uptake. In conclusion, this study shows that several cationic modulators of P-glycoprotein inhibit EMT-mediated transport. As a consequence, the interaction of P-glycoprotein modulators with EMT must be taken into account, and the consequences of this interaction must not be forgotten when using such drugs in vivo.

Keywords: EMT (Extraneuronal monoamine transporter); P-glycoprotein; Organic cation; MPP^+ (1-methyl-4-phenylpyridinium)

1. Introduction

The physiological actions of released catecholamines are terminated by transmembrane transport proteins. These transporters actively remove catecholamines from the extracellular space, thus decreasing their concentration at the receptor level. Several transport systems for these neurotransmitters have been described. The first to be recognised were $uptake_1$, a Na^+-driven, high-affinity, cocaine-sensitive neuronal transporter, and $uptake_2$, a Na^+-independent, low-affinity, high-capacity, corticosterone-sensitive extraneuronal transporter. Recently, other Na^+-independent transmembrane transporters were also found to accept catecholamines as substrates. These include the organic cation transporter type 1 (OCT1; Breidert et al., 1998) and the organic cation transporter type 2 (OCT2; Gründemann et al., 1998a).

$Uptake_2$ was first described more than 30 years ago (Iversen, 1965), but its primary structure was only recently elucidated (Gründemann et al., 1998b; Kekuda et al., 1998). After the molecular cloning of $uptake_2$, this transporter (now renamed extraneuronal monoamine transporter, EMT) was found to be a member of the amphiphilic solute facilitator (ASF) family. The ASF family of transporters constitutes a recently described protein family that includes transmembrane transporters of organic anions (OAT1) and organic cations (e.g. OCT1, OCT2) (Gründemann et al., 1998b; Schömig et al., 1998).

EMT has a broad tissue distribution. It is found in sympathetically innervated tissues (e.g. myocardium, vascular and non-vascular smooth muscle and glandular cells; Trendelenburg, 1988), in central nervous system glia (Staudt et al., 1993; Russ et al., 1996; Streich et al., 1996) and in the retina (Rajan et al., 2000). This transporter strongly contributes to the inactivation of circulating catecholamines (Eisenhofer et al., 1996; Friedgen et al., 1996). EMT transports a wide array of organic compounds, all of which are organic cations (review by Trendelenburg, 1988). After analysis of the substrate specificity of EMT, this

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carrier was classified as a transporter for monoamine transmitters. Known endogenous substrates for EMT include catecholamines such as adrenaline and noradrenaline, histamine and serotonin (Trendelenburg, 1988; Gründemann et al., 1998a, 1999).

P-glycoprotein, also known as the multidrug transporter, belongs to a large family of membrane transport proteins known as the ATP-binding cassete (ABC) superfamily of transporters. Considerable evidence has accumulated indicating that P-glycoprotein plays a role in the development of simultaneous resistance to multiple cytotoxic drugs in cancer cells. P-glycoprotein confers resistance against a wide spectrum of compounds, but most of the compounds that are substrates for P-glycoprotein are hydrophobic organic cations of low molecular weight (reviews by Bellamy, 1996; Ambudkar et al., 1999).

The aim of this study was to investigate the possible effect of agents that can modulate P-glycoprotein-mediated transport on EMT-mediated transport. Two lines of evidence led us to this investigation. First, EMT is a transporter of organic cations, and substrates for P-glycoprotein are mostly hydrophobic organic cations (see above). Second, previous experiments from our group showed that some agents that can modulate P-glycoprotein-mediated transport interfere with the inward membrane transport of catecholamines and of the prototypical organic cation 1-methyl-4-phenylpyridinium (MPP^+) in different cell types (rat hepatocytes, human intestinal epithelial (Caco-2) cells and rat brain microvessel endothelial (RBE4) cells) (Martel et al., 1996, 1998a,b, 1999, 2000, 2001a).

2. Methods

2.1. Cell culture

HEK 293 cells (ATCC CRL-1573, Rockville, MD, USA) stem from human embryonic kidney cells which have been transformed by human adenovirus type 5 (Graham et al., 1977). Cells stably transfected with pcDNA3-hEMT (293_{hEMT}) or with pcDNA3 only ($293_{control}$) were a kind gift from E. Schömig. Cells were maintained in a humidified atmosphere of 5% CO_2–95% air and were grown in Dulbecco's Modified Eagle's Medium (Gibco BRL, Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal calf serum, 100 units ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 0.25 μg ml^{-1} amphotericin B (Sigma, St. Louis, MO, USA). Culture medium was changed every 2–3 days and the culture was split every 7 days.

