Absorption of amino acids in kidney and intestine involves a variety of transporters for different groups of amino acids. This is illustrated by inherited disorders of amino acid absorption, such as Hartnup disorder, cystinuria, iminoglycinuria, dicarboxylic aminoaciduria, and lysinuric protein intolerance, affecting separate groups of amino acids. Recent advances in the molecular identification of apical neutral amino acid transporters has shed a light on the molecular basis of Hartnup disorder and iminoglycinuria.

Protein absorption in the intestine is accomplished by a combination of proteases, peptidases, and peptide and amino acid transporters (35). Together, they accomplish absorption of 95% of the nutritional protein intake. After being absorbed, amino acids are distributed to all organs, including the kidney. Unbound amino acids pass into the glomerular filtrate and are subsequently reabsorbed using similar transport processes as those observed in the intestine. Although the principles of metabolite absorption are similar in kidney and intestine, different transporter isoforms are often expressed in the two organs (15). In the intestine, amino acid transporters are expressed from the duodenum to the ileum, but the highest capacity is observed in the jejunum. Little absorption is thought to occur in the colon. In the kidney, more than 95% of all amino acids are reabsorbed in the proximal tubule. Apical transport processes have been the focus of investigation for their ease of accessibility; basolateral transport processes, however, are equally important for amino acid absorption. This is particularly well illustrated by the absorption defect lysinuric protein intolerance, which is caused by a defect of basolateral transport (44). This review will, however, focus on apical transporters for neutral amino acids.

Absorption of Amino Acids in Intestine and Kidney

Physiological studies performed between 1950 and 1970 suggested the presence of five different transport systems for amino acids in the intestine (reviewed in Refs. 40, 67, 76). These systems were defined by competition experiments at the apical membrane and thus represent apical transport activities. System 1 is a low-affinity transporter for almost all neutral amino acids; system 2 is a transport system for basic amino acids shared by cystine; system 3 is a transporter for proline and hydroxyproline; system 4 is a transporter for glycine, proline, and β-amino acids; and system 5 is specific for anionic amino acids. This classification has stood the test of time (Table 1) with some minor modifications. Further studies of these systems using brush-border membrane vesicles defined the substrate specificity more closely. The broad substrate specificity of system 1, including all neutral amino acids, was confirmed, resulting in it being named system B0 (broad neutral) or NBB (neutral brush border) (34). System 2 was further characterized as an antiporter, which takes up cationic amino acids in exchange for neutral amino acids (system b0,+) (38). System 3 was intensively investigated in brush-border membrane vesicles derived from rabbit intestine and coined the IMINO system (64). System 4 was intensively studied in rat intestine, where system 3 appeared to be lacking, and was called the Imino acid carrier (37). Systems 3 and 4 have often been confused, because species differences were neglected (68). System 5 has been found in many cell types and tissues and has been termed system XAG (33). In the intestine system, B0 appears to have a higher capacity than other amino acid transporters (39). It has to be kept in mind, however, that 16 amino acids compete for system B0, whereas only 2–3 amino acids compete for each of the other systems. From a mixture of amino acids, large neutral amino and cationic acids are absorbed the fastest, whereas small neutral and acidic amino acids are absorbed more slowly (35).

In the kidney, similar transport systems were identified as in the intestine (see Refs. 57, 62, 79). Further studies, however, showed that amino acid transport in kidney might be more complex than in the intestine. For a number of amino acids, low-affinity transporters were identified in the proximal convoluted tubule and high-affinity transporters in the proximal straight tubule (27). This situation is reminiscent of glucose transporters, where the low-affinity SGLT2 is found in the proximal convoluted tubule, which is complemented by the high-affinity SGLT1 in the proximal straight tubule (77). More detailed physiological and genetic studies also revealed the presence of three different systems for proline, hydroxyproline, and glycine (proline and hydroxyproline are transported in the same way; in the following, only proline will be...
mentioned standing for both amino acids). These are a shared system for glycine and proline (low affinity) and a specific system for each glycine and proline, having a higher affinity (59). Evidence was also presented for two transport systems for cationic amino acids, one of which was shared with cystine, whereas the other was not (62).

