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Characterization of MPP^+ secretion across human intestinal Caco-2 cell monolayers: role of P-glycoprotein and a novel Na^+-dependent organic cation transport mechanism

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1 In the kidney, a number of transport proteins involved in the secretion of permanently charged organic cations have recently been cloned. To evaluate the possible similarities between intestine and kidney in the handling of organic cations we investigated the transport of 1-methyl-4-phenylpyridinium (MPP^+) across monolayers of intestinal Caco-2 cells. MPP^+ is a prototypic substrate of the cloned organic cation transporters hOCT1 and hOCT2.

2 In Caco-2 cell monolayers, the basolateral to apical flux of MPP^+ was significantly greater than the apical to basolateral flux, consistent with net secretion of MPP^+.

3 Net secretion of MPP^+ was abolished by addition of either 10 μM cyclosporin A or 100 μM verapamil to the apical membrane. In contrast, secretion of MPP^+ was unaffected by addition of either TEA (2 mM) or decynium-22 (2 μM) to either apical or basolateral membranes. These results suggest that MPP^+ secretion is mediated primarily by P-glycoprotein located at the apical membrane. We found no evidence of a role for hOCT1 or hOCT2 in the secretion of MPP^+.

4 In addition to net secretion of MPP^+, we found evidence of a Na^+-dependent MPP^+ uptake mechanism at the apical membrane of Caco-2 cells.

5 Na^+-dependent MPP^+ uptake was sensitive to inhibition by the organic cations; decynium-22 (2 μM), TEA (2 mM) and cimetidine (5 mM) but not by carnitine, guanidine or proline.

6 These results suggest that net secretion of MPP^+ across the apical membrane of Caco-2 cells is a function of the relative contributions of MPP^+secretion mediated by P-glycoprotein and MPP^+ absorption mediated by a novel Na^+-dependent transport mechanism.

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Keywords: Caco-2 cells; organic cation transport; MPP^+; P-glycoprotein

Abbreviations: bp, base pair; DMEM, Dulbecco's modified Eagle's medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; MPP, 1-methyl-4-phenylpyridinium; NMDG, N-methyl-D-glucamine; OCT, organic cation transporter; PCR, polymerase chain reaction; TEA, tetraethylammonium; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol

Introduction

The secretion of xenobiotics and endogenous metabolites is a primary physiological function of the intestine (Israili & Dayton, 1984). In concert with xenobiotic metabolism and elimination by the liver and kidney, the intestine provides an important route for drug clearance and an important means to modulate drug absorption across the gastrointestinal tract. Intestinal clearance of cationic drug molecules involves the uptake of organic cations across the basolateral membrane into the cell driven by an inside negative membrane potential, followed by exit from the cell across the apical membrane. At least two transport mechanisms are important in the secretion of organic cations across the apical membrane of enterocytes: P-glycoprotein and organic cation proton antiport. P-glycoprotein mediates the active secretion of a wide range of lipophilic drug molecules across the apical membrane (Hunter *et al*., 1993; Hsing, *et al*., 1992; Saitoh *et al*., 1995; Thiebaut *et al*., 1987). Organic cation/proton antiport, in which organic cations efflux across the apical membrane in exchange for protons, is important in the intestinal elimination of less hydrophobic organic cations that are not substrates for P-glycoprotein (Turnheim & Lauterbach, 1977; Miyamoto *et al*. 1988). In addition to P-glycoprotein and organic cation proton antiport, there is also substantial evidence for the uptake of organic cations across the apical membrane. Apical uptake of organic cations is an important mechanism for the absorption of a number of important organic cations from the lumen of the gut and as a mechanism to modulate drug secretion (Kuo *et al*., 1994; Tamai *et al*., 1997).

In the kidney, in addition to P-glycoprotein (Horio *et al*., 1989; Simmons *et al*., 1997), a number of transport proteins involved in the transport of a range of organic cations have recently been cloned. For a prototypic organic cation such as the potent neurotoxin; 1-methyl-4-phenylpyridinium (MPP^+), the potential sensitive transport mechanism involved in its accumulation across the basolateral membrane (Ullrich *et al*., 1992) has been identified, at the molecular level, as OCT1 (Grundemann *et al*., 1994). At the apical membrane, extrusion of MPP^+ from the cell is mediated by organic cation/proton antiport (David *et al*., 1995). Recently Grundemann and colleagues (1999) provided evidence that MPP^+/proton antiport was the result of the expression of the organic cation transporter OCT2 in the apical membrane of rat proximal tubule. The expression of both OCT1 and OCT2 has been demonstrated in human intestinal Caco-2 cells (O'Neill *et al*., 1999), a cell line often used as a model of small intestinal function. This raises the possibility that intestinal expression of OCT1 and OCT2 may play an important role in the intestinal handling of organic cations like MPP^+.