For the experiments, 293 cells were seeded on 24-well plastic cell culture clusters (2 cm^2; Ø 15.6 mm; Corning Costar, Corning, NY, USA) precoated with 0.1 g/l poly-L-ornithine (in 0.15 M boric acid–NaOH, pH 8.4). After 2 days in culture (75–100% confluence), the cells were used in uptake experiments. Each square centimeter contained about 200–250 μg cell protein.

2.2. Transport studies

The transport experiments were performed in medium with the following composition (in mM): 125 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.2 $CaCl_2$, 25 HEPES–NaOH, and 5.6 D(+)glucose, pH 7.4. Initially, the growth medium was aspirated and the cells were washed with medium at 37°C. Then the cell monolayers were pre-incubated for 20 min in medium at 37°C. Uptake was initiated by the addition of 0.3 ml medium at 37°C containing 200 nM [^{3}H]MPP^+. Incubation was stopped by placing the cells on ice and rinsing the cells with 0.5 ml ice-cold medium. The cells were then solubilized with 0.3 ml 0.1% (v/v) Triton X-100 (in 5 mM Tris–HCl, pH 7.4), and placed at 37°C overnight. Radioactivity in the cells was measured by liquid scintillation counting.

Drugs to be tested were present during both the pre-incubation and incubation periods, except where indicated (*trans*-stimulation experiments).

2.3. Protein determination

The protein content of cell monolayers was determined as described by Bradford (1976), with human serum albumin as standard.

2.4. Calculations and statistics

For the calculation of IC_{50}'s, the parameters of the Hill equation for multisite inhibition (Segel, 1975, Eq. VIII-9) were fitted to the experimental data by a non-linear regression analysis, using a computer-assisted method (Motulsky et al., 1994). The IC_{50}'s are given as geometric means.

Arithmetic means are given with S.E.M. and geometric means are given with 95% confidence limits. Statistical significance of the difference between various groups was evaluated by one-way analysis of variance (ANOVA test) followed by the Bonferroni test. For comparison between two groups, Student's *t*-test was used. Differences were considered to be significant when $P < 0.05$.

2.5. Materials

[^{3}H]MPP^+ (*N*-[methyl-^{3}H]-4-phenylpyridinium acetate; specific activity 82 Ci $mmol^{-1}$) (New England Nuclear Chemicals, Dreieich, Germany); digitoxin (K&K Laboratories, Plainview, NY, USA); cyclosporine A, MPP^+ (1-methyl-4-phenylpyridinium iodide) (Research Biochemicals International, Natick, MA, USA); daunomycin hydrochloride, Triton X-100 (Merck, Darmstadt, Germany); L-arginine hydrochloride, choline chloride, decynium22 (1,1′-diethyl-2,2′-cyanine iodide), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), L-histidine hydrochloride, poly-L-ornithine hydrobromide, Tris (tris-

(hydroxymethyl)-aminomethane hydrochloride), tyramine hydrochloride, verapamil hydrochloride, vinblastine sulphate (Sigma); rhodamine123 hydrate (Aldrich Chemical, Milwaukee, WI, USA).

Digitoxin was dissolved in ethanol; the final concentration of the solvent in the buffer was 1%. In experiments using cyclosporine A, the final concentration of dimethylsulfoxide and fetal calf serum in the buffer was 1% and 0.5% (v/v), respectively. Controls for these drugs were run in the presence of the respective solvents.

3. Results

3.1. Time course of [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ and 293$_{control}$ cells

In previous experiments using the same cell lines, we verified that 293$_{hEMT}$ cells efficiently remove [^{3}H]MPP$^+$ from the medium, and that uptake of [^{3}H]MPP$^+$ by 293$_{control}$ cells is almost negligible (Martel et al., 2001b). Moreover, [^{3}H]MPP$^+$ uptake by 293$_{hEMT}$ cells was linear with time for the first 1–2 min of incubation. So, in this work, 293$_{hEMT}$ cells were incubated with [^{3}H]MPP$^+$ for 1 min, in order to measure initial rates of uptake.

3.2. Effect of agents that can modulate P-glycoprotein-mediated transport

The effect of several agents that can modulate P-glycoprotein-mediated transport on hEMT-mediated transport was studied by testing the effect of increasing concentrations of these compounds on [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ cells. Agents that can modulate P-glycoprotein-mediated transport to be tested were grouped into two categories: organic cations (verapamil, rhodamine123, daunomycin and vinblastine) and non-organic cations (cyclosporine A and digitoxin).