**Energetics of Amino Acid Absorption**

It was initially assumed that, similar to glucose transport, uptake of amino acids across the apical membrane was driven by the Na\(^+\)-electrochemical gradient (52). This notion was supported by the demonstration that system B\(^0\) is a Na\(^+\)-neutral amino acid cotransporter. However, cationic amino acid transport was demonstrated to be mediated by an antiport activity in the intestine (38, 71). The IMINO system was also shown to be Na\(^+\)-dependent (64), but the amino acid carrier was insensitive to Na\(^+\) replacement and was shown to be driven by the proton-motive force (49, 50). As a result, three different driving forces are observed in amino acid absorption, namely the Na\(^+\)-electrochemical gradient, the proton-motive force, and the substrate gradients.

**Inherited disorders of amino acid absorption**

Rare inherited disorders were particularly influential in the definition of amino acid transport activities in kidney and intestine (Refs. 36, 57; Table 1). Cystinuria is a disorder affecting the absorption of cationic amino acids and cystine in both kidney and intestine. It is caused by mutations in either of the two subunits [rBAT(SLC3A2) and b\(^0\),AT(SLC7A9)] forming the apical transporter for cationic amino acids system b\(^0\)+. Mutations in the basolateral transporter for cationic amino acids cause lysinuric protein intolerance. The transporters involved in these disorders have been reviewed recently in this journal and are not further discussed here (45). Hartnup disorder has been characterized as an aminoaciduria affecting all neutral amino acids but proline. The aminoaciduria is the defining hallmark of the disorder. Clinical symptoms include a light-sensitive skin rash, cerebellar ataxia, and occasional psychotic symptoms (5). They are not observed in all patients and usually cease after childhood when the demand of amino acids for protein biosynthesis decreases. Inminoglycinuria is a benign syndrome, defined by elevated amounts of proline, hydroxyproline, and glycine in the urine (47). The disorder is genetically complex (18). Because of the lack of clinical symptoms, inminoglycinuric individuals have only been identified during urine mass screening programs in the US, Canada, and Australia (2, 47). Dicarboxylic aminoaciduria also appears to be a largely benign syndrome and is thought to arise from mutations in the apical aspartate/glutamate transporter EAAC1 (28).

There are a few less well known inherited disorders of amino acid absorption, such as methionine malabsorption, tryptophan malabsorption (blue diaper syndrome), and benign hyperdibasic aminoaciduria (15). Due to the lack of patients, these disorders are still ill defined and are not further discussed here.

**Molecular Cloning of Apical Neutral Amino Acid Transporters in Kidney and Intestine**

The gene encoding the general neutral amino acid transport system B\(^0\) was identified in a bioinformatic screen for genes involved in Hartnup disorder (13) after the disorder was mapped to the tip of chromosome 5 (43). Screening for membrane proteins in the syntenic region of the mouse genome revealed the presence of a new member of the solute carrier family 6 (SLC6), also known as the neurotransmitter transporter family. The gene received the identifier SLC6A19 and has the common name B\(^0\)AT1. Expression of mouse B\(^0\)AT1 in Xenopus oocytes subsequently revealed its activity as a Na\(^+\)-dependent transporter for a broad range of neutral amino acids. Sequence comparison identified B\(^0\)AT1 as a new member of the so called “orphan transporter” branch of the SLC6 family. The branch received its name because of an apparent lack of neurotransmitter transport activity associated with its members (42). The identification of B\(^0\)AT1 for the first time suggested a possible function for the “orphans” as amino acid transporters in epithelial cells and brain neurons. Subsequent functional screening of other orphan transporters revealed that XT3 (alias SIT, rB21a, XTR3, SLC6A20) was the long-sought apical system IMINO transporter (30, 66), whereas v7-3 (SLC6A15) encoded the proline and branched-chain amino acid transporter B\(^0\)AT2 (14, 65). The kidney-specific transporter XT2 (alias Rosit, SLC6A18) is likely to be involved in glycine transport (48). Despite intensive attempts, no substrates have been identified for the neuronal transporter NTT4 and for the testis-specific transporter NTT5.

