Amino Acid Transport Across Mammalian Intestinal and Renal Epithelia

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Broër S. Amino Acid Transport Across Mammalian Intestinal and Renal Epithelia. Physiol Rev 88: 249–286, 2008; doi:10.1152/physrev.00018.2006.—The transport of amino acids in kidney and intestine is critical for the supply of amino acids to all tissues and the homeostasis of plasma amino acid levels. This is illustrated by a number of inherited disorders affecting amino acid transport in epithelial cells, such as cystinuria, lysinuric protein intolerance, Hartnup disorder, iminoglycinuria, dicarboxylic aminoaciduria, and some other less well-described disturbances of amino acid transport. The identification of most epithelial amino acid transporters over the past 15 years allows the definition of these disorders at the molecular level and provides a clear picture of the functional cooperation between transporters in the apical and basolateral membranes of mammalian epithelial cells. Transport of amino acids across the apical membrane not only makes use of sodium-dependent symporters, but also uses the proton-motive force and the gradient of other amino acids to efficiently absorb amino acids from the lumen. In the basolateral membrane, antiporters cooperate with facilitators to release amino acids without depleting cells of valuable nutrients. With very few exceptions, individual amino acids are transported by more than one transporter, providing backup capacity for absorption in the case of mutational inactivation of a transport system.

I. INTRODUCTION

Protein forms up to 30% of the typical western human diet. After its digestion, the resulting peptides and amino acids are efficiently absorbed by the enterocytes of the small intestine (1). Inside enterocytes peptides are hydrolyzed, and the resulting amino acids are released together with those absorbed by amino acid transporters. Through the blood, amino acids are delivered to all tissues, where they serve as building blocks for protein synthesis, as
precursors for a wide variety of bioactive molecules, and as energy metabolites. In the kidney, unbound amino acids are filtered and subsequently reabsorbed to avoid wastage of valuable metabolites. Although amino acid transport has been studied in many types of epithelial cells, this review focuses on mammalian kidney and intestine, because the knowledge is much more advanced in these two tissues. Excellent reviews have covered the nonmolecular aspects of this field (209, 224, 233, 305, 315, 320, 339, 343, 356, 375, 418, 419). More recently, certain molecular aspects of epithelial transport have been presented (75, 97, 98, 113, 254, 256–261, 393–396, 398). This review aims to summarize our knowledge of the mediators of amino acid transport in the apical and basolateral membrane. Transport processes in model organisms such as Danio Rerio (zebrafish), Drosophila melanogaster, and Caenorhabditis elegans are increasingly studied; however, so far they have contributed little to the understanding of mammalian epithelial transport and as a result are not covered in this review. It is impossible to cover all aspects of this field, and the author apologizes for any omission of references that are relevant to the many aspects of this research area.

Amino acid transport activities are frequently referred to as “systems”; the term indicates a functionally identified transport activity that appears to be similar in a variety of cell types. Pioneered by studies of Halvor Christensen (reviewed in Refs. 76, 77) in nonepithelial cells, it was recognized that amino acid transport systems accept groups of amino acids rather than individual amino acids. Christensen’s work established the presence of neutral amino acid transporters that prefer leucine and other large hydrophobic neutral amino acids (system L) (252), prefer alanine and other small and polar neutral amino acids (system A), or prefer alanine, serine, and cysteine (system ASC). A separate nomenclature (x for anionic, y for cationic, z for neutral) has been applied to systems mediating transport of cationic amino acids (system y\(^+\)) and anionic amino acids (system X\(^-\)). With a few exceptions (system L, system T), lowercase acronyms indicate Na\(^+\)-independent transporters, whereas uppercase acronyms are used for Na\(^+\)-dependent transporters. These amino acid transport systems have subsequently been identified in many cell types and were confirmed by molecular cloning of the mediators of these transport activities (Table 1). Apical transport systems in epithelial cells, however, did not appear to match the properties of the systems characterized by Christensen’s group. As a result, new systems were defined for epithelial cells, which are described below. Systems similar to those of nonepithelial cells were identified in the basolateral membrane. Because the nomenclature has remained inconsistent and confusing, Table 1 provides an overview of systems and their mediators.

Studies performed in the 1950s and 1960s revealed that peptides and amino acids are actively absorbed in the intestine. Amino acids and peptides from 300 g of protein from endogenous and exogenous sources are absorbed every day, of which only the equivalent of 10 g appears as fecal nitrogen (122). The proximal jejunum is the major site of amino acid and peptide absorption, but other parts of the small intestine also have significant transport capacity (345). In the kidney nephron, the proximal tubule is the major site of nutrient reabsorption. About 95–99% of all amino acids are reabsorbed in the proximal convoluted tubule and proximal straight tubule (reviewed in Refs. 343, 418). With the exception of histidine, glycine, and taurine, the clearance rate of all amino acids in humans is <1 ml/min \(\times 1.73 \text{ m}^2\), in contrast to a value of \(\approx 120 \text{ ml/min \times 1.73 m}^2\) for substances that are neither reabsorbed nor secreted. Based on functional studies in kidney and intestine and the amino acid profile in the urine of individuals with different aminoacidurias, five transport activities were proposed (209, 320, 375, 418):

1. the “neutral system” or “methionine preferring system” transporting all neutral amino acids; 2) the “basic system” transporting cationic amino acids together with cystine; 3) the “acidic system” transporting glutamate and aspartate; 4) the “iminoglycine system” transporting proline, hydroxyproline, and glycine; and 5) the \(\beta\)-amino acid system. This review adopts this classification and will discuss each group of amino acids, summarizing the inherited disorders associated with them, functional studies, and the molecular mediators involved in their transport.

II. NEUTRAL AMINO ACIDS

A. Inherited Disorders of Neutral Amino Acid Transport

1. Hartnup disorder (OMIM 234500)

Hartnup disorder was first described in 1956 (22) as a renal aminoaciduria of neutral amino acids associated with a pellagra-like skin rash and episodes of cerebellar ataxia. The disorder is autosomal recessive with an estimated frequency of \(~1:20,000\) (187). It is now well established that most patients will remain clinically asymptomatic when provided with a protein-rich diet. Most likely the lack of uptake of neutral amino acids can be compensated by the transport of peptides (10, 210). Hartnup disorder affects both renal and intestinal uptake of neutral amino acids. Impaired intestinal uptake can be inferred from the presence of bacterial degradation products, such as indole and tryptamine (11, 212). An almost complete lack of intestinal tryptophan absorption was demonstrated in vivo (155) and in biopsy material in vitro.

Physiol Rev • VOL 88 • JANUARY 2008 • www.prv.org
### Epithelial amino acid transport systems and their mediators

<table>
<thead>
<tr>
<th>System</th>
<th>cDNA</th>
<th>SLC</th>
<th>Amino Acid Substrates</th>
<th>Analogs</th>
<th>Affinity</th>
<th>Mechanism</th>
<th>Ions</th>
<th>Expression*</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>SNAT2</td>
<td>SLC38A2</td>
<td>G,P,A,S,C,Q,N,H,M</td>
<td>MeAIB</td>
<td>Medium</td>
<td>S</td>
<td>Na+</td>
<td>Ub</td>
</tr>
<tr>
<td></td>
<td>SNAT4</td>
<td>SLC38A4</td>
<td>G,A,S,C,Q,N,M,AA*</td>
<td>MeAIB</td>
<td>Medium</td>
<td>S</td>
<td>Na+</td>
<td>K</td>
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<tr>
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<td>ASC1T</td>
<td>SLC1A4</td>
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<td>Na+</td>
<td>K</td>
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<td>ASC2T</td>
<td>SLC1A5</td>
<td>A,S,C,T,Q</td>
<td></td>
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<td>A</td>
<td>Na+</td>
<td>K</td>
</tr>
<tr>
<td>asc</td>
<td>4F2hc/asc1</td>
<td>SLC3A2/SLC7A10</td>
<td>G,A,S,C,T</td>
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<td>High</td>
<td>A</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td>B⁰</td>
<td>B⁰AT1</td>
<td>SLC6A19</td>
<td>AA²</td>
<td>BCH</td>
<td>Low</td>
<td>S</td>
<td>Na+</td>
<td>K</td>
</tr>
<tr>
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<td>SLC6A15</td>
<td>P,L,V,M</td>
<td>BCH</td>
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<td>Na+</td>
<td>K</td>
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<tr>
<td>B⁰⁺⁺⁻</td>
<td>rBATB⁰⁺⁺⁻AT</td>
<td>SLC3A1/SLC7A0</td>
<td>R,K,O,cystine</td>
<td>High</td>
<td>A</td>
<td>K</td>
<td></td>
<td></td>
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<tr>
<td>β</td>
<td>TauT</td>
<td>SLC6A6</td>
<td>Tau, β-Ala</td>
<td>High</td>
<td>S</td>
<td>Na+, Cl⁻</td>
<td>K (AM,BM)</td>
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<td>Gly</td>
<td>XT2</td>
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<td>NR</td>
<td>K (AM)</td>
<td></td>
<td></td>
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<tr>
<td>IMINO</td>
<td>IMINO</td>
<td>SLC6A20</td>
<td>P, HO-P</td>
<td>MeAIB</td>
<td>Medium</td>
<td>S</td>
<td>Na⁺, Cl⁻</td>
<td>K (AM)</td>
</tr>
<tr>
<td>L</td>
<td>4F2hc/LAT1</td>
<td>SLC3A2/SLC7A5</td>
<td>H,M,L,I,V,F,Y,W</td>
<td>BCH</td>
<td>High</td>
<td>A</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4F2hc/LAT2</td>
<td>SLC3A2/SLC7A8</td>
<td>AA² except P</td>
<td>BCH</td>
<td>Medium</td>
<td>A</td>
<td>K (BM)</td>
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<td>SLC43A1</td>
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<td>BCH</td>
<td>Low</td>
<td>U</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td>LAT4</td>
<td>SLC43A2</td>
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<td></td>
<td>BCH</td>
<td>Low</td>
<td>U</td>
<td></td>
<td></td>
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<tr>
<td>N</td>
<td>SNAT3</td>
<td>SLC38A3</td>
<td>Q,N,H</td>
<td>Low</td>
<td>S</td>
<td>Na⁺(S), H⁺(A)</td>
<td>K (BM)</td>
<td></td>
</tr>
<tr>
<td>SNAT5</td>
<td>SLC38A5</td>
<td>Q,N,H,G</td>
<td>Low</td>
<td>S</td>
<td>Na⁺(S), H⁺(A)</td>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAT (Imino acid)</td>
<td>PAT1</td>
<td>SLC36A1</td>
<td>P,G,A GABA, β-Ala</td>
<td>MeAIB</td>
<td>Low</td>
<td>S</td>
<td>H⁺</td>
<td>K (AM)</td>
</tr>
<tr>
<td>PAT2</td>
<td>SLC36A2</td>
<td>P,G,A</td>
<td>MeAIB</td>
<td>Medium</td>
<td>S</td>
<td>H⁺</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>TXT1</td>
<td>SLC16A10</td>
<td>F,Y</td>
<td>Low</td>
<td>U</td>
<td>K (BM)</td>
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<td></td>
</tr>
<tr>
<td>X⁻AG</td>
<td>EAAT2</td>
<td>SLC1A2</td>
<td>E,D</td>
<td>High</td>
<td>S</td>
<td>Na⁺,H⁺(S), K⁺(A)</td>
<td>K (BM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EAAT3</td>
<td>SLC1A1</td>
<td>E,D</td>
<td>High</td>
<td>S</td>
<td>Na⁺,H⁺(S), K⁺(A)</td>
<td>K (AM)</td>
<td></td>
</tr>
</tbody>
</table>