Table 1
Inhibition by various organic compounds of [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ cells. Shown are the IC$_{50}$'s with the corresponding 95% confidence intervals

Substance	IC$_{50}$ (μmol/l)	*n*
Rhodamine123	3.6 (1.7–7.5)	3
Verapamil	37 (7–206)	5
Daunomycin	130 (55–306)	7
Tyramine	470 (250–870)	5

Interestingly enough, the cationic agents that can modulate P-glycoprotein-mediated transport verapamil, daunomycin and rhodamine123 produced a marked and concentration-dependent inhibition of [^{3}H]MPP$^+$ uptake (Fig. 1). IC$_{50}$'s of these compounds in relation to [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ cells are shown in Table 1. The ranking order of potency of these drugs was found to be rhodamine123 > verapamil > daunomycin. Vinblastine, also an organic cation, was found to be an exception: at the highest concentration used (300 μM), this compound decreased [^{3}H]MPP$^+$ uptake only to 71 ± 2% of control (Fig. 2).

On the contrary, the non-cationic agents that can modulate P-glycoprotein-mediated transport cyclosporine A

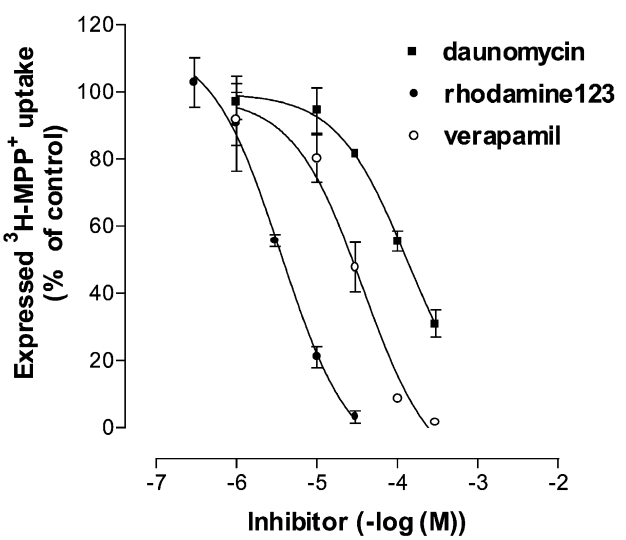

Fig. 1. Inhibition of expressed uptake of [^{3}H]MPP$^+$ into 293$_{hEMT}$ cells by rhodamine123, verapamil and daunomycin. Cells were incubated at 37°C with 200 nM [^{3}H]MPP$^+$ for 1 min, in order to measure initial rates of uptake. Expressed uptake was calculated by subtraction from total uptake into 293$_{hEMT}$ cells of uptake into 293$_{control}$ cells. Shown are means ± S.E.M. of the uptake in the presence of inhibitor relative to control (n = 3–7).

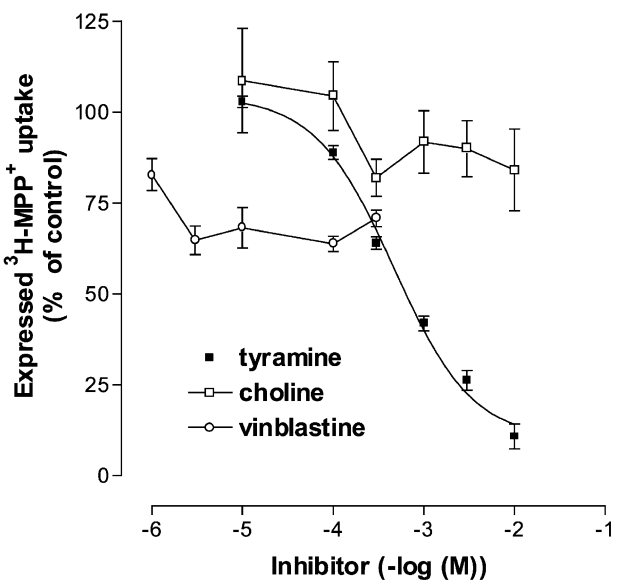

Fig. 2. Effect of vinblastine, tyramine and choline on expressed [^{3}H]MPP$^+$ uptake into 293$_{hEMT}$ cells. Cells were incubated at 37°C with 200 nM [^{3}H]MPP$^+$ for 1 min, in order to measure initial rates of uptake. Expressed uptake was calculated by subtraction from total uptake into 293$_{hEMT}$ cells of uptake into 293$_{control}$ cells. Shown are means ± S.E.M. of the uptake in the presence of inhibitor relative to control (n = 5–7).

