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H^+-coupled nutrient, micronutrient and drug transporters in the mammalian small intestine

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Abstract

The H^+-electrochemical gradient was originally considered as a driving force for solute transport only across cellular membranes of bacteria, plants and yeast. However, in the mammalian small intestine a H^+electrochemical gradient is present at the epithelial brush-border membrane in the form of an acid microclimate. Over recent years a large number of H^+-coupled cotransport mechanisms have been identified at the luminal membrane of the mammalian small intestine. These transporters are responsible for the initial stage in absorption of a remarkable variety of essential and non-essential nutrients and micronutrients including protein digestion products (di/tripeptides and amino acids), vitamins, short-chain fatty acids and divalent metal ions. Proton-coupled cotransporters expressed at the mammalian small intestinal brush-border membrane include: the di/tripeptide transporter PepT1 (SLC15A1); the proton-coupled amino-acid transporter PAT1 (SLC36A1); the divalent metal transporter DMT1 (SLC11A2); the organic anion transporting polypeptide OATP2B1 (SLC02B1); the monocarboxylate transporter MCT1 (SLC16A1); the proton-coupled folate transporter PCFT (SLC46A1); the sodium-glucose linked cotransporter SGLT1 (SLC5A1); and the excitatory amino acid carrier EAAC1 (SLC1A1). Emerging research demonstrates that the optimal intestinal absorptive capacity of certain H^+-coupled cotransporters (PepT1 and PAT1) is dependent upon function of the brush-border Na^+/H^+ exchanger NHE3 (SLC9A3). The high oral bioavailability of a large number of pharmaceutical compounds is due, in part, to absorptive transport via these same H^+-coupled cotransporters. Drugs undergoing H^+-coupled cotransport across the intestinal brush-border membrane include those used to treat bacterial infections, hypercholesterolaemia, hypertension, hyperglycaemia, viral infections, allergies, epilepsy, schizophrenia, rheumatoid arthritis and cancer.

The role of the Na^+-electrochemical gradient in solute transport

It is nearly 50 years since Halvor Christensen and colleagues proposed that movement of uncharged solutes across mammalian cell membranes might occur in the form of a complex between the carrier, the solute (the example being the amino acid glycine) and sodium (Riggs *et al.* 1958). They suggested that solute uptake could be driven by the energy stored in the transmembrane ionic gradients for cations present due to the asymmetric distribution of the alkali metals sodium and potassium (Riggs *et al.* 1958). Around the same time, Ricklis & Quastel (1958) and Csáky & Thale (1960) demonstrated that intestinal sugar transport was dependent upon the presence of mucosal sodium. These and other observations were unified by Crane and colleagues (Crane *et al.* 1961; Crane, 1962) under what became known as the "Na^+ gradient hypothesis". This hypothesis describes Na^+

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gradient dependent uphill accumulation of glucose across the intestinal brush-border membrane and has been adapted to describe the accumulative transport of many other solutes (both nutrients and drugs) (Fig. 1*A*). The energy for the whole process is in the form of ATP which is utilized by the basolateral Na^+,K^+-ATPase to drive three Na^+ out of the cell in exchange for the inward movement of two K^+, thus generating transmembrane and transepithelial concentration gradients for Na^+ and K^+. The resultant inside-negative membrane potential and high luminal Na^+ concentration generate a large inwardly-directed Na^+-electrochemical gradient across the intestinal mucosal surface. It is the energy stored within this transmembrane Na^+-electrochemical gradient that can be used to drive the uphill (concentrative) accumulation of many solutes across the luminal surface of the small intestinal epithelium. A large body of evidence is now available to support the role of such secondary active Na^+-coupled membrane transporters in the uphill transport of a wide variety of solutes across the intestinal brush-border membrane including: the excitatory amino acid carrier EAAC1 or EAAT3 (SLC1A1) which transports the anionic amino acids glutamate and aspartate (Kanai & Hediger, 2004); the sodium-glucose linked cotransporter SGLT1 (SLC5A1) (Wright & Turk, 2004); the multivitamin transporter SMVT (SLC5A6) which transports biotin, lipoate and pantothenate (Wright & Turk, 2004); the monocarboxylate transporters SMCT1 (SLC5A8) and SMCT2 (SLC5A12) which transport short-chain fatty acids, nicotinate and many other related compounds (Gopal *et al.* 2004; Coady *et al.* 2004; Srinivas *et al.* 2005); the serotonin transporter SERT (SLC6A4) (Martel *et al.* 2003); the taurine transporter TAUT (SLC6A6) (Chen *et al.* 2004); the neutral and dibasic amino acid transport system $B^{0,+}$ ($ATB^{0,+}$/SLC6A14) (Hatanaka *et al.* 2004); the neutral amino acid transport system B^0 (B^0AT1/SLC6A19) (Bröer *et al.* 2004); the IMINO transport system (SIT1/XT3s1/SLC6A20) (Kowalczuk *et al.* 2005; Takanaga *et al.* 2005); the bile salt transporter ASBT (SLC10A2) (Hagenbuch & Dawson, 2004); the dicarboxylate transporter NaDC-1 (SLC13A2) (Markovich & Murer, 2004); the ascorbic acid transporter SVCT1 (SLC23A1) (Takanaga *et al.* 2004); the nucleoside transporter CNT1 (SLC28A1) (Gray *et al.* 2004); the inorganic phosphate transporter NaPi-IIb (SLC34A2) (Murer *et al.* 2004).

The role of the H^+-electrochemical gradient in solute transport

Around the same time that the Na^+ gradient hypothesis was formulated, Peter Mitchell proposed the “chemiosmotic hypothesis” (Mitchell, 1961). This hypothesis has been used (Mitchell, 1963; Mitchell, 1973) to propose that sugar transport across microbial cell membranes can be energized by a transmembrane H^+-electrochemical gradient. This theory was confirmed with the demonstration of rheogenic 1:1 H^+:lactose cotransport into *Escherichia coli* containing the lactose permease LacY (West, 1970; West & Mitchell, 1972; 1973). The H^+-electrochemical gradient is now considered an essential driving force for transmembrane transport of many solutes in yeast, plants and bacteria (Henderson, 1990; Bush, 1993; Hediger, 1994; Wipf *et al.* 2002). Perhaps it was due to the origins of the various hypotheses regarding ion-driven solute transport that Na^+-coupling became accepted generally as the primary means of solute movement in mammalian tissues whereas H^+-coupling was considered to be specific for plants, yeast and bacteria. Unfortunately once such a doctrine becomes enshrined within the literature it is often difficult to dislodge from the scientific psyche. This paper reviews current evidence for the existence of a H^+-electrochemical gradient at the brush-border surface of the mammalian small intestinal epithelium and the role this gradient plays in driving nutrient, micronutrient and drug absorption via numerous H^+-coupled membrane transporters.