| x⁻⁺⁺ | 4F2hc/xCCT | SLC3A2/SLC7A11 | E, cystine⁻ | High | A | K |
|       | CAT-1 | SLC7A1 | | | | |
| y⁺⁺   | 4F2hc/y⁺LAT1 | SLC3A2/SLC7A7 | K,R,Q,H,M,L,A,C | High | A | Na⁺(S-AA²) | K (BM) |
| y⁺⁺⁻ | 4F2hc/y⁻LAT2 | SLC3A2/SLC7A6 | K,R,Q,H,M,L,A,C | High | A | Na⁺(S-AA²) | K (BM) |

| NR, not reported; A, antiport; AA², neutral amino acids; AA⁻, cationic amino acids; U, uniport; S, symport; S-AA², symport together with neutral amino acids; K, kidney; I, intestine; AM, apical membrane; BM, basolateral membrane; Ub, ubiquitous. Amino acids are given in one-letter codes. O, ornithine; HO-P, hydroxyproline. Affinity: high, <100 μM; medium, 100 μM to 1 mM; low, >1 mM. * Expression in epithelial cells of kidney and intestine. |

These results suggest that the transporter affected in Hartnup disorder is the major mediator of tryptophan uptake in the intestine. However, other neutral amino acids are still absorbed to some extent. In the kidney, there is strong evidence for additional neutral amino acid transporters. The renal clearance for some neutral amino acids (serine, threonine, glutamine, tyrosine, tryptophan, histidine) in Hartnup disorder ranges between 60 and 80 ml·min⁻¹·1.73 m⁻²; for other neutral amino acids, it may be as little as 20 ml·min⁻¹·1.73 m⁻² (84). Intriguingly, excretion of proline and glycine, although both being substrates of the Hartnup transporter (see sect. uC), is not elevated. As a result, it appears that in the renal tubule additional transport systems for neutral amino acids exist, most notably for proline and glycine. It has been suggested that the lack of intestinal tryptophan transporter is responsible for most if not all clinical phenotypes of Hartnup disorder (375). This is supported by the observation that clinical symptoms are mainly observed in individuals with lower than normal plasma amino acid concentrations (323). The pellagra-like skin rash most likely results from nicotinamide deficiency caused by the lack of its metabolic precursor tryptophan. In support of this notion, the rash often responds to nicotinic acid supplementation (209). The episodes of cerebellar ataxia remain unexplained. It has been suggested that bacterial degradation products of tryptophan could be toxic (209). However, a protein-rich diet usually abolishes the symptoms of Hartnup disorder, although breakdown products of tryptophan are still detectable. Hartnup disorder may be a multifactorial disease (323). However, apart from its clinical variability, there is little evidence for it being a multigenic disorder. For example, the urinary amino acid profile appears to be similar in most cases. Thus far, only the neutral amino acid transporter B⁰⁰AT1 (SLC6A19) is known to harbor mutations in Hartnup disorder (see sect. uC and Table 2).

### 2. Methionine malabsorption (OMIM 250900)

Hooft et al. (145) reported a case of a 2-year-old girl suffering from diarrhea and convulsions. Large quantities of methionine and branched-chain amino acid (BCAA) were found in the feces but not in the urine, suggesting an intestinal transport defect. Bacterial degradation products of methionine, such as α-hydroxybutyrate, were found in the urine. A low-methionine diet reduced the symptoms, whereas oral methionine loading caused diarr
rhea and methioninuria (375). This suggests that a similar system might be operating in the kidney, but that it is only visible at increased plasma levels of methionine. In agreement with the notion of an additional transport system for methionine in the kidney, methionine excretion in Hartnup disorder is usually limited (212). A defect similar to methionine malabsorption was reported by Smith and Strang (349). The infant showed edema, convulsions, and mental retardation, together with excretion of α-hydroxybutyrate and phenylpyruvate. The disorder can be discriminated from Hartnup disorder because amino acids are usually not found in increased amounts in the urine. The severity of the disorder points to an expression of the transporter in other organs such as liver and brain. A system Phe transporter has been described in intestinal brush-border membrane vesicles (BBMV) (357), which also transports phenylalanine and methionine and may be affected in this disorder. The molecular correlate of this transporter is unknown, and the existence of system Phe has been disputed (see sect. II B).

3. Tryptophan malabsorption: blue diaper syndrome (OMIM 211000)

Drummond et al. (101) initially reported two cases of an inborn error of intestinal tryptophan transport. In these cases, symptoms were quite severe, including failure to thrive, recurrent fever, hypercalcemia, and nephrocalcinosis. The feces contained increased amounts of tryptophan, and its bacterial degradation products were often referred to as indoles. Indoles are absorbed in the colon and are converted into indoxylsulfate (indican) in the liver. Indican, when released into the urine or the feces, oxidizes to become indigo, causing the blue color of the diapers (208). Renal excretion of amino acids was measured in one case and appeared to be normal; however, it was not measured at an increased amino acid load. Given that the bulk of tryptophan is transported by the neutral amino acid transporter involved in Hartnup disorder, it appears likely that the transporter responsible for blue diaper syndrome has a basolateral location. A candidate for this disorder is the basolateral transporter for aromatic amino acids TAT1 (see sect. II C and Table 2).

B. Neutral Amino Acid Transport in Kidney and Intestine

1. Transport of neutral amino acids across the apical membrane of renal epithelial cells

The accumulation of neutral amino acids in kidney is driven by the Na⁺-electrochemical gradient. Removal of Na⁺ abolishes the accumulation of neutral amino acids in slices (120, 324, 329), abolishes transepithelial transport of neutral amino acids in perfused tubules (386), and prevents accumulation in BBMV (105, 107). A 1 Na⁺:1 amino acid was reported for phenylalanine and alanine (107, 147, 178), which is consistent with the observation of inward currents during neutral amino acid transport in intact rat proximal tubule (308). Interestingly, it appears that increased extracellular Na⁺ mostly affects the $K_m$ for alanine rather than the $V_{max}$ of the transport process. The $K_m$ for Na⁺ decreases when the membrane hyperpolarizes (147).

The substrate specificity of the transporter for neutral amino acids has been studied in some detail. Infusion

TABLE 2. Transport systems in kidney and intestine defined by inherited aminoacidurias

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Kidney</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apical</td>
<td>Basolateral</td>
</tr>
<tr>
<td>Neutral amino acids, cystine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cationic amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cationic amino acids</td>
<td>+</td>
<td>(?)</td>
</tr>
<tr>
<td>Methionine, BCAA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycine, proline, hydroxyproline</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FADIC</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

AA, amino acid; BCAA, branched-chain amino acids; BCH, 2-amino-2-norbornane-carboxylic acid; BBMV, brush-border membrane vesicles; BLMV, basolateral membrane vesicles; GFR, glomerular filtration rate; LPI, lysinuric protein intolerance; MeAIB, α-N-acetyl-l-methylaminoisobutyric acid; NEM, N-ethylmaleimide; pCMBS, p-chloromercuribenzenesulfonate; PCT, proximal convoluted tubule; PST, proximal straight tubule; PT, proximal tubule.
of a single neutral amino acid into the blood influences secretion and clearance of other neutral amino acids (161, 343). Histidine reabsorption is inhibited by almost all neutral amino acids including imino acids but also by cationic amino acids (416, 417), suggesting it can also be transported by the cationic amino acid transporter. Infusion of alanine, surprisingly, not only inhibits reabsorption of other neutral amino acids but also of cationic amino acids (400) and at higher concentrations even of anionic amino acids. Infusion of anionic amino acids, in contrast, does not increase excretion of neutral amino acids. Most likely this “nonspecific” inhibition is caused by competition of several transporters for the sodium electrochemical gradient. For example, D-glucose also inhibits amino acid uptake (213, 237). The substrate specificity of the renal proximal neutral amino acid transporter was significantly refined by studies using BBMV. These demonstrate that uptake of L-alanine is strongly inhibited by L-valine, L-leucine, L-serine, L-phenylalanine, L-glycine, L-proline, and L-hydroxyproline. In contrast, cationic or anionic amino acids were ineffective as inhibitors (107, 338). Later, mutual inhibition of alanine, glutamine, leucine, phenylalanine, serine, tryptophan, valine, and cysteine was observed in BBMV from bovine kidney (190). The combined evidence from these and other studies resulted in the definition of a transport system B0 (for broad neutral) (100), which is equivalent to system NBB (for neutral brush border) as defined in studies of intestinal transport (see below). The substrate specificity of system B0 is similar to the pattern of amino acids identified in the urine of individuals with Hartnup disorder (see sect. II A1). Neutral amino acids show \( K_m \) values ranging from 1 to 10 mM, but individual values vary significantly between studies (100, 105, 107, 147, 157, 178, 190, 308, 338).