A second group of apical amino acid transporters was identified by their homology to members of the amino acid auxin permease (AAAP) superfamily (8). This family was initially identified in plants, where it mediates proton-dependent uptake of auxin and amino acids (22). Similarly, mammalian homologs of this family either translocate protons or are strongly pH-dependent. They either reside on vesicular compartments, such as the vesicular GABA transporter, or are expressed in the plasma membrane, such as the neutral amino acid transporters of the system A and N family (32). In an attempt to identify new members of this family, Sagne et al. identified a lysosomal transporter for small neutral amino acids (53). Subsequently, it was shown that this transporter is also...
found on the plasma membrane when expressed in HeLa cells and *Xenopus* oocytes. Further studies showed that it had characteristics similar to the Imino acid carrier identified in rat intestine and Caco-2 cells (11). In the following, the biochemical properties of the major apical neutral amino acid transporters are briefly described.

**B⁰AT1 (SLC6A19)**

B⁰AT1 is the molecular correlate of the major apical neutral amino acid transport system B⁰ (or NBB) (13) (FIGURE 1). It transports all neutral amino acids. Large aliphatic amino acids such as methionine, leucine, valine, etc. are the preferred substrates of the transporter. The preference is mainly caused by differences of the $K_m$ rather than the maximum transport velocity. A stoichiometry of 1Na⁺/amino acid was determined. In contrast to many other members of the SLC6 family, B⁰AT1 is a chloride-independent transporter. Interestingly, the substrate $K_m$ is highly dependent on the concentration of Na⁺ and vice versa (7). This behavior is now elegantly explained by the putative structure of B⁰AT1 (see below).

Immunohistochemical analysis shows strong expression of B⁰AT1 in the early segments of the proximal convoluted tubule (S1, S2), even reaching into the glomerulus (51). In the intestine, the transporter was found in all segments of the small intestine with the highest expression occurring in duodenum, jejunum, and ileum. In both tissues, the transporter is restricted to the apical membrane. The human B⁰AT1 shows little activity in heterologous expression systems, but its partial characterization is consistent with the properties of the murine isoform outlined above (29, 61).

**B⁰AT2 (SLC6A15, v7-3, SBAT1)**

B⁰AT2 was initially identified as a brain neurotransmitter transporter of unknown function (74). Functional studies demonstrated it to be a Na⁺-dependent neutral amino acid transporter (14, 65). B⁰AT2 transports branched-chain amino acids and proline with high affinity and has the same mechanism as B⁰AT1. High-affinity Na⁺-dependent transporters for neutral amino acids have been reported in the proximal straight tubule. B⁰AT2 is expressed in the kidney, but localization studies have not yet been reported.

**IMINO (SLC6A20, SIT, XT3, XTRP3, rB21A)**

IMINO, the molecular correlate of system IMINO (FIGURE 1), is a Na⁺- and Cl⁻-dependent transporter for proline and other N-methylated amino acids and analogs (30, 66). L-amino acids are transported preferentially. N-methyl aminoisobutyric acid (MeAIB), an amino acid analog frequently used to identify system A activity, is also a substrate of the IMINO transporter. IMINO is expressed in the apical membrane of both kidney and intestine (51). In the mouse and rat genome, two highly homologous genes are found at the syntenic location where in humans a single SLC6A20 gene is located (16). In mouse and rat, these are known as XT3 (XTRP3, rB21A) and XT3s1 (XTRP3s1), respectively. Unfortunately, the genes have been named in different order in the two species. As a result, mouse XT3 corresponds to rat XT3s1 and vice versa. In all species, only one gene shows activity in heterologous expression systems, namely rat XT3 (rB21A), mouse XT3s1, and human SLC6A20. To unify the nomenclature, it has been suggested calling the transporters IMINOB and IMINOK (30). IMINOB in all species is highly expressed in the brain, kidney, and intestine, whereas IMINOK is only expressed in kidney. In all species, IMINOB is the functional transporter.