The acid microclimate

The H^+-electrochemical gradient consists of both chemical and electrical gradient components (intestinal enterocytes have an inside-negative membrane potential). The first evidence for the existence of an area of low pH adjacent to the luminal surface of the small intestinal epithelium was produced almost 50 years ago (Hogben *et al.* 1959). It was suggested that a microclimate with a "virtual pH" of 5.3 existed at the luminal surface which was required to explain the differences between predicted rates and experimental measurements of absorption of various drugs (weak acids and bases) according to pH-partition theory (Schanker *et al.* 1958; Hogben *et al.* 1959). The acid microclimate has since been measured directly between pH 6.1-6.8 at the luminal surface of the mammalian small intestine both *in vivo* and *in vitro* by use of pH-sensitive microelectrodes (Lucas *et al.* 1975; Daniel *et al.* 1985; Shimada, 1987; McEwan *et al.* 1988; McKie *et al.* 1988; Shimada & Hoshi, 1988; Daniel *et al.* 1989). Surface pH measurements within the range of the "virtual pH" have only been observed using *in vitro* tissues in the presence of 10mM glucose (Lucas *et al.* 1980). These unusually low values have been attributed to a stimulatory effect of glucose on H^+ secretion *in vitro* which may reflect an increase in H^+/lactate efflux (Daniel & Rehner, 1986; Daniel *et al.* 1989). In addition, Hogben and colleagues (1959) may have simply overestimated the acidity of the microclimate because many of the weak acids used in their study (e.g. salicylic acid, benzoic acid) are now known to be substrates for H^+-coupled transporters at the intestinal brush-border membrane (see subsection on OATP2B1 and MCT1 below). The apparent increase in passive non-ionic penetration of weak acids following a decrease in luminal pH may, at least partly, reflect an increase in uptake via pH gradient dependent carrier-mediated transport.

Exactly how the acid microclimate is generated is still a matter for debate. Many studies favour transmembrane H^+ secretion into the negatively-charged mucus layer which will act as a diffusion barrier so that ion (H^+) concentrations at the surface are different from those in the bulk lumen. In contrast, Shiau and colleagues (1985) suggest that the microclimate exists solely due to the presence of the mucus layer and is not due to H^+ secretion although if this is correct it is not clear why the microclimate varies along the length of the small intestine and crypt-villus axis (Daniel *et al.* 1985; McEwan *et al.* 1988; Daniel *et al.* 1989). A role for membrane transport in maintenance of the acid microclimate seems certain as the microclimate is alkalinized by the removal of extracellular Na^+, the presence of amiloride or by addition of various factors that increase intracellular cAMP or cGMP (Lucas *et al.* 1980; Shimada, 1987; McEwan *et al.* 1988; McKie *et al.* 1988; Shimada & Hoshi, 1988; Daniel *et al.* 1989). These characteristics all point towards a role for the apical Na^+/H^+ exchanger NHE3 (SLC9A3) (Murer *et al.* 1976; Brant *et al.* 1995; Hoogerwerf *et al.* 1996) in maintenance of the acid microclimate. Although NHE3 may play a role in acid microclimate maintenance it seems unlikely to be solely responsible for pH microclimate generation as NHE3 functions poorly at typical microclimate surface pH values (Orlowski, 1993; Thwaites *et al.* 2002; Anderson *et al.* 2004).

Here we review the current evidence for a diverse range of H^+/solute cotransporters at the luminal membrane of the small intestinal epithelium. It should be noted that, by convention, these transporters are described here as H^+/solute cotransporters rather than OH^-/solute antiporters although there is little experimental evidence to distinguish between these two modes of transport.

SLC15A1: the di/tripeptide transporter PepT1

Protein is digested within the lumen of the mammalian small intestine to release small di- or tripeptides (2-3 amino acids in length) and individual amino acids. Protein is absorbed from

diet in these two distinct forms. The first solid evidence for H^+-coupled di/tripeptide transport (Fig. 2) came from a series of studies by Ganapathy & Leibach (Ganapathy & Leibach, 1983, 1985; Ganapathy *et al.* 1984) where dipeptide uptake into intestinal brush-border membrane vesicles (BBMV) was shown to be independent of Na^+ but stimulated by a pH gradient (which was abolished in the presence of a protonophore). This pH-dependent stimulation of dipeptide uptake was associated with a depolarization of the membrane potential and was increased in the presence of a valinomycin-induced K^+-diffusion potential. Boyd & Ward (1982) had been the first to suggest that dipeptide transport may be coupled to movement of a cation other than Na^+. The identity of this cation and the H^+-coupled, pH-dependent, rheogenic nature of dipeptide transport was eventually confirmed in 1993 in a series of studies using human intestinal epithelial Caco-2 cell monolayers in which H^+/dipeptide cotransport was observed (Thwaites *et al.* 1993a; 1993b; 1993c; 1993f). Two earlier studies had demonstrated uptake and transepithelial transport of the aminocephalosporin antibiotics cephalexin (Dantzig & Bergin, 1990) and cephradine (Inui *et al.* 1992).

A cDNA was isolated from rabbit small intestine which, when expressed in *Xenopus laevis* oocytes was able to induce H^+-coupled, Na^+-independent, di and tripeptide uptake (Fei *et al.* 1994; Boll *et al.* 1994). This transporter was named PepT1 (Fei *et al.* 1994) and was later isolated from human ileum (Liang *et al.* 1995) and human intestinal Caco-2 cells (Walker et al. 1998). PepT1 mRNA is found along the length of the small intestine and is localized to the absorptive enterocytes in the duodenum, jejunum and ileum (Freeman *et al.* 1995). PepT1 protein is immunolocalized to the brush-border membrane of human small intestine and human intestinal Caco-2 cell monolayers (Walker *et al.* 1998). PepT1 represents the first member (SLC15A1) of solute carrier family 15 which also includes three other related sequences (Daniel & Kottra, 2004). PepT1 is a low-affinity, high-capacity, transport system which has a very broad substrate specificity and plays a unique role in nutrient absorption, transporting most of the potential 400 dipeptides and 8000 tripeptides which result from enzymatic breakdown of dietary protein. PepT1 can also transport a large number of hydrophilic drugs and is, therefore, responsible for the high levels of oral bioavailability of many pharmaceutical compounds. Examples of PepT1 substrates include (Table 1) penicillin and cephalosporin antibiotics, angiotensin-converting enzyme (ACE) inhibitors, anti-cancer drugs, and pro-drugs of levodopa or 3,4-dihydroxy-L-phenylalanine (L-DOPA) and the anti-viral agents acyclovir, ganciclovir and azidothymidine or zidovudine (ZT) (Rubio-Aliaga & Daniel, 2002).