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Methods

Materials

Cell culture media, supplements, and tissue culture plastics were supplied by Life Technologies (Paisley, U.K.). Tissue culture filters (Transwells) were from Costar (High Wycombe, U.K.). [^{3}H]-MPP and [^{14}C]-mannitol were purchased from NEN. All other chemicals and biochemicals were obtained from Sigma (Poole, U.K.) or BDH (Lutterworth, U.K.) and were of the highest quality available.

Reverse transcription (RT) polymerase-chain reaction (PCR)

Poly-A$^+$ RNA extracted from Caco-2 cells (21 days after confluency was reached, passage 102) was reverse transcribed (MMLV reverse transcriptase, Helena Biosciences U.K.) and the resulting cDNA subjected to PCR analysis using the Expand Long Template PCR System (Boehringer-Mannheim, Germany) according to the manufacturer's instructions, using an annealing temperature of 56°C. For amplification of hOCT1 the primers used were 5′-CAT GAG CAT GCT GAG CCA T-3′ (positions 53–71) and 5′-GGG TAG GCA AGT ATG AGG-3′ (positions 1828–1845) and for hOCT2 5′-GCC CTC CTG CCT GCA GGA T-3′ (positions 125–143) and 5′-TAG ATG CTC CTC TCC ACC-3′ (positions 2188–2206). Negative controls, excluding cDNA from the reaction, were carried out in all cases. The resulting products were isolated from the agarose gel, and subjected to automated sequencing (ABI PRISM 377 DNA sequencer).

Cell culture

Caco-2 cells (passage 100–109) were grown in Dulbecco's modified Eagle's medium (DMEM) (with 4.5 g l^{-1} glucose) supplemented with 10% (v v^{-1}) foetal calf serum (FCS), 2 mmol l^{-1} glutamine, 1% non-essential amino acids and gentamycin (60 μg ml^{-1}). Cells were maintained in culture at 37°C in a 5% CO_2, 95% air atmosphere, with the culture media replaced every 2 days. For transport studies, cell monolayers were prepared by seeding at high density (9.0×10^5 cells cm^{-2}) onto 12 mm diameter Transwell polycarbonate filter supports (Costar). Monolayer confluence was estimated by microscopy and measurement of transepithelial electrical resistance (R_T). Experiments were performed 14–21 days after confluence was achieved and 18–24 h after feeding.

Transport measurements

Bidirectional transepithelial flux measurements were performed essentially as previously described by Simmons (1990). Briefly, the cell monolayers (grown on 12.5 mm diameter filters) were extensively washed four times in 500 ml of modified Krebs' buffer (mmol l^{-1}): NaCl, 140; KCl, 5.4; $MgSO_4$, 1.2; KH_2PO_4, 0.3; NaH_2PO_4, 0.3; $CaCl_2$, 2; Glucose, 5; HEPES, 10, Na$^+$-free Krebs' buffer (composition as above, except that either N-methyl-D-glucamine (NMDG) Cl$^-$ or choline Cl$^-$ replaced NaCl and KH_2PO_4 replaced NaH_2PO_4) or Cl$^-$ free Krebs' buffer (composition as above, except that Cl$^-$ salts were replaced with gluconate salts). All solutions were buffered to the desired pH at 37°C, with Tris base. The filters were then placed in 12-well plastic plates, each well containing 1 ml of prewarmed unlabelled substrate (MPP$^+$ or vinblastine) and mannitol with a further 0.5 ml added to the upper chamber (apical solution) and preincubated for 1 h. Apical to basolateral flux and basolateral to apical flux of either MPP$^+$ or vinblastine were measured in resistance paired monolayers. To begin the flux, [^{3}H]-MPP$^+$ (0.5 μCi ml^{-1}) or [^{3}H]-vinblastine (0.5 μCi ml^{-1}) and [^{14}C]-mannitol (0.25 μCi ml^{-1}) were added to either the apical or basolateral chamber. 100 μl aliquots were removed from the appropriate chamber at 60 min intervals and the volume replaced with unlabelled Krebs' buffer. ^{3}H or ^{14}C activity in the samples was determined by liquid scintillation spectrometry using a Beckman LS 5000CE liquid scintillation counter. Fluxes across the monolayers into the contralateral chamber are expressed as nmol cm^{-2} h^{-1} and are corrected for flux *via* the paracellular route. Initial rates of MPP$^+$ uptake across the apical membrane of Caco-2 cell monolayers was measured in an almost identical manner except that the cells were pre-incubated in Krebs' buffer not unlabelled substrate. Uptake was initiated by the replacement of the Krebs' buffer in the apical chamber with Krebs' buffer containing [^{3}H]-MPP$^+$ (0.5 μCi ml^{-1}) and [^{14}C]-mannitol (0.25 μCi ml^{-1}). At the end of the incubation period, cell monolayers were washed four times in 500 ml volumes of ice-cold Krebs' buffer (or Na$^+$-free or Cl$^-$-free Krebs' buffer) at pH 7.4 to remove extracellular isotope. The cell monolayers were then excised from the filter insert and the cell associated isotope determined by liquid scintillation counting. Initial rates of uptake are expressed as pmoles cm^{-2} min^{-1}.