Evidence has been provided for additional neutral amino acid transport systems in the kidney. Kragn-Hansen and Sheikh (179) reported a low-affinity uptake of serine in the proximal convoluted tubule and a high-affinity system in the proximal straight tubule. A similar observation was made for the transport of phenylalanine (178) and the BCAA (157). The common pattern observed for serine, phenylalanine, and the BCAA suggests that a high-affinity variant of system B0 is expressed in the proximal straight tubule (Fig. 1). These results confirm the general notion that low-affinity transporters are found in the proximal convoluted tubule, whereas high-affinity transporters of similar substrate specificity are expressed in the proximal straight tubule. Tryptophan transport appeared to be more complex. It is mediated by system B0 in the proximal convoluted tubule, but two transport systems were observed in BBMV derived from proximal straight tubule, namely, a \( \text{Na}^+ \)-dependent system of low affinity and a \( \text{H}^+ \)-dependent system (152). In addition, it has been reported that neutral amino acid transport is partially inhibited by lysine (213), which most likely reflects the activity of the major mediator of cationic amino acid transport in kidney and intestine (see sect. III B).

Solubilization of membrane transporters from rat kidney BBMV followed by reconstitution into liposomes, in addition, revealed the presence of an antiporter with similar characteristics to system ASC (251). Taken together, there is ample evidence for the presence of a common low-affinity \( \text{Na}^+ \)-dependent neutral amino acid transporter (system B0) in the proximal tubule (Fig. 1). System B0 is different from the \( \text{Na}^+ \)-dependent neutral amino acid transporters in nonepithelial cells, such as system A and system ASC (77), because it transports BCAA and aromatic amino acids. Molecular cloning of these transporters has confirmed that system B0 (B0AT1) is different from system A (SNAT isoforms) and ASC (ASCT2). A candidate for the high-affinity transporter of the proximal straight tube is B0AT2. ASCT2 appears to mediate system ASC-like transport activity in the apical membrane. These transporters are described in section II C.
2. Transport of neutral amino acids across the apical membrane of intestinal epithelial cells

The transport of neutral amino acids across the intact intestinal epithelium has been studied in considerable detail, in particular that of alanine (83). Transport of neutral amino acids is almost entirely Na\(^+\)-dependent, and no accumulation is observed in the absence of Na\(^+\) (79, 290, 316). As in the kidney, increased Na\(^+\) concentrations decrease the \(K_m\) of alanine but do not affect \(V_{\text{max}}\). A cotransport stoichiometry of 1:1 was determined (266). Further analysis of the transport process suggested that alanine binds preferentially to the transporter. It was proposed that alanine can be transported in the absence of Na\(^+\), albeit with much lower affinity (83). A correlation was determined showing a decrease of the substrate \(K_m\) with larger side chains (280), which is particularly striking for linear side chains from glycine to norleucine. As a result, phenylalanine, methionine, and leucine have low \(K_m\) values for the neutral amino acid transporter (ranging from 1.5 to 4 mM), whereas alanine (16 mM) and particularly glycine have a very low affinity. Similar to the kidney, a single transporter with broad specificity for neutral amino acids was proposed by several groups (188, 246, 280, 410). Sepulveda and Smith (334), in contrast, proposed two transport systems for neutral amino acids in the rabbit ileum. System 1 transports most neutral amino acids, whereas system 2 has a preference for large neutral amino acids. System 1 appears to be the equivalent of system L and may represent a common transport system for neutral amino acids in both systems. As a result, glycine, alanine, and serine are almost exclusively transported by system 1, whereas large neutral amino acids are transported by both systems. System 1 appears to be the equivalent of system B\(^0\), in the kidney proximal convoluted tubule. The \(K_m\) values for this system ranged from 0.5 mM for large neutral amino acids to \(~3\) mM for short-chain amino acids. System 2 appears to be a Na\(^+\)-independent transport activity for neutral amino acids (265). Unfortunately, inhibition of neutral amino acids by cationic amino acids was not tested in this study, opening the possibility that the Na\(^+\)-independent transport reflected the activity of the cationic amino acid transport system b\(^0\), (see sect. \(\mu\)).

Studies using BBMV confirmed the presence of a dominant Na\(^+\)-dependent transporter for most if not all neutral amino acids (193, 312) in the apical membrane. This system received the name system B (or NBB), indicating the similarity to the transport activity observed in kidney. A Na\(^+\)-independent neutral amino acid transport activity was also detected in BBMV (193, 357), which was not inhibited by cationic amino acids. This activity has strong similarity to system L and may represent a contamination of the BBMV by basolateral membrane vesicles (BLMV). In addition to the system B-like activity, a system ASC-like activity has been detected in the mucosal membrane of the rabbit ileum (192, 227). Its assignment was based on the fact that L-glutamate transport at low pH was inhibited by neutral amino acids (76). An interaction with glutamate at low pH has also been proposed for system B\(^0\) (315).

Munck (223) was the first to demonstrate an interaction of cationic and neutral amino acids in the intestine. As in the kidney, this appears to be the major transport activity for cationic amino acids (see sect. \(\mu\)). Munck and Munck (226, 236) also described a Na\(^+\)- and Cl\(^-\)-dependent transport activity for neutral and cationic amino acids in the rabbit distal ileum, which has been termed the \(\beta\)-alanine carrier or system B\(^0\), (388). The acronym B\(^0\), indicates a Na\(^+\)-dependent transporter with high affinity for neutral and cationic amino acids (Table 1) (389). To avoid confusion with system \(\beta\), which transports taurine and \(\beta\)-alanine, the term \(\beta\)-alanine carrier is no longer used. The transporter is Na\(^+\) and Cl\(^-\)-dependent (234, 390). In the rabbit, system B\(^0\), appears to be restricted to the distal ileum and has not been detected in rat, guinea pig, hamster, or human (233). However, a similar activity was observed in Caco-2 cells derived from human colon (148). A Na\(^+\)-dependent phenylalanine transporter (often referred to as system Phe) that is shared by methionine, but not by other amino acids, has been proposed by Stevens et al. (357) using rabbit jejunal BBMV. Its existence is based on the observation that phenylalanine uptake is partially inhibited by most amino acids (system B\(^0\) activity) but completely inhibited by methionine and phenylalanine itself. With the use of intact intestinal tissue, the existence of system Phe was disputed by two subsequent studies (167, 229). A specific transporter for glutamine was identified by del Castillo et al. (92). The localization of the transporter was not established.

In summary, there is strong evidence for the presence of system B\(^0\) in the intestine; the role of system ASC in amino acid absorption remains to be determined. System B\(^0\), has a specialized role in rabbit ileum but appears to be more widely expressed in the colon. These transporters are described in section \(\mu\)C. System Phe and the glutamine-specific transporter have been reported in isolated publications and require further confirmation.

3. Transport of neutral amino acids across the basolateral membrane of epithelial cells

Only a limited number of studies have analyzed the properties of amino acid transport across the basolateral membrane. Vesicles derived from the basolateral membrane were introduced by Hopfer et al. (146). In BBMV derived from rat small intestine, a Na\(^+\)-independent transporter of broad specificity was identified (214, 406). The transporter was similar to the system L transporter defined in nonepithelial cells (252); however, it appeared to accept a large variety of neutral amino acids with the
exception of α-(methylamino)isobutyric acid (MeAIB) and proline. This contrasts to classical system L (252), which prefers BCAA and aromatic amino acids. The following $K_I$ values (in mM) were determined for alanine transport: glutamine (0.3), threonine (0.4), alanine (0.6), phenylalanine (1.0), valine (1.2), methionine (1.3), and glycine (1.6). The uptake of neutral amino acids into BLMV was trans-stimulated by intravesicular substrates. The transporter is sensitive to inhibition by p-chloromercuribenzenesulfonate (pCMBS) and N-ethylmaleimide (NEM). Evidence was also provided for a $Na^+$-dependent uptake of alanine in intestinal BLMV. Three different $Na^+$-dependent components were identified, namely, a system A-like activity, a system ASC-like activity, and a novel activity. All $Na^+$-dependent transporters have affinities in the micromolar range (356). They most likely serve to recruit nutritional amino acids when the intestine is inactive or starved. System ASC activity was also detected in the brush-border membrane in both kidney and intestine (see above). The intestine in particular metabolizes significant amounts of glutamine from the circulation (408, 409); in the postabsorptive state, about one-third of arterial glutamine is removed in a single pass of the small intestine (413). As a result, $Na^+$-dependent transporters may be more prominent in the basolateral membrane of the intestine than in the kidney. Glutamine uptake across the basolateral membrane in the intestine is $Na^+$ dependent and has properties of system A (5, 371, 406). Glutamine uptake is inhibited by other small neutral amino acids and also by the amino acid analog MeAIB.

$Na^+$-dependent and $\text{V}^*$-independent transport activities were also found for tryptophan (152) in BLMV derived from rabbit kidney; however, it remains unclear whether the $Na^+$-dependent tryptophan transporter reflects a contamination with brush border. Such a contamination was considered likely in a study on phenylalanine transport in intestinal BLMV. Three different $Na^+$-dependent components were identified, namely, a system A-like activity, a system ASC-like activity, and a novel activity. All $Na^+$-dependent transporters have affinities in the micromolar range (356). They most likely serve to recruit nutritional amino acids when the intestine is inactive or starved. System ASC activity was also detected in the brush-border membrane in both kidney and intestine (see above). The intestine in particular metabolizes significant amounts of glutamine from the circulation (408, 409); in the postabsorptive state, about one-third of arterial glutamine is removed in a single pass of the small intestine (413). As a result, $Na^+$-dependent transporters may be more prominent in the basolateral membrane of the intestine than in the kidney. Glutamine uptake across the basolateral membrane in the intestine is $Na^+$ dependent and has properties of system A (5, 371, 406). Glutamine uptake is inhibited by other small neutral amino acids and also by the amino acid analog MeAIB.

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sistent with the expression of the transporter in differentiated enterocytes (46). The transporter is mainly expressed in kidney and intestine, but small amounts have been detected in spleen and lung.