(25–100 μM) and digitoxin (1–100 μM) were both devoid of effect upon [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ cells. In the presence of cyclosporine A or digitoxin (100 μM) [^{3}H]MPP$^+$ uptake was found to be 106 ± 10% ($n = 3$) and 93 ± 6% ($n = 4$) of control, respectively.

3.3. Effect of cationic amino acids

The effect of basic amino acids on hEMT-mediated transport was studied by testing the effect of L-arginine and L-histidine (10 μM–10 mM) on [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ cells. L-arginine and L-histidine (up to 1 mM) had no significant effect on [^{3}H]MPP$^+$ uptake. However, for concentrations higher than 1 mM, both compounds produced an inhibition of [^{3}H]MPP$^+$ uptake. At 10 mM, L-arginine and L-histidine decreased [^{3}H]MPP$^+$ uptake to 74 ± 4% and 58 ± 4% of control, respectively.

3.4. Effect of endogenous cationic compounds

The effect of the endogenous organic cations choline and tyramine was investigated. Tyramine produced a concentration-dependent inhibition of [^{3}H]MPP$^+$ uptake (Fig. 2). Its IC_{50} was calculated to be 470 (250–870) μM (Table 1). On the other hand, choline showed a very small inhibitory effect upon [^{3}H]MPP$^+$ uptake, evident only with very high concentrations of this compound (Fig. 2).

3.5. Effect of drugs on 293$_{control}$ cells

Uptake of [^{3}H]MPP$^+$ was also measured in 293$_{control}$ cells, in the absence or presence of the tested compounds. The compounds to be tested were vinblastine, daunomycin, rhodamine123 and cyclosporine A (100 μM), verapamil (300 μM) and choline, tyramine, L-arginine and L-histidine (1 mM). We were thus able to determine the influence of these compounds on the endogenous [^{3}H]MPP$^+$ uptake activity. The endogenous uptake (0.92 ± 0.05 pmol/mg protein; $n = 14$) was only a fraction (< 15%) of total [^{3}H]MPP$^+$ uptake in 293$_{hEMT}$ cells. Moreover, uptake of [^{3}H]MPP$^+$ in 293$_{control}$ cells was not affected by any of the tested compounds (results not shown).

3.6. Trans-stimulation experiments

The efficiency of MPP$^+$, verapamil, tyramine, rhodamine123 and daunomycin to accelerate the counter-transport of [^{3}H]MPP$^+$ was examined. Cells expressing hEMT were pre-incubated for 20 min with unlabeled solutes. The concentration of unlabeled solutes corresponded to ≅ 3× their IC_{50} (see Table 1), i.e. 120 μM verapamil, 1.5 mM tyramine, 10 μM rhodamine123 and 300 μM daunomycin or to ≅ 3× its K_m (Russ et al., 1992), i.e. 100 μM MPP$^+$. Controls were pre-incubated in the absence of drugs. After thorough washing, uptake of [^{3}H]MPP$^+$ was measured as described. Cells preloaded with 100 μM MPP$^+$ or 1.5 mM tyramine showed a significant increase (of 45.5% and 33.5%, respectively) in [^{3}H]MPP$^+$ uptake relative to control (Fig. 3). On the other hand, pre-treatment of the cells with 120 μm verapamil or 300 μM daunomycin caused a strong inhibition (apparent "*trans*-inhibition") in [^{3}H]MPP$^+$ uptake (to 8% and 48% of control, respectively), and pre-treatment of the cells with rhodamine123 was devoid of effect on [^{3}H]MPP$^+$ uptake (Fig. 3).