**XT2 (SLC6A18, XTRP2, Rosit)**

XT2 is strongly expressed in the kidney proximal tubule, particularly its S3 segment, but does not occur in other tissues (FIGURE 1). Functional transport of XT2 has not yet been demonstrated, although numerous substrates were tested (75). Expression at the cell surface was experimentally verified (41); later, localization in the apical membrane of the proximal straight tubule was shown. To identify the
physiological role of XT2, a knockout mouse was generated by Quan et al. (48). The mouse showed elevated levels of glycine in the urine; however, other amino acids appeared to be elevated as well (48). Vesicles prepared from kidney medulla lacked a high-affinity glycine transport activity, whereas leucine transport appeared to be normal. The mouse displayed elevated blood pressure, pointing to a role of glycine in blood pressure regulation. Consistent with this observation, XT2 is upregulated in hypernatremic rats, suggesting a role of glycine in the downregulation of blood-pressure. In mouse, six different splice variants of the gene have been identified (41). Because the first 10 helices are critical for function in the SLC6 transporter family, most of the splice variants are unlikely to be functional and thus may not have a physiological role.

**PAT1 (SLC36A1, LYAAT-1, TRAMD3)**

PAT1 (proton amino acid transporter 1) is the molecular correlate of the intestinal imino acid carrier (1) (**FIGURE 1**). PAT1 is a proton-amino acid cotransporter having a stoichiometry of 1:1 (11). Due to its mechanism, PAT1 activity increases at acidic pH, which correlates well with acidic micromilieu close to the brush-border in the intestine. Glycine, proline, and alanine are the preferred substrates of the transporter. The maximum velocity is similar for the three substrates. All substrates are transported with low affinity, showing $K_m$ values in the range of 2–10 mM. The transporter does not discriminate between L- and D-isoforms of these amino acids; in addition, β-alanine is transported with similar affinity as α-alanine. Similar to the IMINO transporter, the amino acid analog MeAIB is recognized by PAT1. The transporter is strongly expressed in the small intestine, colon, kidney, and brain. The system IMINO (SLC6A20) transporter can be discriminated from PAT1 (SLC36A1) by its substrate specificity, particularly the exclusion of glycine and its stereospecificity (9).

**PAT2 (SLC36A2)**

PAT2 (proton amino acid transporter 2) has significant functional and sequence similarity to PAT1 (10). PAT2 is a proton-amino acid cotransporter with the same stoichiometry as PAT1 (11). The pH dependence is less steep, and as a result the transporter is more active at neutral and alkaline pH than PAT1. Proline, glycine, and alanine are the preferred substrates of the transporter, but glycine has a significantly higher maximum velocity (23). The transporter does not strongly discriminate between D- and L-isoforms. The $K_m$ values for the preferred substrates are below 1 mM. The transporter is thus considered as the high-affinity isoform of the Imino acid carrier. In contrast to PAT1, β-alanine has significantly lower affinity for the transporter than α-alanine. Similarly γ-aminobutyric acid is a reasonable substrate for PAT1 but is hardly recognized by PAT2. The transporter is strongly expressed in heart and lung and more weakly in kidney and muscle.