Statistical analysis

Data are expressed as mean ± s.e.mean. Statistical comparison of mean values was made using a Student's *t*-test (2-tailed solution) for paired or unpaired data as appropriate. For multiple comparisons, a one-way analysis of variance (ANOVA) test was employed and significance assigned using a Dunnett post-test.

Results

hOCT1 and hOCT2 are expressed in Caco-2 cell monolayers

The expression of hOCT1 and hOCT2 in polarized monolayers of Caco-2 cells was assayed by RT–PCR. Figure 1a shows the

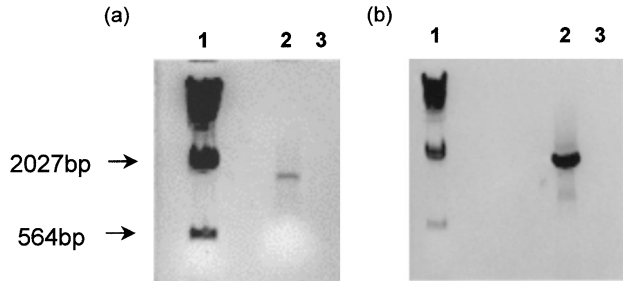

Figure 1 Expression of hOCT1 and hOCT2 mRNA in Caco-2 cells was identified by RT–PCR. (a) 0.8% agarose gel, Lane 1; λ*Hind*III DNA marker. Lane 2; 1792 bp RT–PCR product from Caco-2 cells, using primers specific to hOCT1. Product is consistent with expected size of hOCT1. Lane 3; Negative PCR control. (b) 0.8% agarose gel, Lane 1; λ*Hind*III DNA marker. Lane 2; 2081 bp RT–PCR product from Caco-2 cells, using primers specific to hOCT2. Product is consistent with expected size of hOCT2. Lane 3; Negative PCR control.

1792 bp product isolated using primers specific to hOCT1 and Figure 1b shows the 2081 bp product isolated using primers specific to hOCT2. When sequenced these products were found to be 100% identical at the nucleotide level to the published sequences of hOCT1 and hOCT2 respectively (Gorboulev *et al*., 1997). Thus, similar to native intestine (Gorboulev *et al*., 1997), both hOCT1 and hOCT2 appear to be expressed in polarized monolayers of Caco-2 cells.

Transepithelial fluxes of MPP+ across Caco-2 monolayers

The bi-directional transepithelial fluxes of 25 μM MPP^+ across monolayers of Caco-2 cells showed a marked asymmetry (Figure 2). Basolateral to apical flux of MPP^+ was significantly greater than the corresponding apical to basolateral flux, resulting in a significant net secretory flux of MPP^+ from the basolateral to apical chamber (0.87 ± 0.05 nmol cm^{-2} h^{-1}, $P<0.01$, $n=6$). In order to test the involvement of OCT1 or OCT2 in this process, we measured the net flux of 25 μM MPP^+ in the presence of the potent inhibitor of OCT-mediated transport, decynium-22 or the competitive substrate TEA. Apical addition of 2 μM decynium-22 (Figure 3a), had no significant effect upon the magnitude of either the apical to basolateral (0.9 ± 0.15 nmol cm^{-2} h^{-1} *vs* 0.77 ± 0.1 nmol cm^{-2} h^{-1}, $P>0.5$, $n=6$) or the basolateral to apical flux of MPP^+ (2.05 ± 0.08 nmol cm^{-2} h^{-1} *vs* 2.25 ± 0.1 nmol cm^{-2} h^{-1}, $P>0.5$, $n=6$). Equally, addition of 2 μM decynium-22 to the basolateral membrane had no significant effect upon MPP^+ flux in either the apical to basolateral (0.75 ± 0.1 nmol cm^{-2} h^{-1}, $P>0.5$, $n=6$) or basolateral to apical (2.08 ± 0.1 nmol cm^{-2} h^{-1}, $P>0.5$, $n=6$) direction. Similarly, 2 mM TEA had no effect upon the magnitude of MPP^+ secretion when added to either the basolateral or apical bathing solution (Figure 3b).