SLC36A1: the proton-coupled amino-acid transporter PAT1

Protein is also absorbed across the intestinal brush-border membrane in the form of individual amino acids. Transepithelial transport of amino acids across the intestinal epithelium is mediated via a number of amino acid transport systems arranged in parallel and series at the apical and basolateral membranes of the intestinal enterocyte. For many years it was considered that intestinal amino acid transport was solely a function of Na^+-dependent transport systems although there was never much evidence against the involvement of other transmembrane ionic gradients (see Thwaites & Anderson, 2007).

A H^+-coupled, pH gradient dependent, Na^+-independent, rheogenic amino acid transporter was identified at the brush-border membrane of the human intestinal epithelial cell line Caco-2 (Fig. 2) (Thwaites *et al.* 1993d; 1993e; 1994b; 1995a; 1995b; 1995c; Thwaites & Stevens, 1999; Thwaites *et al.* 2000). This transporter was named system PAT (Thwaites *et al.* 1994b; Thwaites & Anderson, 2007). The related cDNA has recently been isolated from rat (Sagné *et al.* 2001), mouse (Boll *et al.* 2002), human (Chen *et al.* 2003a) and rabbit (Miyauchi *et al.* 2005). The cloned transporter is known as PAT1. PAT1 mRNA is

expressed in all regions of the small intestine and PAT1 protein is immunolocalised to the brush-border membrane of the human and rat small intestine, and human intestinal Caco-2 cell monolayers (Chen *et al.* 2003a; Anderson *et al.* 2004). PAT1-like transport has been detected at the brush-border membrane of rat jejunum (Anderson *et al.* 2004; Iñigo *et al.* 2006). Like, PepT1, PAT1 is also a low-affinity, high-capacity, transporter which plays a role in both nutrient and drug absorption (Thwaites & Anderson, 2007). PAT1 has a broad substrate specificity (Table 1) transporting both D- and L-imino and amino acids, ß- and γ-amino acids, and a large number of neuromodulatory and anti-bacterial agents (Thwaites *et al.* 1993d; 1993e; 1994b; 1995a; 1995b; 1995c; Thwaites & Stevens, 1999; Thwaites *et al.* 2000; Boll *et al.* 2002; Chen *et al.* 2003a; Boll *et al.* 2003; Anderson *et al.* 2004; Metzner *et al.* 2004; 2005; Abbot *et al.* 2006; Thwaites & Anderson, 2007). PAT1 can also function in two different modes either as an electrogenic H^+/amino acid cotransporter or as an electroneutral H^+/anion cotransporter for short-chain fatty acids (Foltz *et al.* 2004a) (Table 1).

PAT1 is the first member (SLC36A1) of solute carrier family 36 which includes three other related sequences in all mammalian genomes (Chen *et al.* 2003b; Bermingham & Pennington, 2004; Boll *et al.* 2004). PAT2 (SLC36A2) is also a H^+-coupled amino acid transporter but has a distinct substrate specificity and tissue distribution from PAT1 and is not expressed in the small intestine (Boll *et al.* 2002; Chen *et al.* 2003b; Foltz *et al.* 2004b; Rubio-Aliaga *et al.* 2004; Kennedy *et al.* 2005a). Iminoglycinuria is a disorder characterised by defective renal tubular reabsorption of amino acids (glycine, proline and hydroxyproline) and in some cases there is also defective transport in the small intestine (Online Mendelian Inheritance in Man (OMIM) database, OMIM 242600, www.ncbi.nlm.nih.gov). Bröer has recently suggested (Bröer, 2006) that iminoglycinuria is likely to be a multigene disorder which may include several transporters such as PAT1, PAT2 and IMINO. The ancient nature of the H^+-electrochemical gradient is emphasised, by the PAT-related transporters, as there are more than four related sequences in the genomes of lower eukaryotes (Sagné *et al.* 2001). For example, there are eight PAT-related sequences in *Drosophila melanogaster*. The *D. melanogaster* transporter CG1139 has similar functional characteristics to PAT1 (following heterologous expression in *X. laevis* oocytes). Overexpression of CG1139 is associated with modulation of eye and wing growth suggesting that PAT transporters might regulate growth *in vivo* (Goberdhan *et al.* 2005).

SLC11A2: the divalent metal transporter DMT1

The H^+-coupled divalent metal transporter DMT1 (Fig. 2) was originally cloned and named NRAMP2 (Vidal *et al.* 1995) because of its sequence similarity to the natural resistance-associated macrophage protein NRAMP1. The function of the protein remained unknown until the mRNA was isolated by expression cloning and shown to transport divalent metals, for example Fe^{2+}, when expressed in *X. laevis* oocytes (Gunshin *et al.* 1997). The transporter was renamed DCT1 (divalent cation transporter). This transporter is the major route for intestinal absorption of non-haem iron and transports iron in the ferrous Fe^{2+} form following reduction of the ferric Fe^{3+} form by an apical ferrireductase (Mackenzie & Garrick, 2005). The role of this transporter in intestinal Fe^{2+} absorption was confirmed by the identification of a G185R mutation in the *Nramp2* gene in both the microcytic (*mk*) mouse and Belgrade (*b*) rat that have abnormal intestinal iron absorption (Fleming *et al.* 1997; 1998). Inclusion of the G185R mutation in the NRAMP2/DCT1 clone leads to a loss of function (Su *et al.* 1998). The transporter is now known as DMT1 or SLC11A2, the second member of solute carrier family 11 (Mackenzie & Hediger, 2004). DMT1 has been immunolocalised to the brush-border membrane of the proximal small intestine and human intestinal Caco-2 cells (Canonne-Hergaux *et al.* 1999; Tandy *et al.* 2000). Knockdown of DMT1 in Caco-2 cells leads to a reduction in Fe^{2+} uptake (Bannon *et al.* 2003). Targeted

deletion of the *Slc11a2* gene in mouse intestine is associated with anaemia once the animals become dependent upon intestinal nutrition (Gunshin *et al.* 2005). As well as H^+/Fe^{2+} cotransport (Gunshin *et al.* 1997), DMT1 can also mediate transport of a range of divalent metals (e.g. Mn^{2+}, Ni^{2+}, Co^{2+})(Table 1).

