The Transport of Organic Cations in the Small Intestine: Current Knowledge and Emerging Concepts

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A wide variety of drugs and endogenous bioactive amines are organic cations (OCs). Approximately 40% of all conventional drugs on the market are OCs. Thus, the transport of xenobiotics or endogenous OCs in the body has been a subject of considerable interest, since the discovery and cloning of a family of OC transporters, referred to as organic cation transporter (OCTs), and a new subfamily of OCTs, OCTNs, leading to the functional characterization of these transporters in various systems including oocytes and some cell lines. Organic cation transporters are critical in drug absorption, targeting, and disposition of a drug. In this review, the recent advances in the characterization of organic cation transporters and their distribution in the small intestine are discussed. The results of the *in vitro* transport studies of various OCs in the small intestine using techniques such as isolated brush-border membrane vesicles, Ussing chamber systems and Caco-2 cells are discussed, and *in vivo* knock-out animal studies are summarized. Such information is essential for predicting pharmacokinetics and pharmacodynamics and in the design and development of new cationic drugs. An understanding of the mechanisms that control the intestinal transport of OCs will clearly aid achieving desirable clinical outcomes.

Key words: Organic cations, Organic cation transporter, Small intestine, Knock-out mice

INTRODUCTION

"Organic cations (OC)" are organic molecules with a transient or permanent positive net charge. This class (Table I) includes:

(1) many endogenous, physiologically important compounds, such as monoamine neurotransmitters and coenzymes, as exemplified by choline, acetylcholine, dopamine, norepinephrine and histamine.

(2) numerous drugs (Koepsell *et al.*, 2003) from a wide array of clinical classes such as antihistamines, skeletal muscle relaxants, antiarrhythmics and β-adrenoceptor blocking agents. Approximately 40% of all conventional drugs in the process of drug discovery and drugs that are currently on the market are cationic molecules (Neuhoff *et al.*, 2003). Among them, anisotropine, isopropamide, methantheline, atracurium, pancuronium, bretylium, and

glycopyrrolate, which are quaternary amines, are positively charged at physiological conditions, whereas other amines can be protonated and acquire a positive charge, depending on their pKa values (Zhang *et al.*, 1998).

(3) xenobiotics, ingredients and model drugs, as represented by tetraethylammonium (TEA), tributylmethyl ammonium (TBuMA), and MPP^+ (1-methyl-4-phenylpyridinium).

The homeostasis of such endogenous OCs is determined by their endogenous production, their absorption in the small intestine, and their excretion by the intestine, liver, and kidney. OCs have many important biological functions. They are transported into cells where they act as essential nutrients (i.e., thiamine) or have other influence on cell metabolism (Koepsell, 1998). Their intestinal absorption, tissue distribution, and biliary, renal, and intestinal excretion are likely to be largely dependent on membrane transporters. In addition to membrane transporters, cation channels are known to be permeable for small OCs, although their contribution to these processes is not currently understood (Akaike *et al.*, 1984; McCleskey and Almers, 1985).

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Table I. Categories of organic cations

Categories	Compound	Molecular weight
Organic Cations		
Biogenic amines	Dopamine	153.2
	Epinephrine	183.2
	Norepinephrine	169.2
	Serotonin (5-Hydroxytryptamine)	176.2
	Histamine	111.2
	Choline[a]	104.2
	Thiamine[a]	301.8
Drugs	Guanidine	59.1
	Amantadine	151.3
	Cimetidine	252.3
	Ranitidine	314.4
	Buformin	157.2
	Metformin	129.2
	Diphenhydramine	255.4
	Tacrine	198.3
	Neostigmine bromide[a]	303.2
	Propantheline bromide[a]	448.4
	Clidinium bromide[a]	432.4
	Bretylium tosylate[a]	414.4
	Carbachol chloride[a]	182.7
	Pralidoxime chloride[a]	172.6
	Pancuronium bromide[a]	732.7
Model compound	TEA (tetraethyl ammonium)[a]	130.3
	TBuMA (tributylmethyl ammonium)[a]	200.4
	MPP[+] (1-methyl-4-phenylpyridinium)[a]	170.0

[a] Quaternary ammonium

ORGANIC CATION TRANSPORTERS AND ITS DISTRIBUTION IN THE SMALL INTESTINE

The transport of OCs has been a subject of interest for more than forty years and numerous studies have used intact animals, isolated organs, tissue slices, perfused renal tubules, and isolated plasma membrane vesicles, in attempts to understand this process. Several OC transporters that are members of a family of transporters termed OCT have recently been cloned from various species (Grundemann et al., 1994; Walsh et al., 1996; Okuda et al., 1996; Gorboulev et al., 1997; Grundemann et al., 1997; Lopez-Nieto et al., 1997; Zhang et al., 1997; Terashita et al., 1998). Schomig and collaborators proposed the name "amphiphilic solute facilitator" (ASF)-family (Schomig et al., 1998; Grundemann et al., 1999), whereas other investigators used the term "organic ion transporter family" (Sekine et al., 2000). However, the notations "ASF" or "organic ion transporter family" merely replace the inconsistencies of the present terminology with new problems, since many substrates are either not amphilphilic (such as TEA), or not ionized at all (cimetidine at pH 8.5).