The effects of inhibitors of P-glycoprotein on transepithelial fluxes of MPP+

In the next series of experiments, we investigated the effects of cyclosporin A and verapamil, both well characterized competitive substrates of P-glycoprotein, on the transepithelial fluxes of [^{3}H]-MPP^+ across Caco-2 cell monolayers. Figure 4a shows that addition of 100 μM cyclosporin A to the apical membrane of Caco-2 cell monolayers abolished the net secretion of MPP^+. Indeed, cyclosporin A reversed the direction of MPP^+ transport from net secretion (0.7 ± 0.1 nmol cm^{-2} h^{-1}, $n=6$) to a net absorption of MPP^+ (0.68 ± 0.1 nmol cm^{-2} h^{-1}, $P<0.001$, $n=6$). Cyclosporin A significantly inhibited the basolateral to apical flux of MPP^+ ($P<0.001$). This was mirrored by an almost identical increase in apical to basolateral flux of MPP^+ ($P<0.001$) (Figure 4a). Taken together these results suggest that the magnitude and direction of MPP^+ flux across the apical membrane is a balance between MPP^+ uptake across the apical membrane, mediated by a cyclosporin A insensitive transporter, and MPP^+ efflux mediated by P-glycoprotein. A similar inhibition of basolateral to apical MPP^+ flux was found with the apical addition of verapamil (100 μM). However, in contrast to the actions of cyclosporin A, verapamil also inhibited apical to basolateral MPP^+ flux (Figure 4b). Inhibition of MPP^+ uptake across the apical membrane probably reflects competition between verapamil and MPP^+ for a common uptake pathway.

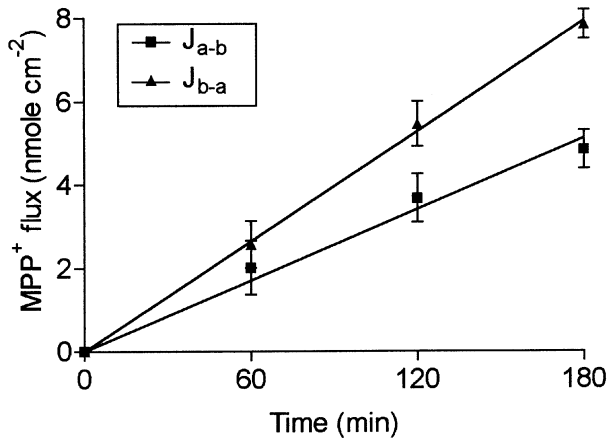

Figure 2 Transport of MPP^+ across Caco-2 cell monolayers. Apical to basolateral (J_{a-b}) and basolateral to apical fluxes (J_{b-a}) of [^{3}H]-MPP (25 μM) were determined over a 3 h period. The results are expressed as the mean ± s.e.mean of six separate determinations.

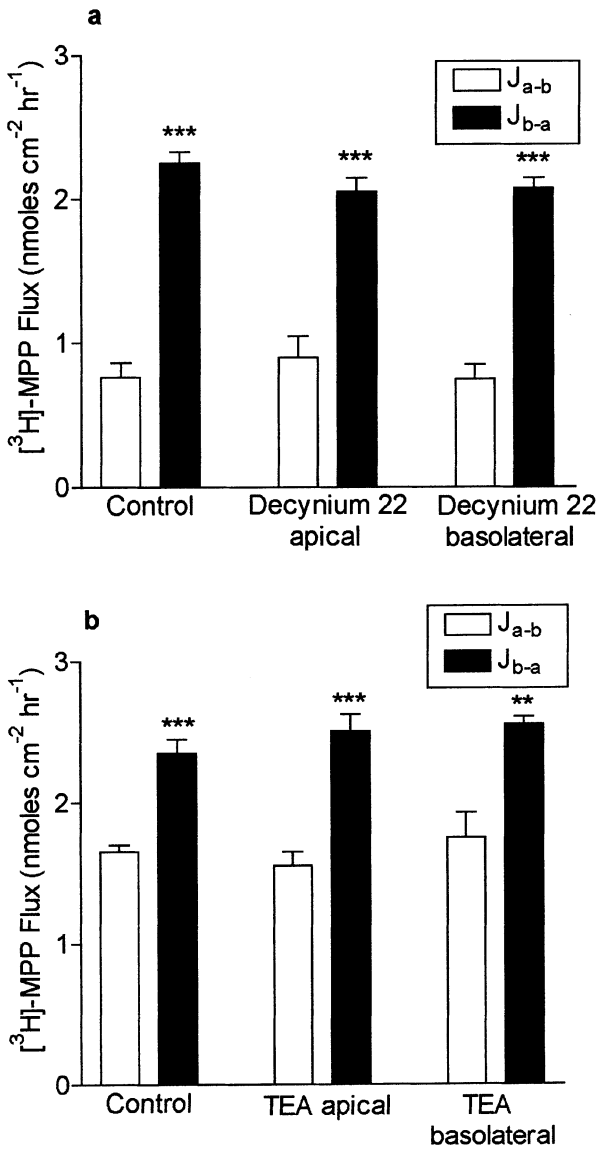

Figure 3 The effect of (a) decynium 22 (2 μM) and (b) TEA (2 mM) upon transepithelial fluxes of [^{3}H]-MPP^+ (25 μM) across epithelial monolayers of Caco-2 cells. Apical to basolateral flux (J_{a-b}) and basolateral to apical (J_{b-a}) were measured over a 2 h period. The results are the mean ± s.e.mean of six separate determinations. ***$P<0.001$, **$P<0.01$ compared to (J_{a-b}) in each data set.