SLC02B1: the organic anion transporting polypeptide OATP2B1

The pH-partition theory (see subsection on acid microclimate above) was proposed to account for the increased absorption of organic anions across the intestinal wall. However, it is now evident that many organic anions are substrates for pH-dependent carrier-mediated transport systems expressed at the intestinal brush-border membrane. The organic anion transporting polypeptide OATP2B1 (Fig. 2) is a member (SLC02B1) of the solute carrier family SLC0 (Hagenbuch & Meier, 2004). This SLC family is relatively unusual as different transport proteins are expressed in different mammalian species and the relationship of human OATPs to rat and mouse oatps is not clear. OATP2B1 was originally isolated from human brain and named OATP-B (Tamai *et al.* 2000; Kullak-Ublick *et al.* 2001) or SLC21A9 (Hagenbuch & Meier, 2003). OATP2B1 mRNA is expressed in the human small intestine (Tamai *et al.* 2000; Kullak-Ublick *et al.* 2001; Sai *et al.* 2006) and OATP2B1 protein is immunolocalised at the brush-border surface of both human small intestine (Kobayashi *et al.* 2003) and human intestinal Caco-2 cell monolayers (Sai *et al.* 2006). Heterologous expression of OATP2B1 produces a Na^+-independent, pH-gradient dependent transporter (Nozawa *et al.* 2004) with a relatively narrow substrate specificity compared to other OATPs. OATP2B1 transports the physiological sulfate-conjugated steroids oestrone-3-sulfate and dehydroepiandrosterone sulfate (DHEAS) (Tamai *et al.* 2000; Kullak-Ublick *et al.* 2001) and at lower pH (consistent with pH within the acid microclimate) broadens its specificity to include taurocholate (Nozawa *et al.* 2004) suggesting that OATP2B1 may play a role in the enterohepatic circulation of both bile acids and oestrogen (Sai *et al.* 2006). OATP2B1 may also play an important role in oral drug delivery as it can transport bromosulphothalein (BSP), the 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors pravastatin and atorvastatin (used clinically to reduce hypercholesterolaemia), the anti-histamine fexofenadine and the anti-diabetic glibenclamide (Kobayashi *et al.* 2003; Nozawa *et al.* 2004; Satoh *et al.* 2005; Grube *et al.* 2006) (Table 1). However, there is some overlap in the apparent substrate specificity between OATP2B1 and the monocarboxylate transporter MCT1 (SLC16A1, see subsection on MCT1 below). Results from studies using intact tissues where these transporters may be coexpressed should be interpreted carefully, in particular in tissues from species where it is not clear which SLC0 members are expressed at the intestinal luminal membrane. Compounds that are potential substrates for both OATP2B1 and MCT1 include nicotinate, benzoate, salicylate, valproate, pravastatin and atorvastatin (Table 1).

OATP2B1-like function is consistent with transport characteristics at the apical surface of human intestinal Caco-2 cell monolayers and BBMV prepared from rabbit jejunum (Tamai *et al.* 1995a; Sai *et al.* 2006) although it is not clear which SLC0 members are expressed in the rabbit small intestine. The ability of grapefruit (and other citrus) juice and constituents to reduce the oral bioavailability of fexofenadine (Dresser *et al.* 2002) seems likely to be mediated by inhibition of intestinal absorption via OATP2B1 (Satoh *et al.* 2005), identifying OATP2B1 as a potential site for diet-drug interactions. The physiological and pharmacological role played by OATP2B1 in intestinal absorption may also vary between individuals. For example, a single nucleotide polymorphism (found in 31% of the Japanese population investigated within the study) leads to an amino acid change in the OATP2B1 protein (S486F) which is associated with a greater than 50% reduction in transport capacity (Nozawa *et al.* 2002).

SLC16A1: the monocarboxylate transporter MCT1

The monocarboxylate transporter MCT1 is the first member (SLC16A1) of solute carrier family 16 (Halestrap & Meredith, 2004). The first SLC16 transporter to be identified at the molecular level was originally isolated from a Chinese hamster ovary (CHO) cell line and named MEV because it caused an increase in mevalonate uptake in transiently transfected cells (Kim *et al.* 1992). MEV includes a single base change in the sequence compared to the wild-type cDNA (MCT1). The hamster wild-type cDNA was identified as a transporter of lactate and pyruvate and named MCT1 (Garcia *et al.* 1994) and later isolated from rat small intestine (Takanaga *et al.* 1995). MCT1 requires coexpression *in situ* of the ancillary protein CD147 for proper cell surface expression and function (Halestrap & Meredith, 2004).

MCT1 functions as a H^+-coupled transporter (Bröer *et al.* 1998) of a wide variety of anions (Table 1, Fig. 2) including the vitamin B_3 nicotinate, the monocarboxylates L- and D-lactate (with a preference for the L-form), pyruvate, acetate, propionate and butyrate, and the ketone bodies acetoacetate and ß-hydroxybutyrate (Tamai *et al.* 1995b, 1999). MCT1 also transports benzoate (a food preservative) and salicylate (which have been used to estimate the acid microclimate and which are also substrates for OATP2B1, see above), D,L-2-hydroxy-(4-methylthio)butanoic acid (a feed supplement), and a number of other pharmaceutical compounds including the anti-convulsant valproate, and the HMG-CoA reductase inhibitors pravastatin and atorvastatin (also OATP2B1 substrates) (Tamai *et al.* 1995b, 1999; Wu *et al.* 2000; Martín-Venegas *et al.* 2007). A key functional characteristic of MCT1 activity is its sensitivity to inhibition by α-cyano-4-hydroxycinnamate (Bröer *et al.* 1998).

The short-chain fatty acids butyrate, propionate and acetate are the major organic anions in the lumen of the large intestine where they are produced by bacterial fermentation of dietary fibre and undigested carbohydrates. Butyrate is a major energy source for colonocytes. There is a physiological role for a butyrate uptake mechanism at the luminal membrane of the colon and there is evidence for both MCT1 (SLC16A1) and SMCT1 (SLC6A8) in the literature (Ritzhaupt *et al.* 1998; Ganapathy et al. 2005). We will not discuss the evidence for colonic transport here in any further detail as the focus of this review is the role of the H^+-electrochemical gradient in absorption across the small intestine where there is substantial evidence for an inward H^+ gradient (see subsection on acid microclimate above). The physiological role for a monocarboxylate transporter in the small intestine must be to mediate monocarboxylate absorption from diet (it is possible that a greater requirement for small intestinal uptake might be needed in animals practicing coprophagy). MCT1-like activity has been demonstrated as pH-gradient dependent lactate uptake and lactate-sensitive nicotinate uptake in rabbit and rat small intestinal BBMV (Tiruppathi *et al.* 1988; Simanjuntak *et al.* 1990; Takanaga *et al.* 1996).