Nozaki et al. (248) allocated the Hartnup disorder gene to the tip of chromosome 5. Screening for amino acid transporter candidates in this region revealed two members of the SLC6 family, namely, the orphan transporter XT2 and a novel gene related to XT2 and located right next to it on chromosome 5. The XT2 related gene was initially identified as B^OAT1 in mouse (46), followed by the identification of the human SLC6A19 as the gene mutated in Hartnup disorder (175, 333) (Table 2). The human B^OAT1 gene is located on chromosome 5p15.33, and its 12 exons span a region of 20,251 bases. A total of 10 mutations have been identified to date that cosegregate with the disease and render the transporter nonfunctional (44). These include the splice mutation analyzed in samples from Eddy Hartnup, who gave the disorder its name (175). In the Australian population, D173N is the most frequent mutation, occurring at a frequency of 0.007. The mechanism by which these mutations inactivate B^OAT1 remains largely unclear. Only the mutation R57C, a mutation of the most highly conserved residue in the SLC6 family, is known to disrupt a critical ion pair in the transporter, which has been proposed as the extracellular gate in a bacterial homolog of the SLC6 family (415). There is some evidence for a second Hartnup disorder gene. Kleta et al. (175) reported two American pedigrees with an apparent lack of mutations in SLC6A19. However, no analysis of the promoter region has been performed. Further analysis of these families will be important to clarify whether Hartnup disorder may be caused by more than one gene. The XT2 gene (SLC6A18) has been implicated in glycine transport and is discussed in section 2C.

Very recently, it has been reported that expression of the B^OAT1 protein in the brush-border membrane requires coexpression of collectrin (86, 194). The collectrin gene has been identified as a homolog of angiotensin converting enzyme II and was initially thought to be mainly expressed in the collecting duct (421). Collectrin-deficient mice, however, release large amounts of amino acids into the urine, showing a urine amino acid profile similar to Fanconi syndrome (194). In agreement with a role of collectrin in amino acid transport, the protein was detected in the proximal tubule in this study. Collectrin-deficient mice had low levels of B^OAT1 and other members of the SLC6 family in the brush-border membrane. Transcript levels, in contrast, were unaltered, suggesting a posttranscriptional mechanism. Collectrin is a single-pass transmembrane protein and may have a similar role for the trafficking of B^OAT1 to the cell surface as 4F2hc has for the surface expression of LAT2 (86) (see below). The phenotype of the collectrin-deficient mice suggests an intriguing connection between Hartnup disorder and renal Fanconi syndrome. Mice deficient for the hepatocyte nuclear factor 1 (HNF-1) display the typical traits of renal Fanconi syndrome such as polyuria, glucosuria, general aminoaciduria, phosphaturia, and bone demineralization (4, 278). Collectrin expression in the proximal tubule and other organs is regulated by HNF-1 (4, 125), suggesting that the neutral aminoaciduria in renal Fanconi syndrome may be caused by downregulation of B^OAT1.

2. The neutral amino acid transporter
B^OAT2 (SLC6A15)

The neutral amino acid transporter B^OAT2 has been analyzed in two studies (47, 367). Although it was initially described as brain specific, RT-PCR suggests expression of the transporter in mouse but not in human kidney (47, 106, 367). B^OAT2 is functionally related to B^OAT1. It is Na^+ but not Cl^- dependent and mainly transports BCAA plus proline with affinities ranging from 40 to 200 μM. Phenylalanine and alanine are recognized with low affinity. The substrate specificity and affinity of B^OAT2 resemble the high-affinity transporter for neutral amino acids detected in the proximal straight tubule (Fig. 1). The localization of the transporter has been studied in brain tissue (151, 195), but data from the kidney have not been reported.

3. The neutral amino acid transporter
ASCT2 (SLC1A5)

The ASCT2 transporter is the molecular correlate of the intestinal system ASC (169, 387). A second transporter with system ASC-like properties is ASCT1 (9). It is weakly expressed in the kidney but not in the intestine, and its substrate specificity is more restricted than that of ASCT2 (Table 1). The ASCT2 transporter has frequently been referred to as the molecular correlate of system B^0 (13, 169). This designation is at variance with the substrate specificity of system B^0, which excludes branched-chain and aromatic amino acids (387). The subsequent cloning of B^OAT1 demonstrated that it is different from ASCT2 (46). The ASCT2 transporter is Na^+ dependent but not electrogenic. This apparent discrepancy is explained by the mechanism of ASCT2, which involves an obligatory exchange of substrate amino acids against each other and a nonproductive Na^+/Na^+ exchange (48). It appears that there is no fixed ratio between the number of Na^+ exchanged and the number of amino acids exchanged (176). Because of its antiport mechanism, ASCT2 cannot contribute to net transport of neutral amino acids across the apical membrane. ASCT2 transports small neutral amino acids with K_m values of ~20 μM; glycine, leucine and methionine are transported with K_m values of 300–500 μM (387). Immunohistochemical analysis and reconstitution experiments suggest its presence in the apical membrane in the kidney and intestine (13, 251). In the kidney, expression is confined to the proximal tubule; in the
intestine, expression is high in the jejunum and colon but lower in duodenum and ileum (13) (Fig. 1). Animals undergoing resection of the jejunum and ileum show a loss of amino acid transport capacity in the remaining sections, which is accompanied by a decrease of ASCT2 mRNA. This loss can be prevented by application of growth hormone and epidermal growth factor (14). Whether resection also affects B0AT1 mRNA has not been reported yet. Glutamine transport and ASCT2 mRNA are upregulated by the phorbol ester phorbol 12-myristate 13-acetate (PMA) in Caco-2 cells involving stimulation of mitogen-activated kinases and protein kinase C (262).

A key feature of ASCT2 is its interaction with anionic amino acids at low pH (Fig. 5). Glutamate is transported with a $K_m$ value of 1.6 mM (387). ASCT2 shows variable substrate specificity. In mouse and rat, BCAA and aromatic amino acids do not inhibit ASCT2 activity (43, 387; see Ref. 277 though for a variant with broader substrate specificity), whereas rabbit and human ASCT2 (also called ATB0$^+$) are inhibited by almost all neutral amino acids to some extent (169, 170). It is likely that ASCT2 corresponds to a glutamate transport activity described in rabbit ileum, which is inhibited by neutral amino acids at low pH (192, 227). However, similar properties have also been proposed for system B0 (192, 315). It has been argued that the pH dependence of B0AT1 renders it largely inactive at pH 5.5 (227), whereas glutamate uptake via ASCT2 increases at low pH (387). In the intestine, which has an acidic microclimate, ASCT2 could contribute to glutamate uptake. A tertiary active transport can be proposed, involving the uptake of glutamate in exchange for efflux of neutral amino acids via ASCT2, followed by recapture of the neutral amino acids by B0AT1. In agreement with this notion, glutamate excretion is often elevated in patients with Hartnup disorder (279). ASCT2 could also help to equalize neutral amino acid composition in epithelial cells, but ASC-like activity is only about 1/10 of system B0 activity in the intestine (227).

5. The system L-like neutral amino acid transporter 4F2hc/LAT2 (SLC3A2/SLC7A8)

The system L transporter isoform LAT2 forms a heteromeric amino acid transporter with the 4F2 heavy chain (hc) (273, 282, 301, 331). The 4F2hc can associate with a number of light chains to form different transporters (Table 1) (45, 394). The main role of 4F2hc is the trafficking of the complex to the membrane (240). Depending on the species, the 4F2hc protein is comprised of 520–530 amino acid residues. It is heavily glycosylated, resulting in an apparent molecular mass of ~85 kDa. All light chains discovered so far lack glycosylation (331). The light chains are connected to the 4F2hc heavy chain by a disulfide bridge. The two connected cysteines are located just outside the plasma membrane of the 4F2hc molecule and in the extracellular loop between transmembrane helix 3 and 4 of all light chains (271). Although the light chains are similar in size to 4F2hc (500–530 amino acid residues), their apparent molecular mass in SDS gels is ~40 kDa due to their increased hydrophobicity. The 4F2hc/LAT2 heterodimer is found in the basolateral membrane of the intestine and the kidney (301). Expression of both 4F2hc and LAT2 decreases along the proximal tubule (24), in agreement with the absorption capacity for neutral amino acids along the nephron. In the intestine, 4F2hc and LAT2 show the highest expression in jejunum and ileum (89). LAT2 has a broad substrate specificity including all neutral amino acids except proline (273, 331). This substrate specificity is consistent with the Na$^+$-dependent, with a stoichiometry of 2 Na$^+$:1 Cl$^-$. This association is probably unrelated to the absorption of amino acids in the colon. Instead, the association may be related to the expression of ATB0$^+$ in the pituitary gland where it could be involved in the control of eating behavior. Alternatively, it has been proposed that a defective ATB0$^+$ transporter could result in embryo undernutrition causing obesity later in life (391).
independent neutral amino acid transporter defined in vesicle studies (see sect. uB). However, apart from observations in one study (331), LAT2 appears to carry out obligatory antipor (205, 273). As a result, it cannot mediate net transport across the basolateral membrane. Further analysis of the transport properties of LAT2 showed that substrates bind with micromolar affinity to the outside (range 40–200 μM, Ref. 331), whereas cytosolic affinities were in range of 3–30 mM (205). To understand the physiological function of LAT2, Fernandez et al. (111) depleted the transporter in OK cells by an antisense strategy. In LAT2-depleted cells, the intracellular concentration of cysteine was increased whereas the intracellular concentration of other amino acids was either decreased or remained unaltered. This suggested that LAT2 is likely to play a role in cysteine efflux, which is generated from cysteine after reduction by glutathione in the cytosol (81) (Fig. 2). A role of LAT2 in cysteine efflux was also suggested by overexpression of 4F2hc and LAT2 in MDCK cells (23). In summary, it appears that 4F2hc/LAT2 is not the major efflux pathway for neutral amino acids in epithelial cells, but rather has a more specialized role exchanging intracellular cysteine for other extracellular neutral amino acids. Accordingly, it could be a candidate for isolated cystinuria (Table 2 and sect. II A). LAT2 has the same substrate specificity as the efflux pathway characterized in BLMV, but in contrast to this pathway is only partially sensitive to inhibition by NEM (see sect. uB). It has not been investigated whether LAT2 may carry out uniport under certain physiological conditions. The only other transport protein that has been identified as an amino acid efflux protein in basolateral membranes is the aromatic amino acid transporter TAT1. As a result, a uniporter for neutral amino acids must be proposed in the basolateral membrane (Fig. 1).