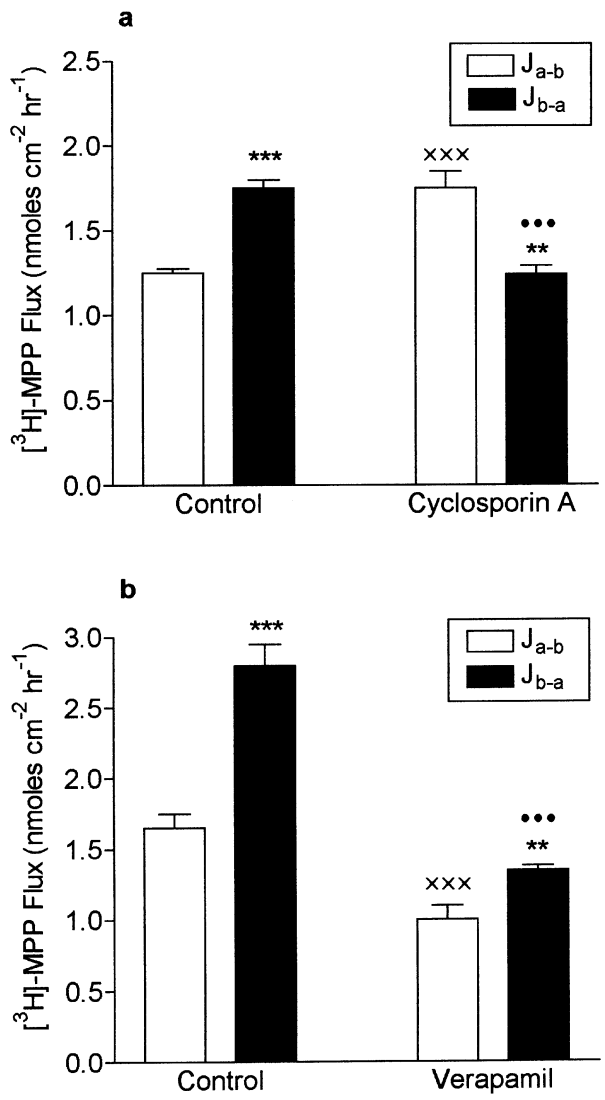

Figure 4 The effect of apical addition of (a) cyclosporin (10 μM) and (b) verapamil (100 μM) upon transepithelial fluxes of [^{3}H]-MPP$^+$ (25 μM) across epithelial monolayers of Caco-2 cells. Apical to basolateral flux (J_{a-b}) and basolateral to apical (J_{b-a}) were measured over a 2 h period. The results are the mean$\pm$s.e.mean of six separate determinations. ***$P<0.001$, **$P<0.01$ compared to (J_{a-b}) in each data set; xxx $P<0.001$ compared to (J_{a-b}) under control conditions; ••• $P<0.001$ compared to (J_{b-a}) under control conditions in both (a) and (b).

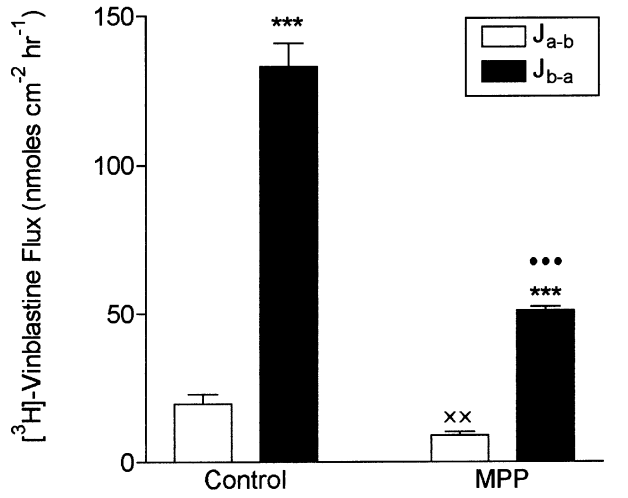

Figure 5 The effect of apical addition of MPP$^+$ (100 μM) upon transepithelial flux of [^{3}H]-vinblastine (1 μM) across epithelial monolayers of Caco-2 cells. Apical to basolateral flux (J_{a-b}) and basolateral to apical flux (J_{b-a}) were measured over a 2 h period. The results are the mean$\pm$s.e.mean of six separate determinations. *** $P<0.001$ compared to (J_{a-b}) in each data set; xx $P<0.01$ compared to (J_{a-b}) under control conditions; ••• $P<0.001$ compared to (J_{b-a}) under control conditions.