Transporters from the OCT family are expressed in epithelial tissues - including kidney, liver, and intestine - and function in the transport of both endogenous amines and various xenobiotics, including a number of clinically important drugs (Busch et al., 1996; Pritchard et al., 1997; Karbach et al., 2000). To date, five different OCTs have been identified: OCT1, OCT2, OCT3, OCTN1, and OCTN2. OCT1~3 are membrane-potential-driven transporters. OCT1 and OCT2 are localized in the basolateral membrane of renal tubules (Urakami et al., 1998; Karbach et al., 2000; Sugawara-Yokoo et al., 2000). OCT1 and OCT2 possess similar multispecifities for various compounds (i.e. TEA, nicotine, thiamine, choline, guanidine; Urakami et al., 1998). Studies in a transfected human retinal pigment epithelial cell line (HRPE) and Xenopus laevis oocytes have demonstrated the OCT3-mediated transport of prototypical OCs including TEA, guanidine, and MPP[+] (Kekuda et al., 1998; Wu et al., 2000a).

Unlike OCT1~3, the functional characteristics of OCTN1 suggest that it is a proton antiporter that is localized in the apical membrane (Tamai et al., 1998; Yabuuchi et al., 1999). OCTN1 is able to transport TEA, and this transport can be inhibited by a variety of compounds, such as cimetidine, procainamide, and verapamil (Yabuuchi et al., 1999; Wu et al., 2000b). OCTN2 transports the zwitterions, carnitine, and TEA (Sekine et al., 1998; Wu et al., 1998a). Its transport of carnitine is inhibited by OCs, such as TEA, choline, and cimetidine, as well as carnitine derivatives (Wu et al., 1999).

Northern blot analyses have shown that rOCT3 specific-specific mRNA transcripts are present in the rat intestine while rOCT1 specific transcripts are present to a lesser extent and rOCT2 is not detectable (Wu et al., 1998b). rOCTN1-specific transcripts are detectable in a wide variety of tissue and are expressed at relatively high levels in the rat intestine (Wu et al., 2000b). Slitt et al. reported that rOCTN2 mRNA was detected in the rat small intestine (Slitt et al., 2002).

In humans, northern blot analyses have demonstrated that signals of hOCT1 (Zhang et al., 1997) and hOCT3 (Wu et al., 2000a) can be detected in intestine. Tamai et al. have shown that hOCTN1 is barely expressed in the human small intestine (Tamai et al., 1997) and hOCTN2 is expressed in the intestine with a weak signal (Tamai et al., 1998). Immunoreactions with cryosections of a normal human jejunum were examined by Muller and co-workers (Muller et al., 2005). OCT1 immunolabeling was observed mainly in the cytoplasm and the lateral membrane of enterocytes. However, no specific immunolabeling was observed when an affinity-purified antibody against hOCT2 was used, despite the expression of OCT2 in the human small intestine by RT-PCR (Gorboulev et al., 1997). Strong OCT3 immunolabeling was found at the apical cell border of the enterocytes. Interestingly the double labeling

Table II. Organic cation transporter (OCT) family

Transporter	Driving force	Substrates[a]	Intestinal expression[b]
hOCT1 (SLC22A1)	Potential	MPP⁺, TEA **Drugs**: acyclovir, ganciclovir	x, #
rOct1 (Slc22a1)		TEA. MPP⁺, NMN, monoamine neurotransmitters **Drugs**: AZT, cimetidine, cladribine, cytarabine, D-tubocurarine	x, #
hOCT2 (SLC22A2)	Potential	TEA, MPP⁺, NMN, agmatine, monoamine neurotransmitters **Drugs**: amantadine, memantine	ND, #
rOct2 (Slc22a2)		TEA, MPP⁺, adrenaline, agmatine, creatinine, monoamine neurotransmitters **Drugs**: amantadine, cimetidine, memantine	NF
hOCT3 (SLC22A3)	Potential	MPP⁺, guanidine, monoamine neurotransmitter **Drugs**: cimetidine, tyramine	x
rOct3 (Slc22a3)		MPP⁺, TEA, guanidine	xx, #
hOCTN1 (SLC22A4)	Proton	TEA, MPP⁺, L-carnitine, acetyl-L-carnitine **Drugs**: pyrilamine, quinidine, verapamil	NF
rOctn1 (Slc22a4)		TEA, MPP⁺	xxx, #
hOCTN2 (SLC22A5)	Sodium (only for carnitine)	TEA, MPP⁺, L,D-carnitine, acetyl-L-carnitine, betaine, choline, cysteine, lysine, methionine	xx
rOctn2 (Slc22a5)		L-carnitine, TEA	#

[a] from Lee and Kim, 2004
[b] from Dresser et al., 2001; Tissue distribution determined by Northern blot (x), RT-PCR (#); (xx) moderate expression level; (x) low expression level; (NF) not found; (ND) not determined.

revealed that the very tips of the microvilli appear to be free of hOCT3.