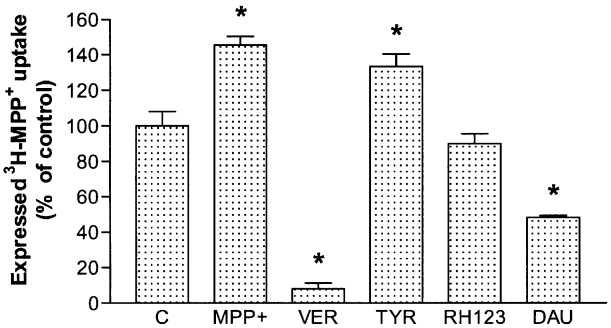

Fig. 3. *Trans*-stimulation of [^{3}H]MPP$^+$ uptake into 293$_{hEMT}$ cells. Cells were pre-incubated for 20 min at 37°C in buffer in the absence (control) or presence of 100 μM MPP$^+$ (MPP$^+$), 120 μM verapamil (VER), 1.5 mM tyramine (TYR), 10 μM rhodamine123 (RH123) or 300 μM daunomycin (DAU). After washing three times with 0.3 ml ice-cold buffer to remove compounds from extracellular space, cells were incubated at 37°C with 200 nM [^{3}H]MPP$^+$ for 1 min. Expressed uptake was calculated by subtraction from total uptake into 293$_{hEMT}$ cells of uptake into 293$_{control}$ cells. Shown are means ± S.E.M. of the uptake in the presence of compound relative to control ($n = 4$). $^*P < 0.05$ vs. respective control.

4. Discussion

The aim of this study was to investigate the possible effect of agents that can modulate P-glycoprotein-mediated transport on hEMT-mediated transport. Moreover, the effect of some endogenous organic cations and of cationic aminoacids was also studied. The experiments were performed using HEK293 cells stably transfected with hEMT, an hEMT-mediated transport model previously characterised (Gründemann et al., 1998b, 1999; Martel et al., 2001b). Radiolabeled MPP$^+$ was chosen as substrate because it is a very good substrate for EMT (Russ et al., 1992; Gründemann et al., 1999), and is not metabolised in vivo (Irwin et al., 1989; Sayre, 1989).

[^{3}H]MPP$^+$ uptake was strongly inhibited by rhodamine123, verapamil and daunomycin, modestly inhibited by vinblastine and unaffected by digitoxin and cyclosporine A. The exogenous organic cations verapamil, vinblastine, daunomycin and rhodamine123 are all P-glycoprotein substrates (review by Ambudkar et al., 1999). The cardiac glycoside digitoxin is also described as a P-glycoprotein substrate (Cavet et al., 1996; Emi et al., 1998; Rebbeor and Senior, 1998). By contrast, the unde-

capeptide cyclosporine A is a non-transported P-glycoprotein inhibitor (review by Ambudkar et al., 1999). So, the difference in the effect of the various P-glycoprotein inhibitors upon hEMT does not seem to be related with the mechanism involved in P-glycoprotein inhibition by these drugs.

Because many of the organic cations tested were found to inhibit hEMT-mediated uptake, we decided to investigate the effect of some endogenous organic cations. Choline, a quaternary ammonium, is an essential precursor for the biosynthesis of acetylcholine and of membrane phospholipids such as phosphatidylcholine (Zeisel et al., 1991). Choline was shown to be a substrate of other members of the ASF family (e.g. rOCT1) (Busch et al., 1996; Gorboulev et al., 1997; Gründemann et al., 1998a,b; Green et al., 1999). In our experiments, this compound had a very discrete inhibitory effect upon $[^3H]MPP^+$ uptake by 293_{hEMT} cells. This observation is in agreement with Gründemann et al. (1998a,b), who showed that [3H]choline was not transported by hEMT. However, we recently verified that choline chloride (125 mM), when substituting NaCl in the extracellular medium, markedly inhibits of $[^3H]MPP^+$ uptake in 293_{hEMT} cells (Martel et al., 2001b). So, it seems that choline, although not a substrate of EMT can, at very high concentrations, inhibit EMT-mediated transport.

The sympathomimetic amine tyramine is widely distributed, although in very low endogenous levels, in invertebrate and vertebrate tissues. In vertebrates, it is partially localized in sympathetic nerves and is present in the brain (Youdim, 1977; Boulton, 1979). Tyramine should therefore be considered as a biogenic amine, and possibly can play a role in sympathetic neurotransmission (Philips et al., 1974; Tallman et al., 1976; Boulton, 1978; Boulton and Juorio, 1979; Saavedra, 1989). Tyramine is a known substrate of EMT (Trendelenburg, 1988; Gründemann et al., 1998b). In the present study, tyramine was found to be an effective inhibitor of $[^3H]MPP^+$ uptake in 293_{hEMT} cells, with an IC_{50} of $\cong 500\ \mu M$.