Transepithelial fluxes of vinblastine across Caco-2 cell monolayers

Vinblastine, a prototypic P-glycoprotein substrate has previously been used to demonstrate the expression of P-glycoprotein in the apical membrane of Caco-2 cells. To demonstrate the importance of P-glycoprotein in the secretion of MPP$^+$ across the apical membrane of Caco-2 cells, we measured the ability of MPP$^+$ to inhibit vinblastine secretion. Figure 5 shows that in the absence of MPP$^+$, the basolateral to apical flux of [^{3}H]-vinblastine was found to be significantly greater than the apical to basolateral flux (133 pmol cm^{-2} h^{-1} *vs* 19.6$\pm$3.2 pmol cm^{-2} h^{-1}, $P<0.001$, $n=12$) consistent with a net secretion of vinblastine across the cell monolayer. Addition of 100 μM MPP$^+$ to the apical membrane of Caco-2 cells resulted in a significant decrease in the basolateral to apical flux of vinblastine ($P<0.001$, $n=12$) consistent with an interaction between vinblastine and MPP$^+$ for P-glycoprotein. Similar to the action of verapamil upon MPP$^+$ flux (Figure 4b), MPP$^+$ also caused a significant decrease in the apical to basolateral flux of vinblastine ($P<0.01$, $n=12$).

Na$^+$-dependent uptake of MPP$^+$ across the apical membrane of Caco-2 cell monolayers

The flux data presented above suggest that both uptake and efflux of MPP$^+$ occur at the apical membrane of Caco-2 cells. We have provided clear evidence that the efflux of MPP$^+$ is mediated by the action of P-glycoprotein. To identify and characterize the transport mechanism responsible for MPP$^+$ uptake, we measured the initial linear uptake rate of MPP$^+$ across the apical membrane of epithelial monolayers of Caco-2 cells grown on permeable filter supports. MPP$^+$ uptake across the apical membrane of Caco-2 was highly Na$^+$-dependent. Replacing NaCl in the uptake buffer with choline Cl$^-$ (Figure 6a) significantly reduced the rate of uptake of 25 μM MPP$^+$ (8.9$\pm$0.3 pmol cm^{-2} min^{-1} *vs* 1.1$\pm$0.1 pmol cm^{-2} min^{-1}, $P<0.001$, $n=6$). A similar reduction in MPP$^+$ uptake was found if NaCl was replaced by NMDG Cl (data not shown). In contrast to the marked effect of Na$^+$ removal upon MPP$^+$ uptake at the apical membrane, replacement of Cl$^-$ ions in the uptake media by the less permeant gluconate anion resulted in only a modest ($\sim$25%) reduction in MPP$^+$ uptake (Figure 6b. 9.1$\pm$0.3 *vs* 6.9$\pm$0.2 pmol cm^{-2} min^{-1}, $P<0.001$, $n=6$).

MPP$^+$ uptake across the apical membrane of Caco-2 cell monolayers is pH-dependent

The uptake of MPP$^+$ across the apical membrane of Caco-2 cells also showed modest pH dependence. Acidification of the extracellular medium from pH 7.4 to pH 6.5 significantly reduced the uptake of MPP$^+$ (9.4$\pm$0.1 *vs* 7.1$\pm$0.1 pmol cm^{-2} min^{-1}, $P<0.001$, $n=6$). In contrast, raising the pH of the extracellular buffer from pH 7.4 to pH 8.4 had no significant effect upon the magnitude of MPP$^+$ uptake across the apical membrane.

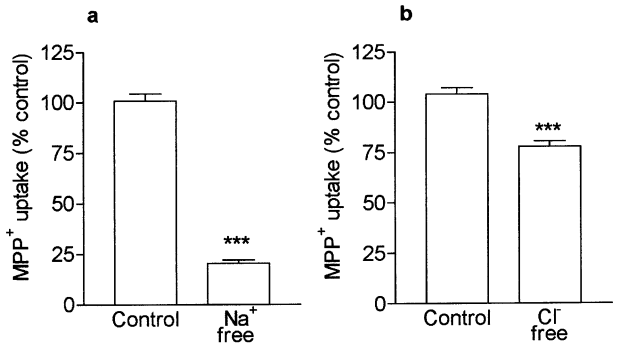

Figure 6 Initial rates of [^{3}H]-MPP$^+$ (25 μM) uptake across the apical membrane of epithelial monolayers of Caco-2 cells grown on permeable filter supports. MPP$^+$ uptake was measured over a 10 m uptake period (a) in normal Krebs' buffer (control condition) and in Na^+-free Krebs' buffer and (b) in normal Krebs' buffer (control condition) and in Cl^--free Krebs' buffer. Uptake is expressed as % of the control condition. The results are the mean$\pm$s.e.mean of six separate determinations. *** $P<0.001$ compared to control condition in both (a) and (b).