The driving force, substrates, and distributions of OCT family in small intestine of both rat and human are summarized in Table II.

THE TRANSPORT OF VARIOUS ORGANIC CATIONS IN THE SMALL INTESTINE

The small intestine is an important site of absorption for orally administered drugs. It also serves as an important route for the clearance of xenobiotics and endogenous metabolites (Israili and Dayton, 1984), in concert with their metabolism and elimination by the liver and kidney.

Transcellular transport occurs either *via* a passive diffusion process or *via* a carrier-mediated process, involving one a large number of carrier proteins (i.e., carrier for amino acids, di/tri peptides, glucose, bile acids, etc.; Tsuji and Takami, 1996). Paracellular transport, involving the passive diffusion of compounds in aqueous solution, is less efficient than the transcellular process because of the much lower surface area available to the compounds entering the intercellular space. Only low molecular weight hydrophilic compounds (e.g. mannitol) cross the intestinal epithelium predominantly *via* the paracellular route.

The mechanism of the intestinal absorption of OCs has been proposed to involve the passive diffusion of non-ionized compounds according to pH-partition theory unless specific transport systems are involved. Several studies have indicated that specific transport systems might facilitate the intestinal absorption of some OCs (Tan et al., 1989; Kuo et al., 1994). In addition, it has been shown that P-glycoprotein (P-gp), which is localized in the intestinal brush-border membrane, is involved in the active secretion of OCs (Hsing et al., 1992; Hunter et al., 1993a, 1993b).

Since the recent cloning of the OCT family, the mechanisms responsible for the absorption and secretion of OCs have received considerable attention. Studies concerning functional properties of OCT have shown that each transporter is selective for some substances, suggesting the existence of multiple transporters in the same tissue. However, information on the physiological mechanisms responsible for OC absorption and secretion in intestine tissue is still limited. Moreover, functional studies performed on transfected cells do not indicate polarized localization (i.e., whether they are apical or basolateral) of the transporters on the membrane (Cova et al., 2002). In comparison to the kidney and liver, much less data are available concerning the mechanisms of OC

transport in the intestine (Zhang et al., 1998).

Guanidine

Guanidine contains three nitrogen atoms, two of which are primary amines and one is a secondary amine. Guanidine hydrochloride has been used in treating the Eaton-Lambert syndrome and botulism. The pharmacokinetics of guanidine chloride in dogs was investigated by Flagstad and coworkers (Flagstad et al., 1986). After intravenous administration, it was rapidly distributed in the organism with a plasma half-life of 7-8 hr and most was eliminated in the urine. Guanidine has shown to be a substrate for rOCT1, rOCT2 and rOCT3 (Kekuda et al., 1998; Grundemann et al., 1999).

The active secretion of guanidine in the intestine was first demonstrated in isolated guinea pig intestinal mucosa (Turnheim and Lauterbach, 1977, 1980). Miyamoto et al. also demonstrated the presence of a guanidine/H^+ antiporter in rabbit intestinal brush-border membrane vesicles (Miyamoto et al., 1988). Cova and co-workers have shown that the influx and efflux of guanidine through the apical membrane were mediated by different transporters, whereas transport across the basolateral membrane was mediated by a member of the OCT family with a high affinity for guanidine (Cova et al., 2002). Studies using cell monolayers and apical membrane vesicles from Caco-2 cells indicate a potential-independent mechanism for guanidine apical uptake and efflux. Conversely, basolateral uptake and efflux are membrane potential dependent and apical efflux is stimulated by an inwardly directed H^+ gradient.

Choline

Choline, a quaternary ammonium compound and a physiological important OC, is essential for the biosynthesis of cell membrane components such as phosphatidylcholine and sphingomyelin, as well as the neurotransmitter acetylcholine (Zeisel, 1981). It also plays a role in nerve signaling, cell signaling and lipid transport and metabolism, and serves as the predominant source of methyl-groups in the body (Zeisel, 2000). Choline has been shown to interact with OCT1 and OCT2 with appreciable affinity (K_m: 620 mM for rOCT1, 210 mM for hOCT2; Gorboulev et al., 1997), but the interaction of this OC with OCT3 was reported to be very weak (Grundemann et al., 1999).