**Molecular Basis of Apical Neutral Amino Acid Transport Disorders**

Two studies have shown that mutations in B0AT1 cause Hartnup disorder (29, 61). To date, a total of 18 mutations have been identified, namely 11 missense mutations, 3 nonsense, 2 frame-shifts, and 2 splice site mutations (**FIGURE 2**). The mutation D173N is the most

![Table 1. Apical amino acid transporters](http://physiologyonline.physiology.org/)

<table>
<thead>
<tr>
<th>System</th>
<th>cDNA</th>
<th>SLC</th>
<th>Amino Acid Substrates</th>
<th>Disorder</th>
<th>Mechanism</th>
<th>Tissue</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B^0$</td>
<td>B0AT1</td>
<td>SLC6A19</td>
<td>$\text{AA}^0, \text{P,L,V,J,M}$</td>
<td>Hartnup</td>
<td>$\text{Na}^+/\text{Sp}$</td>
<td>Ki, St</td>
<td>29, 61</td>
</tr>
<tr>
<td>$b^{0,+}$</td>
<td>rBAT/ $b^{0,+}$AT</td>
<td>SLC3A1/ SLC7A9</td>
<td>$\text{R,K,O,cystine}$</td>
<td>Cystinuria</td>
<td>$\text{Cystinuria}$</td>
<td>Ki, Br</td>
<td>17, 21</td>
</tr>
<tr>
<td>Gly</td>
<td>XT2</td>
<td>SLC6A18</td>
<td>$G$</td>
<td>Iminoglycinuria (candidate)</td>
<td>n.d.</td>
<td>Ki</td>
<td>48</td>
</tr>
<tr>
<td>IMINO</td>
<td>IMINO</td>
<td>SLC6A20</td>
<td>$\text{P, HO-P}$</td>
<td>Iminoglycinuria (candidate)</td>
<td>$\text{Na}^+/\text{Cl}^-\text{Sp}$</td>
<td>Ki, St, Br</td>
<td>30, 66</td>
</tr>
<tr>
<td>PAT1 (Imino acid)</td>
<td>SLC36A1</td>
<td>$\text{P,G,A GABA, }\beta-\text{A}$</td>
<td>Iminoglycinuria (candidate)</td>
<td>$\text{H}^+/\text{Sp}$</td>
<td>Ki, Lu, Ht</td>
<td>11, 53</td>
<td></td>
</tr>
<tr>
<td>PAT2</td>
<td>SLC36A2</td>
<td>$\text{P,G,A}$</td>
<td>Iminoglycinuria (candidate)</td>
<td>$\text{H}^+/\text{Sp}$</td>
<td>Ki, Lu, Ht</td>
<td>11, 53</td>
<td></td>
</tr>
<tr>
<td>$X^-_{\text{AG}}$</td>
<td>EAAT3</td>
<td>SLC1A1</td>
<td>$E,D$</td>
<td>Dicarboxylic</td>
<td>$\text{Na}^+, \text{H}^+/\text{Sp}$</td>
<td>Ki, St, Br</td>
<td>63</td>
</tr>
</tbody>
</table>

Summary of the major apical aminoacid transporters found in kidney and intestine. $\text{AA}^0$, neutral amino acids; HO-P, hydroxyproline; $\beta$-A, beta-alanine; O, ornithine (all other amino acids listed in one-letter code); Br, brain; Ht, heart; Ki, kidney, Lu, lung; St, small intestine.
frequent allele in the Australian and probably the European population (4). Initially, it was suggested that more than one gene might be involved in Hartnup disorder. However, resequencing of pedigrees that could not be explained in the initial studies revealed mutations in B0AT1 in all families (3). This suggests that B0AT1 is the predominant transporter for neutral amino acids in the proximal tubule. The clinical phenotype of Hartnup disorder remains to be explained. It is assumed that most symptoms are caused by a lack of tryptophan, which in humans is a significant precursor of NAD(P)H biosynthesis. In agreement with this notion, it has been reported that the pellagra-like skin rash, which is frequently observed in patients with the disorder, responds to treatment with niacin, the second main precursor of NAD(P)H biosynthesis (58). The clinical variability observed in Hartnup disorder is most likely the result of environmental factors, such as nutrition, or is related to developmental factors such as the maturation of transport processes in kidney and intestine.