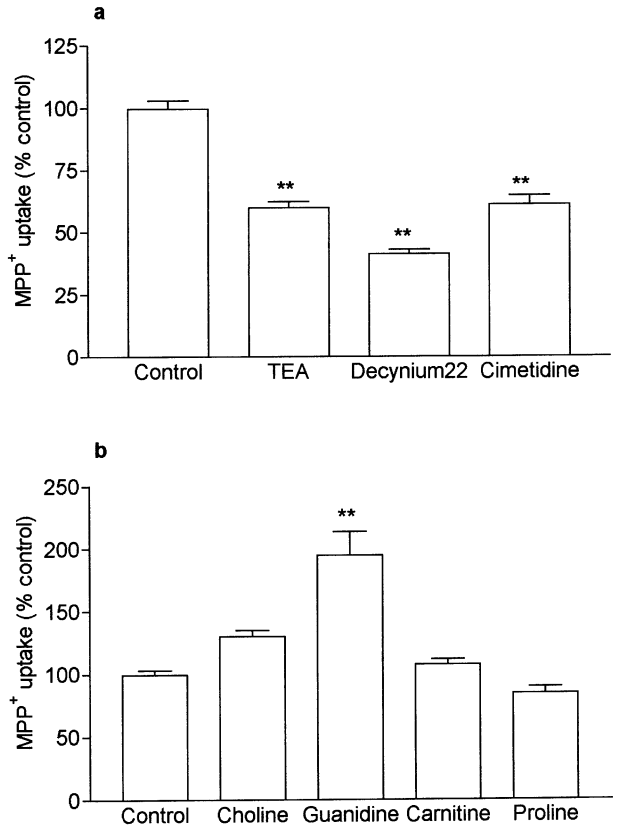

Figure 7 Effects of various compounds on the uptake of [^{3}H]-MPP$^+$ (25 μM) across the apical membrane of epithelial monolayers of Caco-2 cells grown on permeable filter supports. The uptake of MPP$^+$ was measured in the presence of (a) 2 mM TEA; 2 μM decynium 22 or 5 mM cimetidine and (b) 5 mM choline; guanidine; carnitine or proline. Uptake is expressed as % of the control condition. The results are the mean$\pm$s.e.mean of six separate determinations. ** $P<0.01$ compared to control condition in both (a) and (b).

Substrate selectivity of MPP$^+$ uptake across the apical membrane of Caco-2 cell monolayers

The substrate selectivity of apical MPP$^+$ uptake was assessed by measuring the ability of a range of organic cations to *cis*-inhibit [^{3}H]-MPP$^+$ uptake. As shown in Figure 7a, the uptake of MPP$^+$ across the apical membrane of Caco-2 cells was significantly reduced in the presence of 2 mM TEA, 2 μM decynium-22 and 5 mM cimetidine ($P<0.01$, $n=6$, in all cases). However, MPP$^+$ uptake was not sensitive to 5 mM choline, carnitine or proline (Figure 7b). Interestingly, MPP$^+$ uptake was significantly stimulated in the presence of 5 mM guanidine ($P<0.01$, $n=6$). Initially we thought that this might result from a rapid uptake of guanidine into the cell followed by a *trans*-stimulation of MPP$^+$ uptake, however we could not mimic the effect of guanidine upon MPP$^+$ uptake by pre-loading the cells with guanidine before measuring MPP$^+$ uptake (data not shown). It therefore remains unclear as to the mechanism by which guanidine addition stimulates MPP$^+$ uptake.

Discussion

The present study reports the characterization of MPP$^+$ transport across epithelial monolayers of human intestinal Caco-2 cells. MPP$^+$ is a prototypic substrate for a number of cloned organic cation transporters including OCT1 (Grundemann *et al*., 1994); OCT2 (Okuda *et al*., 1996); OCT3/EMT (Kekuda *et al*., 1998; Grundemann *et al*., 1999) and OCTN2 (Tamai *et al*. 1998: Wu *et al*., 1998). Common features of these transporters include inhibition by decynium-22 and competition between MPP$^+$ and TEA (Grundemann *et al*., 1999).

The results of this study show clear differences in the handling of MPP$^+$ by Caco-2 cell monolayers compared with its handling in the kidney. For example, the current model for the renal secretion of organic cations like MPP$^+$ supports a central role for OCT1 in organic cation uptake across the basolateral membrane (Ullrich *et al*., 1992; Grundemann *et al*., 1994). In Caco-2 cells, although we can clearly demonstrate the expression of OCT1 in Caco-2 cells (Figure 1a), basolateral to apical flux of MPP$^+$ was not inhibited by either decynium-22 or TEA. These results argue against OCT1 playing a significant role in the uptake of MPP$^+$ across the basolateral membrane of Caco-2 cells. Since MPP$^+$ is a relatively lipophilic molecule, it is possible that in Caco-2 cells, the contribution of passive diffusion of MPP$^+$ across the basolateral membrane masks the contribution of OCT1 to the total uptake of MPP$^+$. Passive diffusion is known to be important in the basolateral uptake of P-glycoprotein substrates (Hunter *et al*., 1993; Cormet-Boyaka *et al*., 1998). Alternatively, uptake of MPP$^+$ across the basolateral membrane of Caco-2 cells may be mediated by a mechanism distinct from OCT1 or OCT2. Recently, the uptake of cimetidine and ranitidine across the basolateral membrane of Caco-2 cell monolayers was found to be TEA-insensitive. Based on the hydrophilic nature of these drugs, the authors postulated the involvement of a novel TEA-insensitive organic cation transporter (Collet *et al*., 1999).