Studies using Caco-2 cells have indicated that choline uptake is sodium independent, temperature dependent, saturable and demonstrates substrate specificity (Kamath et al., 2003). The uptake of choline in Caco-2 cells is inhibited by the choline analog hemicholinium-3, and the P-gp substrates, daunomycin and verapamil, but TEA and N^1-methylnicotinamide (NMN) had no effect on choline uptake. These results indicate the presence of a carrier-

mediated transport system for choline in Caco-2 cells, which may be independent of OCT.

Thiamine

Thiamine (vitamin B_1) is quaternary ammonium compound, which plays a critical role in normal carbohydrate metabolism and is essential for normal cellular functions and growth. Humans and other mammals cannot synthesize thiamine but instead must obtain the vitamin from exogenous sources via intestinal absorption (Tanphaichirt et al., 1994). Thiamine is absorbed from the gastrointestinal tract and is widely distributed over most body tissues. It is not stored to any appreciable extent in the body and amounts in excess of the body's requirements are excreted in the urine. Thiamine shows nonlinear renal elimination kinetics after the administration of doses of 50, 100 and 200 mg (Weber and Kewitz, 1985).

Laforenza et al. demonstrated a thiamine/H^+ antiport mechanism for thiamine entry into brush border membrane vesicles from the rat small intestine (Laforenza et al., 1998). The transport of thiamine was inhibited by unlabled thiamine, thiamine analogues, spermidine, guanidine, clonidine, and imipramine whereas choline, TEA, and histamine had no effect on the thiamine transport.

In studies using Caco-2 cells, thiamine uptake was found to be temperature and energy dependent, pH sensitive and sodium independent. The uptake of thiamine by Caco-2 cells was found to be inhibited by amprolium and oxythiamine, structural analogs of thiamine, but not by TEA, N-methylnicotinamide (NMN) or choline (Said et al., 1999). This was also confirmed by Dudeja and co-workers using human jejunal brush-border membrane vesicles. Using purified brush-border membrane vesicles (BBMV) isolated from the jejumum of organ donors, thiamine uptake was found to be independent of Na^+ but was markedly stimulated by outwardly directed H^+ gradient and competitively inhibited by the cation transport inhibitor amiloride. The uptake of thiamine by BBM vesicles was sensitive to temperature and significantly inhibited by structural analogs of thiamine but by unrelated OCs (Dudeja et al., 2001).

Diphenhydramine

Diphenhydramine, a tertiary amine with one site of ionization and a pKa value of pH 9.0 (de Roos et al., 1970), is widely used as an antihistamine for the symptomatic relief of hypersensitivity reactions. The oral bioavailability of diphenhydramine in human has been shown to be 40 to 60%, with a half-life of elimination of approximately 4 h (Paton and Webster, 1985).

Mizuuchi et al. investigated the transport mechanisms of diphenhydramine using a Caco-2 cell line (Mizuuchi et al., 1999; Mizuuchi et al., 2000a). The accumulation of

diphenhydramine in the monolayers was influenced by the extracellular pH (pH 7.4 > 6.5 > 5.5). Its uptake was temperature dependent, saturable, and not potential sensitive. The initial uptake of diphenhydramine was competitively inhibited by another antihistamine, chlorpheniramine. On the other hand, cimetidine, TEA, and guanidine, typical substrates for the OCT, had no effect. Moreover, biologically active amines and neurotransmitters, such as histamine, dopamine, serotonin, and choline, also had no effect on the diphenhydramine accumulation. The uptake of diphenhydramine from both the apical and basolateral sides was stimulated by the preloading monolayers with chlorpheniramine (trans-stimulation effect). The transepithelial transport and cellular accumulation of diphenhydramine from the basolateral side was also pH-dependent and inhibited by chlorpheniramine.

These findings indicate that the transport of diphenhydramine in Caco-2 cells is mediated by a specific transport system, a pH-dependent transport system. Mizuuchi et al. concluded that a novel pH-dependent tertiary amine transport system which recognizes N-methyl or N-diethyl moieties, is involved in the transport of diphenhydramine in Caco-2 cells (Mizuuchi et al., 2000b).

Nicotine

Nicotine, a major pharmacologically active component of tobacco smoke is a tertiary amine, the pK_a values of which are 6.2 and 10.9 (Schievelbein and barfour, 1984). It is generally thought to be a primary risk factor in the development of cardiovascular disorders, pulmonary disease and lung cancer (Benowitz, 1988).

Fukuda et al. investigated the transport mechanisms of nicotine across a Caco-2 cell line (Fukuda et al., 2002). Nicotine was predominantly transported across Caco-2 cell monolayers in a unidirectional mode, corresponding to intestinal secretion, by pH-dependent specific transport systems. This pH-dependence of nicotine transport might be partly explained by the passive diffusion of the nonionized form according to pH-partition theory. The unlabled nicotine and its major metabolite cotinine showed potent inhibitory effects on the uptake of labeled nicotine by Caco-2 cells from both sides, whereas TEA, cimetidine, and NMN did not. Thus, the specific uptake systems appeared to be distinct from OCTs. Although dephenhydramine and chlorpheniramine strongly inhibited the accumulation of nicotine from both sides of Caco-2 cells, noncompetitive inhibition was suggested as a result.