Finally, it seemed interesting to study the effect of cationic amino acids on hEMT-mediated transport, as nothing is known in relation to the possible interference of these endogenous compounds with EMT. Our results showed that both L-arginine and L-histidine, up to 1 mM, had no significant effect upon $[^3H]MPP^+$ uptake. The concentration of these amino acids in human plasma is around 100 μM (Scriver et al., 1985; Albert et al., 1986; Mackenzie et al., 1992). So, the observation that these compounds inhibit hEMT only at very high concentrations (3–10 mM) precludes a physiological effect of these amino acids in the extraneuronal inactivation of catecholamines.

$[^3H]MPP^+$ uptake by $293_{control}$ cells was not affected by any of the organic compounds tested (verapamil, daunomycin, vinblastine, rhodamine123, choline, tyramine, L-arginine, L-histidine and cyclosporine A). Thus, hEMT-mediated organic cation uptake is clearly distinct from the organic cation transport process that is constitutively expressed in HEK293 cells.

The ability of a compound to *trans*-stimulate hEMT-mediated transport indicates that hEMT can transport substrates in both directions. Moreover, it also demonstrates that the compound is translocated by hEMT, i.e. that the compound is a substrate for hEMT. The finding that MPP^+ and tyramine *trans*-stimulated $[^3H]MPP^+$ uptake is consistent with previous studies which demonstrated that these compounds are substrates of hEMT. Moreover, the fact that $[^3H]MPP^+$ transport can be driven by the counter-transport of unlabeled MPP^+ or tyramine suggests that hEMT may operate as an organic cation/organic cation exchanger. In contrast, rhodamine123 did not *trans*-stimulate $[^3H]MPP^+$ uptake and daunomycin and verapamil were shown to *trans*-inhibit uptake. This suggests that (1) daunomycin, verapamil and rhodamine123 are not transported by hEMT, (2) these compounds bind hEMT tightly and poorly dissociate from it during the experimental procedures, (3) these compounds bind or are sequestered by intracellular compartments, or (4) hEMT loaded with these compounds returns to a chargeable state more slowly than the unloaded transporter. If the first hypothesis is the correct one, i.e. if daunomycin, verapamil and rhodamine123 are not substrates of this transporter, then this work reinforces the conclusions of Gründemann et al. (1999), who stated that EMT is not a polyspecific organic cation transporter, but rather a transporter for monoamine transmitters.

At present, we have no explanation for the marked differences between the effect of the tested organic cations upon hEMT. It is possible that physical–chemical differences between these compounds might be responsible for their distinct interactions with hEMT. In relation to P-glycoprotein, for instance, the hydrophobic properties of the amphiphilic organic cations are thought to play a crucial role in the accommodation of these agents by the transporter (Smit et al., 1998).

An important conclusion of this study is that several cationic substrates/inhibitors of P-glycoprotein also interact with hEMT, either because they are transported (substrates) or non-transported inhibitors of hEMT. Interestingly enough, verapamil, a substrate for P-glycoprotein, was recently shown to be also a substrate for hepatic and renal organic cation transporters (Ullrich et al., 1993; Zhang et al., 1998; Ohashi et al., 1999; Yabuuchi et al., 1999). So, compounds described as agents that can modulate P-glycoprotein-mediated transport will not only interact with P-glycoprotein in vivo, but also with other organic cation transporters, namely hEMT, also present in the human body. The P-glycoprotein modulators verapamil and daunomycin are therapeutically used as antiarrythmic and as antineoplastic agents, respectively. Moreover, some agents that can modulate P-glycoprotein-mediated transport are currently undergoing clinical trials as chemosensitiser agents, to overcome multidrug resistance in cancer

(Covelli, 1999; Robert, 1999; Sikic, 1999; Sonneveld, 2000). Hence, we think it is important to be aware that these agents, by inhibiting hEMT, will decrease the extraneuronal inactivation of biogenic amines such as catecholamines, therefore increasing their biological effects. Moreover, if agents that modulate P-glycoprotein inhibit not only the removal but also the uptake (through an EMT-related transporter) of antineoplastic agents by cancer cells, their chemosensitiser effect will be reduced. This fact may contribute to their disappointing clinical effects.

Another similarity between P-glycoprotein and the members of the ASF family, to which hEMT belongs, relates with their tissue distribution. P-glycoprotein is a constituent of many tissues, but it is mostly expressed in a polarized manner in the apical membrane of epithelia known to be active in the transport of drugs and other toxins. Similarly, transporters belonging to the ASF family have been described at the hepatic and renal levels. Indeed, hEMT was shown to be present in the human liver (Gründemann et al., 1998b). The similarity between the tissue distribution of P-glycoprotein and hEMT makes it more difficult to ascertain in which transporter a drug is acting on.

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