6. The aromatic amino acid transporter

TAT1 (SLC16A10)

The aromatic amino acid transporter TAT1 is the molecular correlate of system T (173). The protein of 534 amino acids has 12 transmembrane spanning regions (173). System T has been described in erythrocytes as a Na\(^+\)-independent transporter for aromatic amino acids (112). TAT1 is a member of the SLC16 family of monocarboxylate transporters (132). This family is comprised of H\(^+\)-monocarboxylate cotransporters, but in addition contains a number of orphans, the function of which has not yet been established. TAT1 transports aromatic amino acids with low affinity; \(K_m\) values range from 3 to 7 mM (173). Even higher \(K_m\) values were reported in a subsequent study (286). Apart from aromatic amino acids, it also transports the \(N\)-methylated derivatives of these amino acids and L-dopa. In contrast to the basolateral transporters LAT2 and \(y^+\)LAT1 (see sect. II C), this transporter mediates uniport and as a result allows net efflux and influx of aromatic amino acids. Transport of aromatic acids is pH independent and electroneutral, demonstrating that protons are not involved in the transport process. Strong expression is observed in all sections of the small intestine, but not in the kidney (173, 174). A later study, in contrast, showed expression of TAT1 in the basolateral membrane of mouse proximal convoluted tubule (286). In the intestine, expression of TAT1 increases toward the tip of the villi and is observed in the basolateral membrane (173) (Fig. 1). TAT1 could in principle contribute to net efflux of other neutral amino acids by allowing the efflux of aromatic amino acids, which in turn could exchange for other neutral amino acids via LAT2. The low expression in the kidney argues against such a role at least in the kidney.

The TAT1 protein is a strong candidate for blue diaper syndrome (Table 2). As expected from the clinical phenotype (sect. II A), TAT1 is located in the basolateral membrane. In further agreement with the phenotype of blue diaper syndrome, expression is observed in the intestine but not in the kidney (174). The human gene is located on chromosome 6q21–22 and is comprised of six exons (174). Because of a lack of patients, no information is available about the mapping of blue diaper syndrome. The severe phenotype of blue diaper syndrome could be related to the expression of the transporter in the liver. To what extent absorption of tryptophan is impaired remains to be established.

7. The glycoprotein independent L-type transporters (SLC43)

Na\(^+\)-independent transporters for neutral amino acids with properties similar to system L have been identified in two families, namely, SLC7 and SLC43. LAT1 and LAT2 associate with the 4F2hc glycoprotein to form heteromeric neutral amino acid transporters (75, 395, 398). Both LAT1 and LAT2 are obligatory antiporters and cannot mediate the net uptake or efflux of neutral amino acids. LAT3 (15), LAT4 (31), and EEG1 (362), in contrast, form a separate family (SLC43). LAT3 and LAT4 have been shown to mediate Na\(^+\)-independent uniport of neutral amino acids (15, 31); EEG1 reportedly does not induce amino acid transport in oocytes (31). Both LAT3 and LAT4 have narrow substrate specificity, preferring leucine, isoleucine, methionine, and phenylalanine as substrates. LAT4 shows a biphasic relation of substrate concentration to transport activity, resulting in two apparent \(K_m\) values, one of \(\sim 200 \text{ μM}\) and the second of \(\sim 3–4 \text{ mM}\) (31). Both transporters are inhibited by the amino acid analog BCH, a commonly used inhibitor of system L activity. Efflux of substrates from oocytes expressing LAT3 or LAT4 is not stimulated by the presence of extracellular
substrates, discriminating them from the classical system L activity as described by Oxender and Christensen (252).

LAT3 expression is confined to pancreas and liver, whereas LAT4 has a broader tissue distribution but is particularly abundant in the placenta (15, 31). Smaller amounts of the LAT4 protein are found in kidney, intestine, heart, brain adipose tissue, and lymphocytes. In the kidney, LAT4 was expressed in the distal tubule and the collecting duct. In the intestine, the transporter was located in crypt cells. This distribution indicates that LAT4 is not involved in net absorption of amino acids in kidney or intestine.

Two subtypes of system L activity have been described in hepatocytes (402). System L1 is resistant to NEM and has a high affinity for large neutral amino acids. System L2 is sensitive to inhibition by NEM and has a low substrate affinity. Functional data suggest that 4F2hc/LAT1 is the molecular correlate of system L1, whereas LAT3 is the molecular correlate of system L2 (15). Cysteine transport in kidney BLMV was shown to be mediated by two transporters (181) (see sect. II). The high-affinity component most likely is mediated by 4F2hc/LAT2. The low-affinity component could reflect the activity of a glycoprotein-independent neutral amino acid transporter. A candidate for this activity might be EEG1. However, although EEG1 is strongly expressed during embryogenesis, its expression largely ceases in adult animals (362).

8. Amino acid transporters of the SNAT family

SNAT2 (SLC38A2), SNAT3 (SLC38A3), and SNAT5 (SLC38A5)

SNAT3 is the molecular correlate of the Na\textsuperscript{+}-dependent glutamine transport activity observed in kidney BLMV (168). The glutamine transporter SNAT3 was initially isolated from liver (108, 129) and rat brain (62). It is the molecular correlate of system N, a Na\textsuperscript{+}-dependent transporter specific for glutamine, asparagine, and histidine (Table 1) (172). SNAT3 is expressed in kidney but not in small intestine. Further analysis of the distribution of SNAT3 showed its expression in the basolateral membrane (351). In situ hybridization and immunohistochemistry show that in normal kidney SNAT3 is located in the proximal straight tubule, where it is coexpressed with phosphate-activated glutaminase (351). The transporter mediates glutamine transport in cotransport with one Na\textsuperscript{+} in antiport with one H\textsuperscript{+} (42). As a result, it can mediate uptake and release of glutamine.

During chronic acidosis, the kidney actively secretes protons as NH\textsubscript{4}\textsuperscript{+}, which is produced by the phosphate-activated mitochondrial glutaminase (239). The resulting glutamate is further deaminated to generate \(\alpha\)-ketoglutarate. NH\textsubscript{4}\textsuperscript{+} dissociates into NH\textsubscript{3} and protons. NH\textsubscript{3} is thought to diffuse through the membrane; the protons are largely secreted as a result of Na\textsuperscript{+}/H\textsuperscript{+} antiport. SNAT3 mRNA was found to be strongly upregulated in rat kidney cortex after chronic metabolic acidosis (168). These results were further corroborated by immunohistochemical studies (219, 351). During acidosis, SNAT3 mRNA and protein appear in all segments of the proximal tubule, where it cannot be detected in control animals (168, 351). The protein is entirely located in the basolateral membrane.

The mRNA of a second variant of system N (SNAT5) has also been detected in kidney (242, 243). In addition to glutamine, asparagine, and histidine, SNAT5 also transports glycine, alanine, serine, and cysteine. Its transport mechanism appears to be similar to that of SNAT3 (19). The cellular and subcellular localization of SNAT5 and its physiological role have not been determined.

In contrast to the kidney, basolateral uptake of glutamine in the intestine appears to have the characteristics of system A (see sect. II). The system A transporter SNAT2 is expressed in the intestine (364) and most likely reflects the reported system A activity. SNAT2 is also expressed in the renal medulla (289); smaller amounts are found in the kidney cortex. The transporter is upregulated by amino acid depletion (121) and is likely to provide amino acids to intestinal and renal epithelial cells, when recruiting few amino acids from the lumen. The intestine removes significant amounts of glutamine from the circulation for its metabolism. The uptake of glutamine is most likely mediated by SNAT2. A second variant of system A (SNAT4) is expressed in kidney and small intestine (363). Its distribution in these tissues has not been determined. Although related to the neutral amino acid transporter SNAT2, SNAT4 also transports cationic amino acids but not in cotransport with Na\textsuperscript{+} (138). Uptake of glycine via SNAT4 is markedly pH dependent, whereas the uptake of arginine is pH independent. The physiological relevance of SNAT4 for amino acid transport in kidney and intestine remains to be determined.

9. The system asc transporter 4F2hc/asc-1 (SLC3A2/SLC7A10)

The heteromeric transporter 4F2hc/asc-1 (SLC3A2/SLC7A10) mediates the Na\textsuperscript{+}-independent transport of small neutral amino acids (Gly, Ala, Ser, Cys, Thr) with high affinity (\(K_m\) values between 10 and 30 \(\mu\)M) (124, 244). Large neutral amino acids are transported with lower affinity (\(K_m\) values 100–500 \(\mu\)M). Amino acid efflux is strongly \(\alpha\)-stimulated, but some efflux occurs in the absence of extracellular substrates. The transporter is predominantly expressed in brain and lung, but transcripts are also observed in kidney and intestine. The localization of the transporter in the intestine is unknown. Because asc-1 (SLC7A10) is localized next to the non-type I cystinuria gene SLC7A9 (\(b^{\text{cyst}}\) AT, see sect. \(\alpha\)C) on chro-
mosome 19, its role in amino acid absorption was investigated further (274). The transporter is expressed in the loop of Henle, the distal tubule, and collecting ducts. Polymorphisms in the gene were found in cystinuria patients but did not impair the function of the transporter. As a result, a significant role of the transporter in the reabsorption of amino acids was excluded.