MCT1 mRNA has been detected in the small intestine of human, rabbit, rat, hamster and Caco-2 cells (Kim *et al.* 1992; Tamai *et al.* 1995b; Price *et al.* 1998; Hadjiagapiou *et al.* 2000; Englund *et al.* 2006; Seithel *et al.* 2006). An *in situ* hybridisation study of mouse and rat gastrointestinal tract identified MCT1 mRNA throughout from the stomach to the distal colon with greatest intensity in the caecum (Iwanaga *et al.* 2006). In the small intestine the strongest signal was in the rat ileum (Iwanaga *et al.* 2006). Immunolocalization studies of MCT1 within the small intestinal epithelium have provided conflicting observations (Garcia *et al.* 1994; 1995; Tamai *et al.* 1999; Gill *et al.* 2005; Iwanaga *et al.* 2006). MCT1 was immunolocalized to the basolateral membrane in the epithelial cells along the length of the hamster gastrointestinal tract although only images of basolateral staining in stomach and caecum were presented (Garcia *et al.* 1994; 1995). In a more recent study, MCT1-immunoreactivty was absent from the villus epithelium in the mouse ileum although

basolateral immunoreactivity was observed in the crypts (Iwanaga *et al.* 2006). Similarly no immunoreactivity was observed in human terminal ileum (Iwanaga *et al.* 2006). In contrast, MCT1 was found along the length of the rat gastrointestinal tract which in the small intestine showed a greater intensity in the duodenum than the ileum and also in the crypts than the villus (Tamai *et al.* 1999). Staining in the crypts was mainly basolateral but both lateral and brush-border staining was observed in the villus epithelium (Tamai *et al.* 1999). Immunoblotting of apical and basolateral membranes purified from human jejunum and ileum identified MCT1 expression in both membranes with stronger expression in the brush-border (Gill *et al.* 2005).

The human intestinal epithelial cell line Caco-2 was derived from a human colonic adenocarcinoma but expresses a small intestinal enterocyte-like phenotype when grown as confluent polarised monolayers on permeable filters (Delie & Rubas, 1997). A number of investigators (for examples see, Stein *et al.* 2000; Hadjiagapiou *et al.* 2000; Buyse *et al.* 2002) have measured butyrate transport in Caco-2 cell monolayers and identified an MCT1-like transporter at the brush-border membrane. In addition, MCT1 and the ancillary protein CD147 have both been immunolocalised to the brush-border membrane of Caco-2 cells (Buyse *et al.* 2002). Thus, it seems likely, that MCT1 mediates butyrate transport across the brush-border membrane of Caco-2 cells. However, it is not clear (and the purpose for choosing Caco-2 cells is not apparent in some of the studies) whether the MCT1-like transport is representative of butyrate transport across the small intestinal epithelium, large intestinal epithelium or both.

Although it is evident that monocarboxylate transport in the intestine is mediated by a number of transport systems that can be coupled to either H^+ or Na^+ movement, the relative expression of each transporter may vary between the apical and basolateral membranes of epithelial cells in the small and large intestines and may differ between species. Many fascinating questions remain to be answered, for example, what is the relative contribution of SMCT1, SMCT2, PAT1 and MCT1 (and other members of the SLC16 family of MCTs) to monocarboxylate transport and does the contribution of each transporter vary along the crypt-villus and longitudinal axes? The availability of functional clones and transporter specific antisera should provide evidence to elucidate the roles of these transporters in the near future.

SLC46A1: the proton-coupled folate transporter PCFT

Folic acid (vitamin B9) is an essential nutrient and folate deficiency is the most common vitamin deficiency in Western societies. In mammals the sole source of folate is through absorption from diet. A pH-gradient dependent, Na^+-independent, folate transporter has been characterised in a number of studies using BBMV (mainly jejunal) from human, rabbit and rat small intestine (Selhub & Rosenberg, 1981; Schron *et al.* 1985; Said *et al.* 1987; Mason *et al.* 1990). This high-affinity transporter also transports the antineoplastic and immunosuppressive agent methotrexate, 5-methyltetrahydrofolate and 5-formyltetrahydrofolate. A transporter with similar characteristics has been identified at the apical membrane of human intestinal Caco-2 cell monolayers (Vincent *et al.* 1985; Mason *et al.* 1990; Kneuer & Honscha, 2004) and rat intestinal epithelial IEC-6 cells (Said *et al.* 1996; Wang *et al.* 2005). The molecular identity of this pH-gradient dependent, high-affinity, folate transporter has been revealed recently with the isolation of the proton-coupled folate transporter PCFT (Qiu *et al.* 2006) (see Fig. 2, Table 1). PCFT is the first member (SLC46A1) of solute carrier family 46 and this human cDNA was identified using a data mining approach to search for sequences with weak homology to the reduced folate transporter RFT (SLC19A1, see below). In either *Xenopus* oocytes or mammalian cell lines, PCFT has identical characteristics to those previously characterised in intestinal tissues and

it functions as an electrogenic, H^+-coupled, Na^+-independent transporter with a particularly high affinity for the thymidylate synthase inhibitor permetrexed (Wang *et al.* 2004; Qiu *et al.* 2006; Zhao & Goldman, 2007). PCFT mRNA is found throughout the human intestine with greatest abundance in the duodenum and Caco-2 cell line (Qiu *et al.* 2006). PCFT was originally isolated from mouse small intestine as a haem transporter and named heme carrier protein 1 or HCP1 (Shayeghi *et al.* 2005) although the affinity for haem is much lower than for folates (Qiu *et al.* 2006). PCFT/HCP1 protein was immunolocalised to the mucosal surface of mouse duodenum (Shayeghi *et al.* 2005) consistent with a role of this transport protein in absorption of essential components of diet.