Iminoglycinuria is more complex than Hartnup disorder, most likely involving several genes (18). In contrast to Hartnup disorder where the urine amino acid profile is similar in all cases and intestinal transport is always affected, the phenotype of iminoglycinuria is more heterogeneous. In some pedigrees, glycinuria is observed in heterozygotes, whereas in others urine amino acid concentrations are normal. Furthermore, in most cases, intestinal transport appears to be normal, whereas in other pedigrees impaired intestinal transport was reported. Iminoglycinuria also occurs during childhood development. In the first weeks of life, newborns show iminoglycinuria (56). After 3 mo, proline excretion ceases followed by the normalization of glycine excretion after about 6 mo. These observations suggest the presence of specialized transporters for each glycine and proline. The spillover of glycine, hydroxyproline, and proline into the urine in cases of prolinemia (when the plasma proline concentration exceeds 0.8 mM) in addition demonstrates the presence of a common transporter for all three amino acids. These results were confirmed by amino acid infusion studies (55, 60). Taken together, a variety of studies suggest the presence of four different transporters for glycine, proline/hydroxyproline (FIGURE 1), namely 1) a common transporter for all three amino acids in the kidney, 2) a common transporter for all three amino acids in the intestine, 3) a specialized transporter for glycine, and 4) a specialized transporter for proline/hydroxyproline. It should be mentioned that a significant fraction of proline and glycine transport in both kidney and intestine is mediated by the neutral amino acid transporter B0AT1 (SLC6A19). As a result, five transporters contribute to the transport of these three amino acids. The common transporter for all three amino acids in the intestine is the proton amino acid transporter PAT1 (1). The common transporter in the kidney has not been unambiguously identified. Both PAT1 and PAT2 are expressed in the kidney, but immunohistochemical data of their distribution are lacking (11). The pH-dependence of PAT1 renders the transporter less active in the proximal tubule where the extracellular pH is close to neutral. As a result, PAT2 is likely to be the dominant transporter in the proximal tubule. The proline-specific transporter has been identified as the

![Homology model of the human B0AT1 transporter](image2.png)

**FIGURE 2.** Homology model of the human B0AT1 transporter. The homology model was created using the Swiss-Model web server (54). Subsequently, the substrate and Na+ ion were reintroduced at the original coordinates. The figure was generated using PyMOL (DeLano Scientific). A: the view from the side (extracellular side top). B: the transporter from top. Helix 1 is depicted in yellow, and helix 6 is depicted in blue. Putative regions with β-sheet conformation are also depicted in yellow. The backbone of the protein is highlighted. Residues found to be mutated in Hartnup disorder individuals and mentioned in the text are shown in full size.
The Imino activity has been described in rabbit intestine, where it appears to be the dominant transport activity for proline and hydroxyproline together with system B⁰. This has caused considerable confusion because, in rat intestine, the Imino acid carrier (PAT1) and system B⁰ (B⁰AT1) are the dominant transporters (40). This illustrates that species differences can be significant, particularly in the intestine, which is exposed to different diets. The molecular correlate of the glycine-specific transporter most likely is XT2. These transporters can now be combined to hypothesize the different scenarios of iminoglycinuria. In the newborn, PAT1/2 is most likely already expressed, but its capacity is not sufficient to remove all of proline and glycine from the primary urine resulting in iminoglycinuria of the newborn. During the postnatal development of the renal system, the Imino transporter expression increases causing prolinuria to cease. Further development probably results in an increase of XT2 expression, resulting in complete reabsorption of all amino acids after 6 mo. Adult iminoglycinuria, by contrast, is more likely a defect of the common transporter. Iminoglycinuria is an autosomal recessive disorder, suggesting that the different types of iminoglycinuria are caused by different individual genes. It is tempting to suggest that cases with intestinal involvement are caused by mutations in PAT1, whereas cases without intestinal involvement carry mutations in PAT2. How much IMINO and XT2 contribute to the variability of the disorder remains to be determined. Glycinuria in heterozygotes can be explained by differential capacities for proline and glycine transport (12). Even in normal individuals, proline and hydroxyproline are usually undetectable, whereas the concentration of glycine is significant and variable. It has recently been shown that a stop codon in the putative glycine transporter XT2 occurs with a frequency of 0.4 in the Japanese population (20). As a result, about 16% of all individuals in this population are homozygous for this mutation. Unfortunately, urine amino acid concentrations were not reported in this study. It is tempting to speculate that the variable amounts of glycine in the urine of the normal population may be caused by mutations in XT2. Together, these findings suggest that the capacity for reabsorption of glycine is limited, whereas reserve capacity is available for proline. Thus isolated glycinuria is expected for heterozygotes in pedigrees with iminoglycinuria. In view of the multitude of transporters for glycine and proline, it is surprising that of a mixture of amino acids glycine and proline are the slowest to be absorbed in the intestine (35).