Organic cation/proton antiport is thought be the major mechanism involved in the renal and hepatic extrusion of MPP$^+$ across the apical and canalicular membranes respectively (David *et al*., 1995; Moseley *et al*., 1997). Organic cation/proton antiport activity has also been found in the small intestine with the demonstration, in brush border membrane vesicles, of guanidine/proton antiport (Miyamoto *et al*., 1988) and more recently cimetidine/proton antiport (Piyapolrungroj *et al*., 1999). Furthermore, organic cation/proton antiport has been implicated in the secretion of cimetidine across epithelial monolayers of Caco-2 cells (Pade & Stavchansky, 1997). However, the pharmacological profile of MPP$^+$ efflux across the apical membrane of Caco-2 cell monolayers suggests that

P-glycoprotein rather than organic cation/proton antiport is the mechanism responsible for MPP^+ secretion. Thus, MPP^+ exit across the apical membrane of Caco-2 cells is not reduced by either decynium-22 or TEA, but is abolished in the presence of either cyclosporin A or verapamil, both potent competitive substrates of P-glycoprotein (Tiberghein & Loor, 1996). In addition, we were able to demonstrate that, not only was MPP^+ secretion inhibited by P-glycoprotein substrates, but that MPP^+ itself acted as a competitive substrate of vinblastine flux. These results perhaps illustrate the fundamental difference in the importance of P-glycoprotein in the intestinal and renal handling of identical substrates. Definitive evidence in support of this concept has come from a series of elegant experiments using a 'knockout' mdr1a/mdr1b (−/−)- mouse model. Smit *et al*. (1998) showed that, in the absence of functional P-glycoprotein, intestinal clearance of P-glycoprotein substrates was 80% less than in normal animals but that, significantly, whole body clearance of these substrates remained at near normal levels. To explain these results it has been proposed that renal clearance of many hydrophobic substrates does not depend upon the functional expression of P-glycoprotein and that increased renal clearance can compensate for the absence of significant intestinal drug clearance.

The inhibition of P-glycoprotein mediated uptake by cyclosporin A unmasked an apical uptake mechanism for MPP^+. Thus net secretion of MPP^+ reflects a balance between secretion *via* P-glycoprotein and reabsorption. The balance achieved between these two opposing fluxes probably explains in part the variation in asymmetry of MPP^+ flux observed in different batches of control monolayers. We found that the balance between MPP^+ secretion and absorption was most affected by variation in the magnitude of apical MPP^+ uptake (Figures 3 and 4) rather than variation in the magnitude of MPP^+ secretion. The wide variation in apical MPP^+ uptake may reflect a regulation of expression of the transporter by endogenous metabolic substrates in the culture media. The uptake of MPP^+ at the apical membrane showed a marked Na^+ dependence. This distinguishes it from the majority of organic cation uptake mechanisms so far reported at apical membranes of the small intestine, most of which are Na^+-independent (Koepsell, 1998). The Na^+-dependent uptake of MPP^+ was moderately inhibited by both TEA and decynium 22, and had a pH-profile (inhibited at acid pH values) which is a feature of the OCT family of transporters (Grundemann *et al*., 1999). Of the OCT family of transporters so far identified, only OCTN2 is Na^+-dependent. OCTN2 is the Na^+-dependent carnitine transporter and is found at the apical membrane of intestine (Tamai *et al*., 1998). However, the uptake of MPP^+ was not sensitive to *cis*-inhibition by carnitine, suggesting that MPP^+ uptake was not mediated by OCTN2. Recently an extraneuronal dopamine transporter has been reported in COS-7 cells which exhibits strong function homology to the neuronal dopamine transporter DAT (Sugamori *et al*., 1999). The dopamine transporter belongs to a family of Na^+/Cl^- dependent transporters and has high affinity for MPP^+. However, MPP^+ uptake into Caco-2 cells showed only a moderate Cl^- dependence (25%) as opposed to an 80% dependence upon Na^+, thus ruling out the extraneuronal dopamine transporter as the mechanism responsible for MPP^+ uptake. Similarly, the lack of inhibition by proline ruled out the involvement of Na^+-dependent cationic amino acid transport in MPP^+ uptake.

In summary, we report the characterization of MPP^+ transport across epithelial monolayers of human intestinal Caco-2 cells. The major findings of this study are that Caco-2 cell monolayers secrete MPP^+ in a basolateral to apical direction. The secretion of MPP^+ is consistent with passive uptake of MPP^+ across the basolateral membrane followed by active efflux of MPP^+ across the apical membrane mediated by P-glycoprotein. Although Caco-2 cells were found to express both hOCT1 and hOCT2, we found no evidence of their role in the secretion of MPP^+. In addition we found evidence for a Na^+-dependent uptake of MPP^+ across the apical membrane, which served to modulate the magnitude of P-glycoprotein mediated MPP^+ secretion. The identity of this transporter remains to be resolved.

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