For a number of years the reduced folate transporter RFT was considered the prime candidate for proton-coupled folate transport across the intestinal brush-border. RFT (SLC19A1) is the first member of solute carrier family 19 (Ganapathy *et al.* 2004) and is also known as the reduced folate carrier RFC (Dixon *et al.* 1994), folate transporter FOLT (Prasad *et al.* 1995) or intestinal folate carrier 1 (IFC1) (Said *et al.* 1996). RFT was originally cloned from a mouse cDNA library (Dixon *et al.* 1994) and was later cloned from many tissues including the human small intestine (Nguyen *et al.* 1997). The tissue distribution of RFT is consistent with a role in folate absorption as: mRNA is found in human intestinal Caco-2 cells and human and rat small intestine (Prasad *et al.* 1995; Nguyen *et al.* 1997; Said *et al.* 2000); *in situ* hybridisation in human jejunum localises RFT mRNA to epithelial cells particularly in the upper half of the villus (Nguyen *et al.* 1997); RFT protein is immunolocalised to the brush-border membrane of the duodenum, jejunum and ileum (Wang *et al.* 2001); Western blot analysis identifies RFT in jejunal brush-border membranes (Said *et al.* 2000).

The relative contribution of both PCFT and RFT to intestinal folate absorption will require further investigation. In contrast to PCFT, RFT has a strong preference for reduced rather than oxidised folates and RFT functions probably as a folate anion/OH^- antiport transporting folate, methotrexate, and thiamine monophosphate (Zhao *et al.* 2002; Ganapathy *et al.* 2004). RFT is unlikely to be responsible for the pH-gradient dependent (low pH stimulated) uptake observed in intestinal cell lines and BBMV (Selhub & Rosenberg, 1981; Schron *et al.* 1985; Vincent *et al.* 1985; Said *et al.* 1987; Mason *et al.* 1990; Said *et al.* 1996; Kneuer & Honscha, 2004; Wang *et al.* 2005) since RFT expression in oocytes is associated with a decrease in uptake as pH_o is reduced (Nguyen *et al.* 1997; Kumar *et al.* 1998) rather than the increase in uptake observed in PCFT-expressing oocytes (Qiu *et al.* 2006). Goldman and colleagues (Qiu *et al.* 2006) have identified that a loss-of-function mutation in PCFT is the molecular basis of hereditary folate malabsorption (OMIM 229050, www.ncbi.nlm.nih.gov) in a single family. High doses of oral 5-formyltetrahydrofolate relieved the symptoms of the disorder in the two members of the family affected suggesting that a second, lower affinity, folate transporter is also involved in intestinal absorption. Identification of this second transporter as RFT requires further investigation. It will be interesting to identify the nature of the defect in other patients suffering from hereditary folate malabsorption and also if any compensatory changes in RFT expression are observed.

SLC5A1: the sodium-glucose linked cotransporter SGLT1

Thus far we have described a series of intestinal transport systems that function as H^+/solute symporters. There is no evidence to suggest that solute movement through these transporters is linked directly to Na^+ cotransport (although some at least are linked indirectly to Na^+ transport, see subsection on NHE3 below). There are, however, some sugar transporters that can function either as Na^+ or H^+ cotransporters, for example, the *E. coli* melibiose transporter (Tsuchiya & Wilson, 1978). Here we describe an example of a mammalian

transporter that can use either the Na^+ or H^+ electrochemical gradients to drive solute transport.

The sodium-coupled glucose cotransporter SGLT1 is the first member (SLC5A1) of solute carrier family 5 (Wright *et al.* 2004). Although SGLT1 is often considered the archetypal Na^+/solute cotransporter, the Na^+-gradient hypothesis (Crane *et al.* 1961) was after all proposed to account for the Na^+-dependency of sugar absorption, SGLT1 can also function as a H^+/sugar cotransporter (Hirayama *et al.* 1994) (Fig. 2). The evidence for Na^+-glucose cotransport is compelling: the interrelationship between NaCl, water and glucose absorption was demonstrated using rat ileum *in vitro* (Curran, 1960); coupled influx of Na^+ and glucose was demonstrated using flat sheets of rabbit ileum (Schultz & Zalusky, 1964); the brush-border location of the cotransport mechanism was identified unequivocally using rat intestinal BBMV (Hopfer *et al.* 1973); a cDNA (SGLT1) was isolated from rabbit small intestinal mucosa which, when expressed in *X. laevis* oocytes, produced a transport system with all of the characteristics of the intestinal Na^+ driven glucose transporter (Hediger *et al.* 1987). SGLT1 transport is characterised as Na^+-dependent uptake of D-glucose and D-galactose (Table 1) that is inhibitable by phlorizin. However, pH-dependent, Na^+-independent glucose transport has also been observed. Hoshi and colleagues (Hoshi *et al.* 1986) demonstrated that D-glucose uptake into rabbit intestinal BBMV in Na^+-free conditions was stimulated by decreasing extravesicular pH in the presence of an inside-negative vesicular membrane potential. Similarly glucose-stimulated phlorizin-sensitive current in *X. laevis* oocytes expressing rabbit SGLT1 was enhanced by decreasing extracellular pH in the absence of extracellular Na^+ suggesting that SGLT1 can also function in a H^+-coupled mode (Hirayama *et al.* 1994). SGLT1 has an affinity for H^+ that is approximately 500 times greater than Na^+ ($K_{0.5}$ 0.007 and 4mM, respectively) whereas the affinity for glucose is approximately 25 times lower in the H^+-coupled compared to Na^+-coupled mode (Hirayama *et al.* 1994; Quick *et al.* 2001; Wright *et al.* 2004).

In situ hybridisation studies localised SGLT1 to the absorptive villus epithelial cells in duodenum, jejunum and ileum (Hwang *et al.* 1991; Smith *et al.* 1992; Freeman *et al.* 1993). Immunocytochemical studies have identified SGLT1 at the brush-border membrane of the rabbit and rat small intestinal epithelium, human jejunum and Caco-2 cells (Hwang *et al.* 1991; Yoshida *et al.* 1995; Khoursandi *et al.* 2004). The physiological importance of SGLT1 is emphasised in patients with glucose-galactose malabsorption syndrome (OMIM 606824, www.ncbi.nlm.nih.gov) where mutations in SGLT1 are associated with defective intestinal absorption of D-glucose and D-galactose (Wright *et al.* 1991). Thus, SGLT1 is an example of a transport protein that may be able to adapt to maximise sugar absorption by utilising local transmembrane ionic gradients for cations (Na^+ and H^+) along the length of the small intestine which may vary in magnitude both in time and space.