Structure of Apical Amino Acid Transporters

Our understanding of SLC6 transporter structure and function has significantly gained from the recent high-resolution structure of the leucine transporter from *Aquifex aeolicus* (LeuT) (78). This transport protein has significant sequence similarity with all members of the SLC6 family. This allows the generation of homology models (FIGURE 2), which can be used to predict the possible impact of mutations found in inherited disorders. The LeuT structure is characterized by 12 transmembrane helices surrounding a central translocation pathway. Helices 1 and 6 are particularly critical for the functioning of the transporter. Both helices are partially unwound in the center, exposing backbone contacts that form the two Na⁺ binding sites in the transporter. Intriguingly, the carboxylgroup of the substrate amino acid forms part of the Na⁺ binding site 1 in the transporter. This elegantly explains the mutual influence of substrate and Na⁺ ions on each others’ *K₅₀*, which has been observed in B⁰AT1, B⁰AT2, and IMINO (16). It also gives an example of how coupling of substrate transport to the Na⁺ electrochemical gradient can be achieved. The bacterial LeuT transporter is Cl⁻-independent, whereas many of the mammalian members are Cl⁻-dependent. Interestingly, this Cl⁻ binding site is not only conserved in the IMINO transporter but also in the Cl⁻-independent transporters of the SLC6 family such as B⁰AT1 and B⁰AT2, suggesting that these transporters may use a static Cl⁻ ion to stabilize the structure of the transporter. So far, very few of the mutations identified in Hartnup disorder are located in the translocation pathway of the transporter (FIGURE 2). The homology structure provides an immediate explanation as to why an Arg57Cys mutation identified in Japanese families causes Hartnup disorder (29). Arg57 is the most highly conserved residue in the SLC6 family and is thought to provide the extracellular gate of the transporter. It is likely to form a reversible ion pair with residue Asp486. It has been proposed that, during the operation of the transporter, helices 1 and 6 change their tilting angle like a rocker switch (78). The other helices by contrast are likely to move very little, possibly providing a scaffold for the two rocking helices. Thus far, the transporter has only been crystallized in one conformation, which may be a translocation intermediate in which the substrate and cosubstrates are occluded in the center of the protein. In analogy to many other mutations associated with rare disorders, mutations outside the catalytic core of the transporter often affect the trafficking of the protein to the apical membrane; however, experimental evidence has not yet been reported for Hartnup disorder mutations.
Protein-Protein Interactions in the Apical Membrane

Amino acid transporters are likely to have interactions with other proteins in the membrane. The most well described protein-protein interaction of an amino acid transporter in the apical membrane is the heteromeric amino acid transporter rBAT/b0,+AT (45). The rBAT protein is a type II membrane protein with a single transmembrane helix and a large extracellular domain, which has a structure similar to bacterial glycosidases but is catalytically nonfunctional as a glycosidase. The b0,+AT light-chain is a polytopic membrane protein with 12 transmembrane helices and forms the translocation pathway. Both subunits are linked by a disulfide bridge and in addition are likely to form a dimer of heterodimers in the membrane (25). Formation of this heterodimer is required for the complex to be trafficked to the plasma membrane. As a result, mutations in either of the two subunits cause cystinuria. Heteromeric transporters are described in more detail in a recent review in this series (45).