III. CATIONIC AMINO ACIDS AND CYSTINE

A. Inherited Disorders of Cationic Amino Acid and Cystine Transport

1. Cystinuria (OMIM 220100)

Cystinuria is characterized by the occurrence of excessive amounts of arginine, lysine, ornithine, and cystine in the urine. It occurs with a frequency of 1:7,000 (258) and can cause considerable damage to kidneys as a result of stones comprised largely of precipitated cystine. The disease also occurs in dogs (37). Cystinuria was recognized as a disease around 1810 by Wollaston because of the occurrence of cystine precipitates in the urine (412). Garrod (127) then introduced the concept of inherited disorders of metabolism and described cystinuria as one of four examples in his Croonian Lectures. Dent and Rose (94) were the first to demonstrate the existence of a tubular defect in the reabsorption of cystine, arginine, ornithine, and lysine. Later it was shown that the disorder also affected absorption of these amino acids in the intestine (12, 198, 209, 211, 374). Originally, cystinuria was divided into two clinically different phenotypes (136, 137). Type I heterozygotes had no aminoaciduria, whereas type II heterozygotes excreted cystine and lysine into their urine. Rosenberg et al. (299) further subdivided type II on the basis of intestinal biopsy data. In one group (subsequently categorized as type II), intestinal transport of cationic amino acids is defective, but cystine uptake is only partially affected; in type III, the intestinal amino acid transport defect was incomplete for both groups of amino acids (299). The heterogeneity of cystinuria was considered evidence for genetic heterogeneity of the disorder. Cystine clearance is close to the glomerular filtration rate (GFR), whereas clearance of arginine, lysine, and ornithine varies between 40 and 60 ml min⁻¹.1.73 m⁻² (80). This suggests that the transporter mutated in cystinuria carries most of the cystine load in the kidney, whereas additional transporters are likely to exist for cationic amino acids. The absorption capacity for cystine is lost almost completely in all three types of cystinuria, suggesting that although cystinuria appears to be genetically heterogeneous, the mutations affect the same transport activity. Because absorption is defective in the intestine, increased amounts of dibasic amino acids are found in the feces after oral loading. In addition, bacterial degradation products of dibasic amino acids, such as cadaverine, putrescine, and citrulline, are found in feces and urine. In contrast to the kidney, it appears that the intestinal defect is complete for cystine, lysine, and arginine, suggesting that only one transporter for dibasic amino acids is expressed in the intestine and that it also accepts cystine (119). The disease is well explained by mutations occurring in the genes for rBAT and b⁰,⁰⁺AT, jointly forming an apical transporter for cationic amino acids and cystine (see sect. III C and Table 2). An additional transport system for cationic amino acids is hypothesized in the kidney.

2. Isolated cystinuria (OMIM 238200)

An isolated cystinuria was reported by Brodehl et al. (41) that was not accompanied by increased excretion of cationic amino acids. In view of the evidence that there is little if any remaining cystine transport activity in cystinuric patients, this aminoaciduria is difficult to explain. However, the basolateral transporter 4F2hc/LAT2 (see sect. nC) has been shown to be important for cystine release and therefore might be a candidate. There are very few reports in the literature on this disorder, and it has not been clarified whether it may be a type of cystinuria in which a mutation in rBAT or b⁰,⁰⁺AT causes only a partial loss of activity. The residual transport activity could be sufficient to remove dibasic amino acids from the urine to a large extent, whereas cystine would still spill over into the urine.

3. Lysinuric protein intolerance (OMIM 222700)

Lysinuric protein intolerance (LPI) is characterized as a hyperdibasic aminoaciduria accompanied by protein intolerance (346). Plasma levels of dibasic amino acids are significantly lower than in normal adults (346), probably affecting the urea cycle. LPI patients develop hyperammonemia after protein-rich meals causing nausea and vomiting. Patients have an enlarged liver and spleen and suffer from muscle weakness, osteoporosis, protein deposits in the lung, and sparse hair. The reader is referred to a recent review for a discussion of the nonrenal phenotype of this disorder (256). LPI was first discovered in the Finnish population (268), where it is much more prevalent than in other countries (~1:60,000). LPI is characterized by the excretion of lysine, arginine, and ornithine in the urine, lysine being the most prominent (renal clearance ~25 ml min⁻¹.1.73 m⁻²). Both intestinal and renal transport is affected (284), but there is no evidence for genetic heterogeneity. The intestinal transport defect is demonstrated by a failure to increase blood plasma concentrations of cationic amino acids after oral loading (283). In contrast to Hartnup disorder and cystinuria, the
defect cannot be bypassed by peptides containing dibasic amino acids (283). The “cystinuria transporter” cannot compensate for the lack of activity of the “LPI transporter,” suggesting that the two transporters work in series rather than parallel. This has been confirmed by several lines of evidence. 1) Intravenous infusion of citrulline results in dibasic aminoaciduria in LPI patients but not in normal subjects, suggesting that citrulline is converted into arginine and ornithine inside epithelial cells (284). In LPI, the two amino acids accumulate in the cytosol but cannot be released into the blood and therefore appear in the urine together with lysine (283). 2) Accumulation of lysine in enterocytes is impaired in cystinuria (198, 376), but not in LPI (95). 3) Transepithelial flux of lysine is reduced, whereas apical uptake of lysine is normal in biopsies of patients with LPI (95). The renal phenotype of LPI can be explained by a dominant transport system for cationic amino acids in the basolateral membrane of the kidney and intestine, which has been identified as the heteromeric amino acid transporter 4F2hc/y+LAT1 (see sect. IIIC and Table 2).

4. Type I dibasic aminoaciduria (OMIM 222690) and lysine malabsorption syndrome

Several cases of lysinuria have been reported in which protein intolerance was not observed (51, 253, 405). These have been classified as type I dibasic aminoaciduria to discriminate them from LPI (type II dibasic aminoaciduria). Whether some of these cases are mild forms of LPI or reflect mutations of a specific transporter for cationic amino acids remains unclear. Omura et al. (250) reported a specific defect of lysine malabsorption, in which arginine and ornithine transport appeared to be unaffected. The defect appeared to affect both intestinal and renal lysine uptake. Because lysine is the most prevalent amino acid in the urine of LPI patients (346), this case also may reflect an unusual case of LPI. An entirely benign dibasic aminoaciduria has been reported by Whelan et al. (405), which appeared to be of dominant inheritance. Homozygotes did not show hyperammonemia or cystinuria and had normal plasma levels of cationic amino acids, thereby distinguishing the disorder from LPI and cystinuria. The difference of clearance rates observed between cystine and the dibasic amino acids in cystinuria supports the presence of more than one transporter for dibasic amino acids in the apical membrane. Furthermore, the transport defect for dibasic amino acids in cystinuria appears to be complete in the intestine, but only partial in the kidney. A lysine transport activity has been reported in human kidney, which was also present in cystinuric patients (298). Currently, there is no candidate transporter for this disorder.

B. Cationic Amino Acid Transport in Kidney and Intestine

1. Transport of cationic amino acids and cystine across the apical membrane of renal epithelial cells

Cationic amino acids carry a positive charge and as a result are accumulated against a concentration gradient when transported by a uniport mechanism. Such a straightforward mechanism has been reported for a variety of nonepithelial cells, where uptake is mediated by system y+ (78). Transport of cationic amino acids by epithelial cells appears to be different, but the interpretation of data has been inconsistent.

The involvement of Na+ in the transport of cationic amino acids has been discussed controversially. Several studies reported that the initial rate of lysine uptake is not affected by the presence or absence of Na+ (38, 118, 120, 203). However, in micropuncture studies, transport of lysine, arginine, and ornithine was highly Na+ dependent (309, 386). In vesicle studies, cationic amino acid transport is largely Na+ independent (55, 134, 142, 203, 213, 361) and stimulated by an inside-negative membrane potential, suggesting an electrogenic transport mechanism. Uptake of cationic amino acid is strongly trans-stimulated by intracellular or intravesicular cationic amino acids (203, 317). In addition, a Na+-stimulated transport activity was detected in most studies (142, 203, 213, 361). The interpretation of the Na+-stimulated pathway varies. Mircheff et al. (213) found that lysine accumulation into BBMV is stimulated by Na+, but an overshoot phenomenon was not observed. This distinguishes lysine uptake from typical Na+ cotransporters, where this phenomenon is observed. McNamara et al. (203) suggested a single carrier that does not have an obligatory requirement for Na+. The initial rate of lysine uptake was not affected by the Na+ gradient, but higher accumulation and an overshoot were observed in the presence of Na+. Hilden and Saktor (142) found that Na+ only stimulated arginine transport into rabbit BBMV in the presence of an inside-negative membrane potential. Similar results were found by Stieger et al. (361) in rat renal BBMV but interpreted in terms of two transporters for lysine, namely, a Na+-dependent transporter that is membrane potential independent and a Na+-dependent transporter that is driven by the membrane potential. Electrophysiological recordings from rat kidney proximal tubules (309) yielded similar results. In the absence of Na+, cationic amino acids did not induce any currents, whereas depolarization of the membrane potential by cationic amino acids was observed in the presence of Na+. Two different transport mechanisms were also proposed for lysine in human kidney (298) and for arginine uptake in rabbit renal BBMV (55) and in microperfusion studies (341, 342). Hammermann et al. (134), in contrast, proposed that the Na+...
independent system could reflect the activity of the neutral amino acid transport system B₀, where the positive charge of cationic amino acids could replace Na⁺ as the obligatory cotransported ion. However, this interpretation is at variance with the strong trans-stimulation of cationic amino acid transport. Studies of Na⁺ dependence are complicated by the substituent used to replace Na⁺. This can result in changes of the Donnan potential, which in turn can influence the accumulation of cationic amino acids (96).

Ample evidence has been provided in earlier studies that arginine, ornithine, and lysine share a common concentrative transport system in the kidney (161, 341, 400, 407), but the involvement of cystine has been controversial. For example, infusion of lysine or arginine causes increased excretion of both cationic amino acids and cystine, without raising their plasma concentration (293, 304, 400). In kidney cortex slices, mutual inhibition (300) and exchange diffusion were demonstrated for cationic amino acids (317), but not with cystine. Vesicle studies, in contrast, have consistently reported an interaction between cationic amino acids and cystine (55, 142, 203, 361) and also showed that the transporter is highly stereospecific. Two transporters for lysine were defined by Mircheff et al. (213). One is sensitive to alanine, phenylalanine, proline, and β-alanine, and the second is sensitive to alanine and phenylalanine. It appears likely that the system, which is inhibited by phenylalanine and alanine, is identical to system B₀ as defined in the intestine and in mouse blastocysts (388). It is not entirely clear whether the two systems may be one and the same assuming that proline and β-alanine have a very low affinity to the system, which has a high affinity for lysine, phenylalanine, and alanine. Inhibition of lysine transport by neutral amino acids was also detected by Stieger et al. (361) in rat renal BBMV. Lysine transport was inhibited by arginine, cystine, phenylalanine, and methionine, both in the absence or presence of Na⁺ in the incubation medium. No inhibition was observed by glucose or glutamate excluding a nonspecific depolarization as the mechanism of inhibition. Kinetic analysis revealed only one system for lysine transport, because of the presence of a Na⁺-dependent and a Na⁺-independent component the presence of two transporters of similar affinity was favored. Only one system for lysine uptake into rat renal BBMV was observed by McNamara et al. (203) after correcting for uptake by diffusion. The system was shared with cystine but not with other neutral amino acids. A Kₘ of 0.04 mM was determined. Uptake of lysine was strongly trans-stimulated. Trans-stimulation of arginine or lysine uptake by cationic amino acids has consistently been reported in studies using BBMV; however, neutral amino acids failed to stimulate (134).