SLC1A1: the excitatory amino acid carrier EAAC1

The excitatory amino acid carrier EAAC1, also known as EAAT3, is the first member (SLC1A1) of solute carrier family 1 (Kanai & Hediger, 2004). EAAC1 differs from the other transport systems described above as inward solute flux is coupled to the inward movement of both H^+ and Na^+. EAAC1 was originally isolated from a rabbit jejunal cDNA library (Kanai & Hediger, 1992) and is a high affinity transport system for the anionic amino acids L-glutamate, L- and D-aspartate and the dipolar amino acid L-cysteine (Kanai *et al.* 1994; Zerangue & Kavanaugh, 1996a; 1996b). EAAC1 has also been isolated from human ileum and kidney (Kanai *et al.* 1994).

Transport of, for example, glutamate by EAAC1 is accompanied by the inward movement of 3 Na^+, 1 H^+ and the countertransport of 1 K^+ (Fig. 2). Two models have been proposed to

account for the ionic dependency of EAAC1. Zerangue & Kavanaugh (1996a) suggested that the H^+ is cotransported with the anionic amino acid glutamate resulting in an intracellular acidification upon release of H^+ and solute inside the cell. This model is supported by their observation that the zwitterion L-cysteine is transported by the carrier but does not lead to an intracellular acidification because after release cysteine (pK 8.3) will remain protonated. In contrast, Auger & Attwell (2000) suggest that inward H^+ movement occurs during the K^+-countertransport stage of the transport cycle where glutamate is not bound to the carrier.

EAAC1 corresponds to the amino acid transport system X_{AG}^- (Christensen, 1984). The H^+, Na^+ and K^+ dependency of system X_{AG}^- has been demonstrated in the human and rabbit intestinal brush-border membrane using jejunal BBMV (Berteloot, 1984; Harig *et al.* 1987; Maenz *et al.* 1992). Northern blot analysis identifies high levels of expression of EAAC1 in the duodenum, jejunum and ileum and *in situ* hybridisation localises EAAC1 mRNA to the small intestinal epithelium (Kanai & Hediger, 1992). EAAC1 protein has been immunolocalised to the intestinal brush-border membrane in both rat and mouse small intestine although in these studies EAAC1 protein showed greater expression in the lower part of the villus (Rome *et al.* 2002; Iwanaga *et al.* 2005).

The role of the Na^+/H^+ exchanger NHE3 (SLC9A3) in maintenance of the H^+-electrochemical gradient and optimal H^+/solute cotransport

The investigation of intestinal di/tripeptide transport was key to challenging the belief that the Na^+ gradient hypothesis could account for all ion-driven solute absorption in the mammalian small intestine. Until the early 1980s most studies of intestinal absorption used intact tissue preparations (either *in vivo* or *in vitro*) in which it was difficult to control experimental conditions at the mucosal surface. Early studies of intestinal dipeptide uptake using intact tissue preparations identified a Na^+-dependent mechanism (Ganapathy & Leibach, 1985; Ganapathy *et al.* 2006). However, Ganapathy & colleagues (Ganapathy & Leibach, 1983; 1985; Ganapathy *et al.*1984) were able to demonstrate Na^+-independent, pH-gradient dependent uptake of dipeptides using intestinal BBMV. They suggested that the apparent Na^+-tissues was due to a requirement for an apical Na^+/H^+ exchanger, in conjunction with basolateral Na^+,K^+-ATPase, to generate and maintain the inward H^+ gradient (Fig. 1*B*). The role of NHE3 in maintaining intracellular pH and, therefore, the H^+ gradient was demonstrated using Caco-2 cells loaded with the pH-sensitive dye BCECF. Recovery from dipeptide-induced intracellular acidification was via selective activation of apical Na^+/H^+ exchange without any activation of basolateral Na^+/H^+ exchange (Thwaites *et al.* 1993c; 1994a). The Na^+/H^+ exchanger activated following PepT1-mediated H^+/dipeptide cotransport was identified as NHE3 (SLC9A3) by use of selective NHE inhibitors (Thwaites *et al.* 1999; 2002; Kennedy *et al.* 2002; Anderson *et al.* 2003; Kennedy *et al.* 2005b).

The functional relationship between H^+/solute cotransport and NHE3 is not limited to PepT1 as PAT1-mediated H^+/amino acid uptake leads to a similar selective activation of NHE3 (Thwaites *et al.* 1994b; 1995a; 1995b; 1995c; 1999; 2000; Anderson *et al.* 2004; Anderson & Thwaites, 2005). The relationship between PAT1 and NHE3 (Fig. 1*B*) explains the apparent Na^+-dependence of amino acid transport via PAT1 (also known as the imino acid carrier) in intact epithelia (Anderson *et al.* 2004; Anderson & Thwaites, 2005; Thwaites & Anderson, 2007). The extent of the “functional cooperativity” between NHE3 and the other H^+/solute cotransporters reviewed here is not known but may be limited to either the high-capacity, low-affinity, cotransporters or those cotransporters that demonstrate pH-dependence within the range of pH values measured within the extracellular microclimate. Inhibition of NHE3 activity by activators of the protein kinase A pathway (e.g. forskolin, 8-Br-cAMP, vasoactive intestinal peptide, pituitary-adenylate cyclase-activating peptide,

$VPAC_1$ receptor agonists, phosphodiesterase inhibitors) reduces the absorptive capacity of the H^+-coupled cotransporters PepT1 and PAT1 at the intestinal brush-border membrane (Thwaites *et al.* 2002; Anderson *et al.* 2003; 2004; Anderson & Thwaites, 2005; Kennedy *et al.* 2005b; Anderson & Thwaites, 2007) and would presumably reduce the absorptive capacity of any transporter dependent upon maintenance of the H^+-electrochemical gradient.

Conclusion

Absorption across the wall of the small intestine is mediated by a multitude of membrane proteins that allow nutrient, micronutrient and drug transport to proceed against a concentration gradient by coupling uphill movement of solute to downhill movement of ions. There is substantial evidence for the presence of both Na^+ and H^+-coupled solute cotransporters at the brush-border membrane of the mammalian small intestine. The lists presented here are not exhaustive and additional H^+-coupled solute cotransporters are likely to be expressed at the brush-border of the mammalian small intestine. For example, the copper (Cu^{1+}) transporter Ctr1, which is the first member (SLC31A1) of solute carrier family 31 (Petris, 2004), has not yet been demonstrated to be H^+-coupled but is pH-dependent with ^{64}Cu uptake in Ctr1-expressing HEK293 cells increasing as extracellular pH is reduced from pH 7.5 to 5.5 (Lee et al. 2002). Ctr1 protein has been immunolocalised to the apical membrane of human, rat and mouse small intestine and human intestinal Caco-2 cells (Klomp *et al.* 2002; Bauerly *et al.* 2004; Kuo *et al.* 2006; Nose *et al.* 2006).