Therefore, the transport systems for nicotine appear to be independent of the tertiary amine transport system. In additional experiments, the above investigator also confirmed that nicotine is not a substrate for P-gp.

Ranitidine and famotidine

Lee and collaborators characterized the transport of ranitidine and famotidine, histamine H_2-receptor antagonists, across Caco-2 cell monolayers (Lee et al., 2002). Both drugs exhibited a greater permeability in the basolateral to apical direction than in the apical to basolateral direction, indicating apically directed secretion. Basolateral to apical transport was inhibited by P-glycoprotein inhibitors including verapamil and cyclosporine A. The cellular uptake of ranitidine across the basolateral membrane was saturable and temperature dependent, suggesting carrier-mediated transport. TEA-sensitive OCTs were not involved in the uptake of ranitidine and famotidine across the basolateral membrane of Caco-2 cells. These data indicate that the secretory transport of ranitidine and famotidine across Caco-2 cell monolayers is mediated by P-gp in the apical membrane and a carrier in the basolateral membrane which distinct from the TEA-sensitive OCT(s).

MPP+

1-Methyl-4-phenylpyridinium (MPP+) is a quaternary amine and is used as a model compound of OCs. This compound has been extensively employed to study the handling of OCs by various organs or cells including the kidney (Sokol et al., 1987; Lazaruk and Wright, 1990), liver (Martel et al., 1996a, 1996b, 1996c,, 1998a, 1998b, 1999), catecholaminergic neuron (Javitch et al., 1985; Melamed et al., 1985), glial cells (Russ et al., 1996; Streich et al., 1996) and Caki-1 cells (Russ et al., 1992). MPP+ is not metabolized in vivo (Sayer, 1989), and is a typical substrate for OCT1, OCT2, and OCT3 (K_m: rOCT1 for 9.6 μM, hOCT2 for 19 μM, rOCT3 for 25 μM; Gorboulev et al., 1997; Slitt et al., 2002).

Bleasby et al. investigated the transport characteristics of organic cations across Caco-2 cell monolayers using MPP+ as a model substrate (Bleasby et al., 2000). It was demonstrated that the basolateral-to-apical transcellular transport of MPP+ was greater than the apical-to-basolateral transport, indicating a net secretion of MPP+. However, the addition of TEA or decynium-22, inhibitors of OCTs, had no effect on the basolateral-to-apical transport of MPP+, suggesting the lack of involvement of OCT1 and OCT2 in its secretion. They also indicated the Na+-dependent MPP+ uptake from the apical side of Caco-2 cells grown on permeable supports. Although the nature of this Na+-dependent transporter is not clear, the results suggest the existence of a novel Na+-coupled organic cation transporter in the intestinal brush-border membrane.

In contrast, Martel and co-workers reported results that were quite different from the findings of Bleasby et al (Martel et al., 2000). In the presence of the anti-P-gp monoclonal antibody UIC2, the amount of MPP+ present in the Caco-2 cells was not changed. In addition, the uptake of MPP+ was metabolic energy-dependent and

Na⁺-, pH- and potential-independent. Verapamil and rhodamine 123 significantly reduced the transepithelial flux of MPP⁺ in the apical-to-basolateral direction. It was proposed that the absorption of MPP⁺ by the apical membrane occurs through a carrier-mediated mechanism similar to that for the amphiphilic solute facilitator (ASF) family of transporters. Martel and co-workers also proposed that the uptake of OCs at the brush-border membrane of Caco-2 cells may occur through two distinct Na⁺-independent transporters belonging to hEMT and hOCT1 (Martel et al., 2001).

However, this hypothesis was criticized by Katsura and Inui (Katsura and Inui, 2003). Martel et al. evaluated the uptake of MPP⁺ by Caco-2 cells that were grown on plastic dishes and assumed the amount of MPP⁺ accumulated to be the apical uptake (Martel et al., 2000). The accumulation of substrates by epithelial cells grown on plastic dishes does not necessarily reflect apical uptake. Katsura and Inui concluded that it seemed unlikely that OCT1 and OCT3 mediate the apical uptake of organic cations.

Serotonin

Serotonin acts as a neurotransmitter in a subset of mesenteric interneurons (Wade et al., 1996) and serotonin, contained in enterochromaffin (EC) cells, when released in response to chemical or mechanical stimuli, affects gastrointestinal motility and intestinal electrolyte transport (Engel et al., 1984; Imada-Shirakata et al., 1997).