Controversial reports have also been published on cystine transport. McNamara (201) reported that accumulation of cystine into rat renal BBMV was stimulated by the presence of Na⁺, but an overshoot was not observed. The initial velocity was unaffected (202), and as a result, it was suggested that the Na⁺ effect was related to the efflux of cystine but did not affect cystine uptake (202). Cystine uptake was higher in the presence of an imposed membrane potential, but the initial rate of uptake remained unchanged (202). Two kinetic components were observed (201, 330), one with a Kₘ of 0.012 mM and a second low-affinity component with a Kₘ of 6.6 mM. The low-affinity system was not shared by cationic amino acids. A Kₘ value of this dimension has to be considered with caution in view of the fact that the maximum solubility of cystine in aqueous solutions is ~0.2 mM. The high-affinity component was completely inhibited by lysine, but neutral amino acids do not appear to interfere with cystine transport. Cystine uptake was stimulated by trans-lysine, in the absence and presence of Na⁺. Biber et al. (29) also reported a stimulation of cystine uptake by Na⁺. In their study, the initial rate was increased, suggesting a direct coupling of Na⁺ to the uptake of cystine. An inside-negative membrane potential strongly stimulated cystine uptake. In microperfusion studies, cystine uptake was inhibited by both cationic and neutral amino acids (397). It was suggested that cystine may interact with system B₀, which would explain the inhibition of cystine transport by neutral amino acids in the presence of Na⁺. Studies of cystine uptake are complicated by several aspects: 1) its limited solubility; 2) in cortical slices, cystine is converted into cysteine once inside the cell (82); 3) if not converted (i.e., in BBMV), cystine binds to proteins (201); and 4) oxidation products of cystine do show Na⁺-dependent uptake (202). Transport of cysteine occurs via the neutral amino acid transport system B₀ and does not affect the transport of cationic amino acids (360).

The studies on cationic amino acids are difficult to reconcile. All studies agree that cationic amino acid transport is only partially dependent on Na⁺. The strongest evidence has been presented for a Na⁺-independent transporter, which has a high affinity for cationic amino acids, is shared by cystine, and is inhibited by neutral amino acids (Fig. 2). The transporter is functionally related to system B₀⁺ (388). The mediator of this activity has been identified as the heteromeric cationic amino acid transporter rBAT/b₀⁺AT (sect. iiC). Strong evidence has also been presented for a Na⁺-dependent transporter for cationic amino acids or a modulation of the Na⁺-independent transporter by the presence of Na⁺ (Fig. 2). The presence of an additional transporter for cationic amino acids is also supported by the differences in fractional excretion between cystine and the cationic amino acids in cystinuria (sect. iiA). In support of this notion, relatively little interaction has been observed between cystine and cationic amino acids in some ex-
2. Transport of cationic amino acids and cystine across the apical membrane of intestinal epithelial cells

Accumulation of cationic amino acids by the intestinal epithelium was first demonstrated by Hagihira et al. (130). They demonstrated mutual inhibition between arginine, cystine, lysine, and ornithine and observed some inhibition by methionine. The inhibition between cationic and neutral amino acids was subsequently characterized in more detail (225, 291). Lysine transport can be divided into a Na⁺-dependent and a Na⁺-independent fraction (231). Several groups demonstrated counterflow of neutral and cationic amino acids in the intestine. In particular, it could be demonstrated that intracellular leucine accelerates lysine uptake (221, 291). Further investigation showed that all neutral amino acids with the exception of isoleucine are able to trans-stimulate lysine uptake (225). Removal of Na⁺ abolished trans-stimulation, most likely because the neutral amino acids could not be transported into the cell, but affected the net mucosal to serosal transfer of lysine only marginally (225). The interaction between cationic and neutral amino acids in the intestine appears to be at variance with the lack of inhibition of neutral amino acid transport by cationic amino acids (sect. II B). Most likely this is caused by the fact that the capacity of the cationic amino transporter is significantly smaller than the capacity of the neutral amino acid transporter (231). The dominant presence of system B⁰ therefore disguises a small component of neutral amino acid transport by the basic system. However, in some studies a cationic amino acid transport activity has been described resembling system y⁺ in nonepithelial cells (357). A number of studies clearly established the presence of a Na⁺-independent transporter for neutral and cationic amino acids (226, 228, 229, 264). As a result, this transporter has been classified as system b⁰⁰⁺ to indicate its similarity to the system defined in mouse blastocysts (388). More recently, it has been demonstrated that system b⁰⁰⁺ is an obligatory exchanger in BBMV derived from chicken jejunum (380).

In contrast to kidney BBMV, no stimulation of lysine uptake was observed in the presence of a Na⁺ gradient in BBMV derived from rat intestine, suggesting that cationic amino acid transport in the small intestine is less complex than in the proximal tubule (59). However, a protein-rich diet appears to induce a Na⁺-dependent cationic amino acid transporter in rat intestine, which is similar to the one observed in kidney (411). The transport activity was completely inhibited by cationic amino acids and partially inhibited by neutral amino acids. In vesicles, counterflow could only be demonstrated with cationic amino acids. Torras-Llort et al. (379) showed upregulation of a similar transporter in chicken intestine after feeding a lysine-enriched diet. A Na⁺-dependent transport activity for neutral and cationic amino acids, called system B⁰⁺⁺, has been detected in the rabbit distal ileum and is described in section II B. It does not appear to play a major role in the absorption of cationic amino acids in species other than rabbit (233). System B⁰⁺⁺ shows some similarity to the high-protein diet-induced cationic amino acid transporter. The Na⁺-independent a⁰⁺⁺-like transporter for cationic amino acids has been identified as the heteromeric transporter rBAT/b⁰⁺⁺AT (see sect. III C). The induction of a Na⁺-stimulated transporter for cationic amino acids in the intestine by a protein-rich diet lends further support to the notion that the Na⁺-stimulated transporter is a different molecule than rBAT/b⁰⁺⁺AT (Fig. 2).
3. Transport of cationic amino acids across the basolateral membrane of epithelial cells

The absorption of lysine by intestinal epithelial cells is strongly stimulated by leucine added to the luminal side but not if added to the abluminal side. Two different mechanisms have been proposed to explain this stimulation. Munck and Schultz (230) suggested that intracellular leucine acts as an allosteric modulator increasing the basolateral efflux of lysine. Reiser and Christiansen (291), in contrast, refuted an effect of luminal leucine on basolateral lysine efflux and suggested instead that apical heterologous exchange was responsible for the phenomenon. An exchange process across the basolateral membrane involving neutral and cationic amino acids was demonstrated by Cheeseman in frog intestine (63). However, in a later study using basolateral vesicles from rat jejunum, Lawless et al. (184) demonstrated the presence of an electroneutral unipporter for cationic amino acids that was strongly stimulated by micromolar concentrations of leucine. An exchange mechanism was considered unlikely. An amino acid exchanger with high affinity for neutral and cationic amino acids was subsequently discovered by Deves et al. in erythrocytes (99). The transporter was also identified in vesicles derived from placenta (103). The transporter was termed system y\textsubscript{+L} to indicate its interaction with both neutral and cationic amino acids. Neutral amino acids require Na\textsuperscript{+} for interaction with the system, whereas cationic amino acids are transported in the absence of Na\textsuperscript{+}. The transport system is described in more detail in a recent review in this series (98). Cloning of system y\textsubscript{+L} demonstrates that an exchange system as proposed by Cheeseman (63) is expressed in the basolateral membrane (see sect. II\textsubscript{C} and Fig. 2) of kidney and intestine. System y\textsubscript{+}L is affected in lysinuric protein intolerance (see sect. II\textsubscript{A}) and is encoded by the heteromeric transporter 4F2hc/y\textsuperscript{+}LAT1 (see sect. II\textsubscript{C}).