Many of the mammalian H^+-coupled cotransporters described here are also localised in lysosomes in a variety of non-intestinal cell types where they mediate lysosomal efflux using the outward H^+-gradient generated by H^+-ATPase activity. In contrast, the driving forces (Na^+ and H^+-electrochemical gradients) for the intestinal ion-coupled cotransporters are generated ultimately by activity of the basolateral Na^+,K^+-ATPase. Some H^+-coupled cotransporters (perhaps those high-affinity, low-capacity carriers) may be driven solely via the inside-negative membrane potential. In contrast, some of the high-capacity cotransporters (e.g. PepT1 and PAT1) require an additional transporter (NHE3) (see Fig. 1*B*), which utilises the inward Na^+ gradient, to maintain the H^+ gradient across the luminal membrane during absorption. Na^+/H^+ exchangers play key roles throughout evolution (Skulachev, 1994) as “gradient converters” allowing utilisation of both Na^+ and H^+ gradients. These antiport proteins may allow adaptation (at the level of individual transport proteins, cells or tissues) to changes in local ionic microenvironments both in time and space. The primary means by which NHE3 maintains the H^+-electrochemical gradient during solute absorption is through H^+ efflux and regulation of intracellular pH. How the extracellular microclimate is established remains unclear. Overall, the ability to generate and maintain transmembrane ionic gradients during absorption controls the absorptive capacity of the human small intestinal epithelium and, by extension, drug transport via the oral route.

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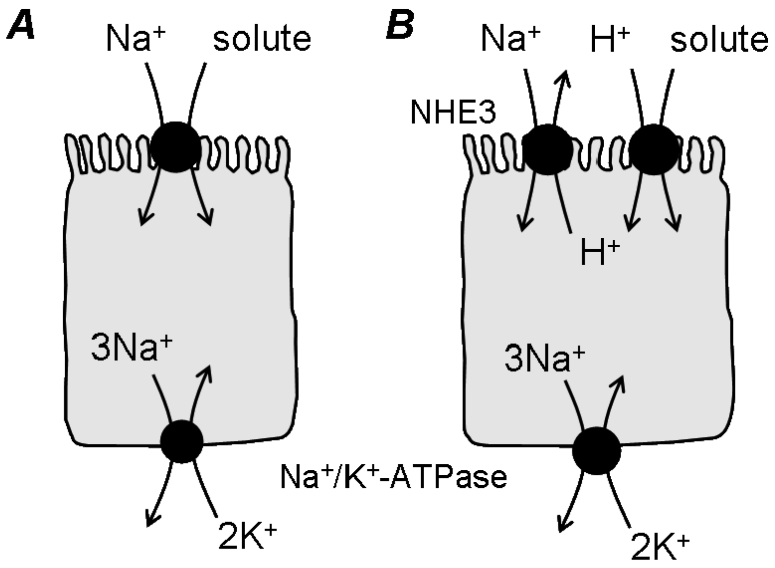

Figure 1.
Schematic representation of Na^+-coupled solute cotransport (the "Na^+ gradient hypothesis") (*A*) and the relationship ("functional cooperativity") between H^+-coupled cotransport (via PepT1 or PAT1) and the Na^+/H^+ exchanger NHE3 (*B*).

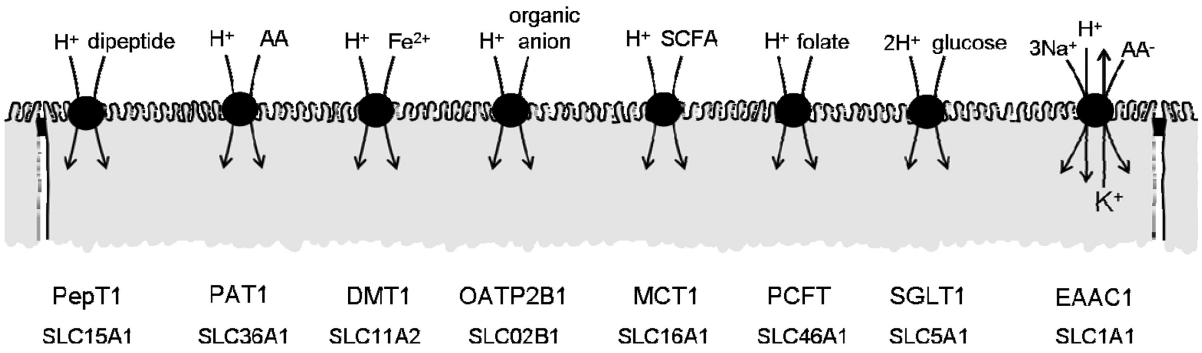

Figure 2.
Schematic representation of the H^+-coupled nutrient, micronutrient and drug transporters at the brush-border membrane of the mammalian small intestine.

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Table 1

Intestinal H^+-coupled nutrient, micronutrient and drug transporters

Gene	Protein	Aliases	Examples of natural substrates, drugs & related compounds
SLC15A1	PepT1		di/tripeptides, β-lactam antibiotics (cephalosporins, penicillins), angiotensin-converting enzyme inhibitors, δ-aminolevulinic acid, bestatin, pro-drugs for L-DOPA and anti-virals (e.g. acyclovir, ganciclovir, AZT)
SLC36A1	PAT1	imino acid carrier LYAAT-1 tramdorin 3	D- and L-amino and imino acids (e.g. proline, alanine, hydroxyproline), D-serine, GABA, MeAIB, taurine, betaine, D- and L-cycloserine, vigabatrin, short-chain fatty acids (e.g. butyrate, acetate, propionate)
SLC11A2	DMT1	NRAMP2 DCT1	Fe^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}
SLC02B1	OATP2B1	OATP-B SLC21A9	oestrone-3-sulphate, DHEAS, benzoate, nicotinate, taurocholate*, BSP, salicylate, valproate, pravastatin*, atorvastatin, fexofenadine, glibenclamide
SLC16A1	MCT1		short-chain fatty acids (e.g. L- and D-lactate, pyruvate, acetate, butyrate, propionate), ketone bodies (acetoacetate, β-hydroxybutyrate), nicotinate, salicylate, benzoate, valproate, atorvastatin, pravastatin
SLC46A1	PCFT	HCP1	folate, methotrexate, permetrexed, 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, heme
SLC5A1	SGLT1		D-glucose, D-galactose
SLC1A1	EAAC1	System X_{AG}^- EAAT3	L-glutamate, L- and D-aspartate, L-cysteine

*OATP2B1 transports taurocholate and pravastatin only at acidic values of pH.