Martel et al. characterized the uptake of serotonin by the apical and basolateral membranes of human intestinal epithelial (Caco-2) cells (Martel et al., 2003). The uptake of serotonin at the apical membrane of Caco-2 cells is specific and Na⁺-, Cl⁺-, and potential-dependent. Its concentration was dependently inhibited by several monoamines (with the following rank order of potency: serotonin >> dopamine > noradrenaline) and tricyclic and nontricyclic antidepressants (with the following rank order of potency: fluosetin > desipramine > cocaine > GBR 12909). In contrast, it was not affected by corticosterone and was only partially inhibited by decynium-22. The uptake of serotonin by the basolateral membranes of Caco-2 cells was Na⁺-dependent and inhibited by desipramine and fluoxetine, but was not affected by corticosterone. Reverse transciptase-polymerase chain reaction data indicates that the mRNA of the neuronal serotonin transporter (SERT) is present in Caco-2 cells and in the human small intestine. They concluded that the OCT family are not involved in either the apical or basolateral uptake of serotonin and that Caco-2 cells functionally express SERT both at their apical and basolateral cell membranes (Martel et al., 2003).

TBuMA

Tributylmethyl ammonium (TBuMA) is a quaternary amine and is used as a model compound for OCs. TBuMA forms lipophilic ion-pair complexes with endogenous bile salts. The formation of a lipophilic ion-pair complex between TBuMA and endogenous bile salts enhances the affinity of TBuMA for P-gp (Song et al., 2001).

The oral bioavailability of TBuMA was reported to show a dose-dependency (i.e., 17, 27 and 35% at doses of 0.4, 4 or 12 mmole/kg, respectively) in the rat (Kim et al., 2005). Relevant mechanisms were investigated in the above study by estimating the mucosal to serosal (m-s) and serosal to mucosal (s-m) transport of TBuMA across the rat ileum in an Ussing chamber experiment (Kim et al., 2005). The m-s permeability rapidly increased with TBuMA concentration in the mucosal side, and then became constant at high TBuMA concentrations. Various studies, including temperature- and potential-dependency and inhibition experiments, revealed that carrier-mediated transport mechanisms (most likely OCTs and P-gp) are involved in the s-m transport of TBuMA, and the saturation of transport at higher concentrations is responsible for the concentration-dependency in the m-s permeability or the dose-dependency for the bioavailability of TBuMA.

A nonlinear regression of m-s transport, based on the assumption of a mixed process of linear diffusion and saturable efflux, exhibited a clearance (CL_{linear}) of 0.343 ml/min/cm² for the passive diffusion, and an apparent K_m of 241 μM for a saturable process. The K_m value is consistent with the concentration range in the intestine that would be expected to be achieved after an oral dosing of TBuMA of 0.4 mmole/kg (i.e., 68~185 μM). Interestingly, the m-s transport of TBuMA was increased by the presence of P-gp substrates or inhibitors in the mucosal side (Fig. 1, Kim et al., 2005), but not by the
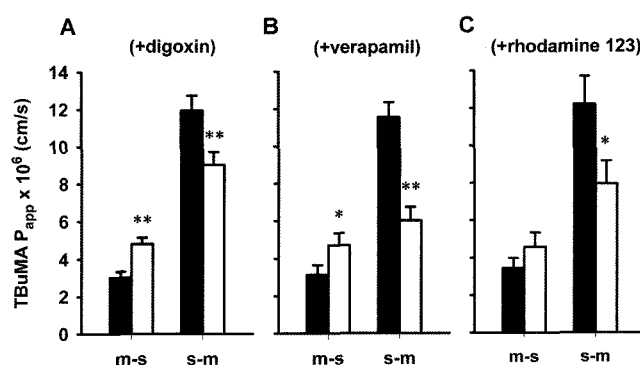

Fig. 1. Effect of the cis addition of (A) digoxin (100 μM), (B) verapamil (100 μM) and (C) rhodamine 123 (100 μM) on the absorptive (m-s) and secretory (s-m) permeabilities of 10 μM TBuMA across the ileum. Each bar represents the mean ± S.D. of 3-4 experiments. (from Kim et al., 2005). **p < 0.01, compared to the control. *p < 0.05, compared to the control.
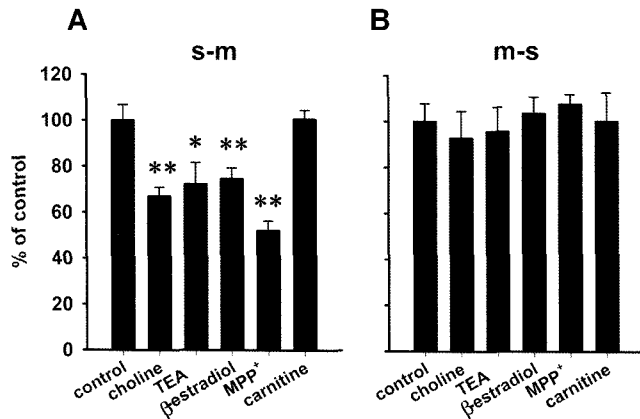
Fig. 2. Effect of the *cis* addition of choline (5 mM), TEA (500 mM), β-estradiol (10 μM), MPP⁺ (1 mM) and carnitine (1 mM) in the serosal (A) or mucosal (B) side on the secretory (s-m) (A) and absorptive (m-s) (B) permeabilities of TBuMA (10 μM) across the ileum. Each bar represents the mean ± S.D. of 3-4 experiments. (from. Kim *et al.*, 2005). **p < 0.01, compared to the control. *p < 0.05, compared to the control.