The limited activity, particularly of human B0AT1, in heterologous expression systems suggested that members of the SLC6 family also might require auxiliary proteins to foster surface expression. Such a protein was discovered recently, when analyzing collectrin-deficient mice (19). It was noticed that these mice, which otherwise have no apparent phenotype, had a precipitate in their urine. Further analysis detected large amounts of neutral amino acids in the urine. In particular, tyrosine levels were very high, causing precipitation of the amino acid as crystals. As the name suggests, collectrin was initially thought to be mainly expressed in the collecting duct (80). Immunohistochemical analysis, however, showed it also to be expressed in the proximal tubule where amino acid reabsorption occurs but also revealed its expression in the pancreas. Collectrin is sequence-related to angiotensin-converting enzyme II (ACE2) but lacks the catalytic domain of the latter (72). Western blot analysis revealed reduced levels of key apical amino acid transporters such as B0AT1, IMINO, and XT2 in the kidney (19). Coexpression of collectrin together with B0AT1 showed increased surface expression in both oocytes and transfected cells. Similar to rBAT, collectrin has only one transmembrane helix and could form a heterodimeric complex with B0AT1, IMINO, and XT2 (FIGURE 1). In the pancreas, collectrin is thought to interact with SNAPIN, a protein involved in the exocytosis of insulin-containing vesicles (26). Thus collectrin could form a bridge between transport proteins and the exocytic machinery, thereby promoting a variety of amino acid transporters to the apical membrane.

The aminoaciduria observed in collectrin-deficient mice suggests a possible link between renal Fanconi syndrome and the apical amino acid transporters. Mice that are deficient in the hepatocyte nuclear factor HNF-1α show symptoms similar to renal Fanconi syndrome (46). In humans, mutations in this transcription factor are associated with Type 3 maturity-onset diabetes of the young (MODY3), which is also accompanied by general aminoaciduria (6). HNF-1α is a tissue-specific transcription factor and is among other tissues highly expressed in the kidney. The collectrin gene is controlled by HNF-1α, suggesting that downregulation of apical amino acid transporters causes the aminoaciduria in these mice (81). A thus-far unexplored interaction is that of ACE2 with apical transporters. Although being named ACE2, its role in the conversion of angiotensin is limited. The enzyme is a general carboxypeptidase with a preference for large neutral amino acids at the carboxyterminus (73). The enzyme is one of many brush-border peptidases that, together with amino acid and peptide transporters, accomplish protein absorption. Interestingly, collectrin is not expressed in the intestine, where it might be replaced by ACE2.

In many studies, proline transport in the intestine has been described as Na+-dependent. As outlined above, this is in part due to the fact that, in some species, IMINO is the dominant proline transporter. However, also in species where this activity is mediated by PAT1, a partial Na+ dependence was noted (1). This is now explained by a functional interaction with the apical Na+/H+ exchanger NHE3 (FIGURE 1). Proton-coupled transport has been established for a variety of nutrients in the apical membrane such as peptides (PEPT1), proline and glycine (PAT1), and folate (HCP) (69). These protons are extruded back into the lumen by NHE3. Coexpression of the apical NHE3 causes PEPT1 and PAT1 to show partial Na+ dependence, whereas the basolateral NHE1 has no effect (70). It is tempting to speculate that these proteins may have a closer association mediated by scaffolding proteins such as NHERF-1 and related proteins (31).

Conclusion

In the past 10 years, all major apical neutral amino acid transporters have been identified on a molecular level. This has considerably improved our understanding of rare inherited aminoacidurias. Although these disorders are clinically not very important, they have opened the door to a better understanding of more complex disorders that affect the kidneys, such as Fanconi syndrome and maturity-onset diabetes of the young.

References