Fig. 3. The currently accepted scheme for the transport of TBuMA in the small intestine of rats (from. Kim *et al.*, 2005)

mucosal presence of OCT substrates or inhibitors (Fig. 2, Kim *et al.*, 2005), suggesting that only efflux transport systems on the apical membrane (e.g., P-gp), but not those on the serosal membrane (e.g., OCTs), of intestinal epithelial cells, are involved in the dose-dependency or concentration dependency. A similar relationship seems likely for drugs that are substrates of efflux transporters on the apical membrane of the intestinal epithelium. As a result of the above findings, a model (Fig. 3, Kim *et al.*,
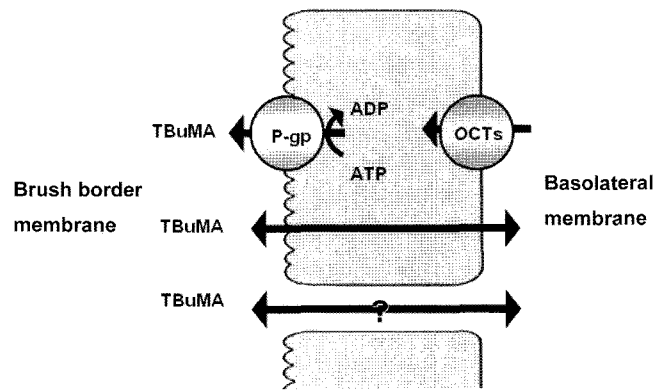
2005) for the intestinal absorption and efflux of TBuMA across the rat small intestine was proposed.

The potential/proton dependency and the TEA-inhibition of various OCs are summarized in Table III.

KNOCK-OUT ANIMAL STUDIES

A knock-out animal model could be useful, to better assess the *in vivo* roles of each transporter in drug elimination. Knock out mice have been generated for OCT1 and OCT3 (Jonker *et al.*, 2001; Zwart *et al.*, 2001). These naturally occurring mutants have been useful for characterizing the role of specific proteins in the transport of endogenous and exogenous compounds, including

Table III. The transport of various drugs and compounds in the small intestine

Organic cation	System	Transport polarity	Dependency		TEA inhibition	References
			Proton	Sodium		
Guanidine	Caco-2 cells	a-b, b-a accumulation	-	Yes	No	Cova *et al.*, 2002
Choline	Caco-2 cells	apical uptake	-	-	No	Kamath *et al.*,2003
Thiamine	Brush border membrane vesicle (rat)	brush border uptake	Yes	No	No	Dudeja *et al.*, 2001
	Caco-2 cells	apical uptake	Yes	No	No	Said *et al.*, 1999
Diphen-hydramine	Caco-2 cells	apical uptake	Yes	No	No	Mizuuchi *et al.*, 1999/2000a
Nicotine	Caco-2 cells	a-b accumulation	Yes	-	No	Fukuda *et al.*, 2002
Ranitidine	Caco-2 cells	b-a transport	-	-	No	Lee *et al.*, 2002
Famotidine	Caco-2 cells	b-a transport	-	-	No	Lee *et al.*, 2002
MPP⁺	Caco-2 cells	a-b, b-a transport	-	Yes	Yes	Bleasby *et al.*, 2000
		apical uptake	-	-	No	Bleasby *et al.*, 2000
		apical uptake	Yes	Yes	No	Martel *et al.*, 2000
Serotonin	Caco-2 cells	apical uptake	-	Yes	No	Martel *et al.*, 2003
TBuMA	Ussing chamber (rat)	m-s, s-m transport	No	Yes	Yes (for s-m)	Kim *et al.*, 2005

– : not determined

drugs and metabolites. Compared to wild-type, OCT1 knock-out mice had similar tissue distributions of choline and cimetidine. In contrast, OCT1 knock-out mice showed greatly reduced concentrations of TEA (Table IV), MPP$^+$ and meta-iodobenzyl-guanidine (MIBG) in the liver, and slightly reduced concentrations in the small intestine. These findings underline the importance of OCT1 in the hepatic and intestinal uptake of several OCs. The importance of OCT1 for the uptake of OCs into the liver and small intestine was confirmed by Sugiyama and coworkers who showed that the concentration of metformin 10 min after an intravenous application was considerably reduced in the liver and small intestine of OCT1 knock-out mice, compared to control animals (Wang *et al.*, 2002). In contrast, the concentration of metformin in the kidney was

constant.

The knock-out of OCT2 had no significant effect on the levels of TEA in the small intestine, liver, and kidney (Table V; Jonker *et al.*, 2003). Pharmacokinetic studies using radioactivity labelled MPP$^+$ in mice with the knock-out of OCT3 did not reveal any significant changes in MPP$^+$ concentration in many tissues including the liver, kidney, small intestine, brain, and placenta. In contrast, the concentration of MPP$^+$ was significantly reduced in the heart. These data suggest that OCT3 is not important for the uptake of MPP$^+$ in the liver, kidney and small intestine, but plays a significant role in the uptake of OCs in the heart.

OUTLOOK AND FUTURE DIRECTION

This overview reviews our present state of knowledge of the major intestinal transport systems of OCs identified to date that mediate intestinal uptake and excretion. During the past decade, significant progress has been in the cloning and molecular characterization of OCTs. Although our knowledge of intestinal transport has increased, we clearly lack a complete understanding of the processes involved in intestinal transport of OCs. Compared with the kidney and liver, molecular mechanisms of organic cation transport in the small intestine currently remain to be elucidated.

Much study remains to be done in order to reach the ultimate goal of understanding the *in vivo* roles of transporters in drug absorption, disposition, and elimination. To accomplish this, it will be necessary to undertake studies at the molecular level, use knock-out animal, perform *in vitro*, *in situ* and *in vivo* studies and to examine species specificity.

Elucidating and characterizing the mechanism involved in the intestinal drug transport of OCs is fundamental to our knowledge of how intestine disposes of xenobiotic and endogenous compounds. An understanding of the mechanisms underlying the drug transporters in small intestine will help us to achieve desirable clinical outcomes in the future.

Table IV. Levels of radioactivity in female wild-type and *Oct1*$^{-/-}$ mice 20 min after an intravenous injection of 0.2 mg of [^{14}C] TEA per kg (from ref. Jonker *et al.*, 2001)

Parameter and tissue of fluid	TEA level		Mutant/ wild-type ratio
	Wild type	*Oct1*$^{-/-}$	
Concentration (ng/mL or g) in:			
Plasma	77 ± 60	41 ± 35	0.53
Brain	2.3 ± 1.5	2.9 ± 2.8	1.24
Spleen	42 ± 6	42 ± 12	1.02
Kidney	418 ± 63	548 ± 483	1.40
Liver	892 ± 283	100 ± 33**	0.11
% Excreted in:			
Small intestine	2.41 ± 1.00	0.21 ± 0.09**	0.09
Cecum	0.30 ± 0.20	0.05 ± 0.04*	0.16
Colon	0.10 ± 0.06	0.01 ± 0.01*	0.13
Urine	45.7 ± 3.4	70.3 ± 12.4**	1.54

Each value represents the mean ± S.D. (n = 4)
** $p < 0.01$, compared to the wild type.
* $p < 0.05$, compared to the wild type.

Table V. Levels of radioactivity in female wild-type and *Oct2*$^{-/-}$ mice 20 min after an intravenous injection of 0.2 mg of [^{14}C] TEA per kg (from ref. Jonker *et al.*, 2003)

Parameter and tissue of fluid	TEA level		Mutant/ wild-type ratio
	Wild type	*Oct2*$^{-/-}$	
Concentration (ng/ml or g) in:			
Plasma	34.2 ± 3.9	38.3 ± 7.6	1.1
Brain	1.8 ± 0.4	1.3 ± 0.1	0.7
Spleen	33.1 ± 3.3	32.7 ± 4.5	1.0
Kidney	552 ± 150	548 ± 230	1.0
Liver	482 ± 52	435 ± 69	0.9
% Excreted in:			
Small intestine	5.1 ± 1.3	3.2 ± 1.6	0.6
Cecum	0.2 ± 0.1	0.2 ± 0.06	0.8
Colon	0.2 ± 0.1	0.12 ± 0.08	0.5
Urine	37 ± 22	39 ± 28	1.1

Each value represents the mean ± S.D. (n = 4)

Abbreviations

The following abbreviations are used: ASF, amphiphilic solute facilitator; BBM, brush-border membrane; TBuMA, Tributylmethyl ammoinium; MPP$^+$, 1-methyl-4-phenylpyridinium; NMN, N^1-methylnicotinamide; P-gp, P-glycoprotein; OCT, organic cation transporter; TEA, tetraethylammonium

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