C. Cationic Amino Acid Transporters

I. The system b\textsuperscript{0,+} transporter rBAT/b\textsuperscript{0,+}AT (SLC3A1/SLC7A9)

The major transporter for cationic amino acids and cystine in the apical membrane of kidney and intestine is the heteromeric transporter rBAT/b\textsuperscript{0,+}AT (260). It is the molecular equivalent of the renal and intestinal cationic amino acid transport system b\textsuperscript{0,+}. The rBAT protein is a type II membrane glycoprotein with a large extracellular domain that is related to bacterial glycosidases. It is comprised of 685 amino acids and is highly glycosylated (28, 370, 404). The b\textsuperscript{0,+}AT protein is a polytopic transmembrane protein comprised of 487 amino acids (molecular mass ~50 kDa) and 12 predicted transmembrane spanning helices (60, 109, 269). Both subunits are linked by a disulfide bridge. Because of their molecular mass, rBAT is called the heavy chain and b\textsuperscript{0,+}AT the light chain. The disulfide bridge links a cysteine residue located just outside the membrane in the rBAT subunit and a cysteine residue in the loop between helix 3 and 4 of the light chain. Both subunits have to be coexpressed to reach the plasma membrane. The rBAT protein alone appears to be relatively unstable and is rapidly degraded (24, 288). Coexpression of b\textsuperscript{0,+}AT stabilizes rBAT and promotes trafficking to the apical membrane. In contrast to the rBAT protein, b\textsuperscript{0,+}AT is stable when expressed alone but remains in the endoplasmic reticulum (23). The rBAT cDNA was isolated by expression cloning using Xenopus oocytes (28, 370, 404). In oocytes, the rBAT protein interacts with an endogenous light chain that has properties which are almost identical to the properties of the mammalian protein. Characterization of the transport properties of the heterodimer has largely relied on its interaction with the oocyte endogenous light chain. The transporter has the substrate specificity of system b\textsuperscript{0,+} as originally described in mouse blastocysts (388). Cationic amino acids bind to the transporter with higher affinity than neutral amino acids. Cystine and the cationic amino acids are transported with $K_m$ values of ~100 $\mu$M. Neutral amino acids have slightly higher $K_m$ values (255). Uptake of neutral amino acids generates an outward current (i.e., an efflux of positive charges) in oocytes, whereas cationic amino acids induce inward currents (3, 54). Efflux of radiolabeled amino acids requires extracellular substrates demonstrating that rBAT/b\textsuperscript{0,+}AT is an obligatory antiporter (73, 218). Reconstitution of the heterologously expressed light chain demonstrated that it is the catalytical subunit and confirmed the obligatory exchange mechanism (288). Similar to the LAT2 light chain (see sect. II\textsubscript{C}), $K_m$ values of the intracellular binding site are several orders of magnitude higher than on the extracellular binding site. In the case of leucine, extracellular binding occurs with a $K_m$ of ~0.5 $\mu$M, whereas intracellular binding occurs with a $K_m$ of ~2.5 mM. In oocytes, outward currents and inward currents are of similar size, suggesting that the transporter is freely reversible (54). Under physiological conditions, however, only cationic amino acids are removed from the medium (275). This preference for the uptake of cationic amino acids is most likely driven by the membrane potential. Thus physiologically, rBAT/b\textsuperscript{0,+}AT acts as an absorption mechanism for cationic amino acids and cystine coupled to the efflux of neutral amino acids. The neutral amino acids are absorbed by B\textsuperscript{AT1} (see sect. II\textsubscript{C}). The counterflow of neutral and cationic amino acids as observed by Munck in rabbit AT1 (see sect. II\textsubscript{C}). The rBAT protein is relatively weakly expressed in the proximal convoluted tubule but strongly expressed...
in the proximal straight tubule (Fig. 2). The $b^{0,+}AT$ protein in contrast shows the highest expression in the early segments of the proximal tubule but is almost undetectable in the proximal straight tubule (60, 269). Further analysis of the distribution showed that all of $b^{0,+}AT$ was bound to rBAT, but that significant amounts of rBAT, particularly in the proximal straight tubule, were associated with an unknown light chain (110). Because cystine is mainly reabsorbed in the early sections of the proximal tubule, this indicates that rBAT/ $b^{0,+}AT$ is the major cystine transporter in the kidney (110). This notion is supported by the clearance of cystine in cystinuria having the same value as that of a solute which is not being reabsorbed. However, anionic cystine may be actively secreted through the paracellular route (see sect. vii) obliterating residual absorption capacity for cystine. As pointed out above, rBAT is rapidly degraded if not complexed with a light chain (24, 275). The strong expression of rBAT in the proximal straight tubule, as a result, is likely to reflect expression of an unknown heterodimer. It is tempting to speculate that this heterodimer is the Na$^+$-stimulated transporter for cationic amino acids (see sect. viiB). Interestingly, rBAT/$b^{0,+}AT$ consistently shows slightly higher transport activity in the presence of Na$^+$ than in its absence (24, 269), demonstrating its potential to mediate Na$^+$-stimulated transport. Expression of rBAT is regulated by nutritional signals. Surprisingly, aspartate is a particularly good inducer of the cationic amino acid transporter subunit rBAT (332).

Linkage and mutation analysis demonstrated that mutations in rBAT and $b^{0,+}AT$ cause cystinuria (56, 57, 109) (Table 2). To date, more than 100 mutations have been described in the rBAT protein and 66 in the $b^{0,+}AT$ protein (117). Mutations in the rBAT protein have also been identified in cystinuria affecting Newfoundland dogs (140). Most mutations in rBAT appear to affect the trafficking of the complex (74); however, some mutations also affect the transport mechanism of the heterodimer (275). Although cystinuria has been divided into three different clinical phenotypes (see sect. viA), these appear to merge into mutations associated with the rBAT/$b^{0,+}AT$ heterodimer. Closer characterization of genotype-phenotype correlation revealed that heterozygotes with mutations in SLC3A1 have urinary amino acid levels within the control range and hence confined to type I (93, 117, 185). Mutations in SLC7A9, in contrast, showed all three clinical phenotypes (117, 185). The majority of the heterozygotes have increased levels of cystine and basic amino acids, and a smaller group (~14%) has amino acid levels within the control range (93). As a result, a new classification of cystinuria based on the genotype has been suggested (93). According to this classification, individuals with mutations in SLC3A1 are categorized as type A, and individuals with mutations in SLC7A9 are categorized as type B. The slc7a9-deficient mouse shows non-type I cystinuria, i.e., elevated excretion of cystine and cationic amino acids is observed in the heterozygotes. Only ~40% of the animals develop kidney stones, suggesting that additional factors are involved in this phenotype. In slc7a9-deficient mice, rBAT expression is significant and the protein appears to be associated with an unknown light chain. There is little indication for additional cysnutria genes. Only 3% of all patients do not have detectable mutations in the SLC3A1 or SLC7A9 gene, but the promoter regions and complete introns have not been sequenced in all cases (117). The rBAT protein is thought to interact with an unknown protein in the proximal straight tubule (Fig. 2), which could be a candidate gene for type I dibasic aminoaciduria. However, amino acid hyperexcretion is similar in SLC3A1/SLC3A1 as in SLC7A9/SLC7A9 genotypes, arguing against an association of rBAT with another cationic amino acid transporter of the proximal straight tubule (see sect. viiB).

2. The cationic amino acid transporter $4F2hc/y^+LAT1$ and $4F2hc/y^+LAT2$ (SLC3A2/SLC7A7 and SLC7A6)

The $y^+LAT1$ transporter is the mediator of cationic amino acid efflux in epithelial cells. It is a protein of 511 amino acids and like LAT2 associates with the 4F2hc to form a heteromeric transporter (270, 381). The role of the 4F2hc is described in section viC. Importantly, 4F2hc guides $y^+LAT1$ to the basolateral membrane (24). Much of our knowledge about the efflux of cationic amino acids across the basolateral membrane has been derived from expression studies of this transporter. In general, it is the molecular correlate of system $y^+$ described in erythrocytes and placenta (99, 103). Transport via $y^+LAT1$ is resistant to inhibition by NEM (99), discriminating it from the widely expressed cationic amino acid transporter CAT-1 (78, 98). The $y^+LAT1$ transporter accepts neutral and cationic amino acids with high affinity ($K_m$ values ~20 $\mu$M) (270, 381). The affinity of neutral amino acids increases by about two orders of magnitude in the presence of Na$^+$. In the absence of Na$^+$, it appears that H$^+$ are cotransported (162). The transport of cationic amino acids, in contrast, is Na$^+$ independent. The transporter carries out an obligate antiport mechanism. Because there is little intracellular Na$^+$, it preferentially mediates efflux of cationic amino acids in exchange for extracellular neutral amino acids. This preference was confirmed by coexpression of rBAT and 4F2hc/$y^+LAT1$ in polarized MDCK cells in the absence of B$^0$AT1 expression (23), resulting in a net flux of leucine from the basolateral side to the apical side. Efflux of cationic amino acids across the basolateral membrane as a result requires the subsequent efflux of neutral amino acids, the mediator of which is still un-
known (Fig. 2). As outlined in section mB, it appears that efflux of cationic amino acids across the basolateral membrane is stimulated by addition of neutral amino acids to the luminal side, but not when added to the abluminal side (230). This effect, which requires uptake of neutral amino acids into the cell, cannot be fully explained by the antiport activity y⁺LAT1.

Linkage studies assigned LPI to chromosome 14q11–13, where the gene encoding y⁺LAT1 is located (182, 183). Two studies subsequently demonstrated that LPI is caused by mutations of the y⁺LAT1 gene (36, 382) (Table 2). To date, a total of 30 mutations have been described in 109 patients, explaining 217 of the 218 alleles (256). The disorder is more prevalent in the Finnish than in any other populations. It appears that the occurrence of LPI in the Finnish population can be explained by a single founder mutation, namely, a splice-acceptor mutation resulting in a 10-bp deletion leading to a premature stop codon. Like many other aminoacidurias, the phenotype of LPI can vary significantly. In LPI even a single mutation can result in different phenotypes, suggesting the lack of a strong genotype/phenotype correlation (238, 352). In contrast to previous reports, no defect of y⁺L-like activity was detected in fibroblasts from LPI patients (39, 85). A second transporter with similar properties to y⁺LAT1 is y⁺LAT2 (49, 270, 381). The transporter is expressed in a wide variety of tissues including small intestine and kidney, but the ratio of y⁺LAT1/y⁺LAT2 is particularly high in the kidney and intestine (337). This explains why y⁺LAT2 cannot replace y⁺LAT1 in the efflux function. It has further been proposed that some y⁺LAT1 mutations may suppress y⁺LAT2 activity perhaps involving larger protein complexes (353).

3. Other transporters involved in cationic amino acid and cystine transport

Cationic amino acid transport in nonepithelial cells is mediated by members of the CAT family. CAT-1 (SLC7A1) is ubiquitously expressed including kidney and intestine (392). Expression of CAT-1 appears to be confined to the basolateral membrane and appears to be more prominent in the medulla (160). This could explain why the cationic amino acid transporter CAT-1 cannot replace the function of y⁺LAT1 (78) in patients with lysinuric protein intolerance.

4F2hc/xCT (SLC7A11) is a heteromeric transporter involved in the defense against oxidative stress (311). It exchanges glutamate and cystine. A recent report suggests the presence of this transporter in the apical membrane of enterocytes and renal epithelial cells (53). These results are in variance with the basolateral distribution of 4F2hc reported in other studies (89, 301).

IV. ANIONIC AMINO ACIDS

A. Inherited Disorders of Anionic Amino Acid Transport

1. Dicarboxylic aminoaciduria (OMIM 222730)

Dicarboxylic aminoaciduria is a rare disorder (206, 366, 372) with an estimated frequency of 1:29,000. Most affected individuals do not show symptoms (186); the inheritance is autosomal recessive. Its hallmark is a profoundly increased excretion of glutamate and aspartate in the urine that reaches or even exceeds the GFR, particularly for glutamate. The disorder is thought to affect the main transport system for glutamate and aspartate. Furthermore, it shows that in the absence of an uptake system, anionic glutamate is actively secreted into the urine. This suggests that the apical transport is defective, whereas accumulation by the basolateral transporter is unaffected (see sect. IVB). Intestinal transport was normal in one study (206), but impaired in another (372). The phenotype of the disorder has not been fully resolved. The first case (372) reportedly had growth retardation, mental retardation, and a tendency toward fasting hypoglycemia and ketoacidosis. The second case (206) was without symptoms but showed lower levels of glutamate in the urine. Mental retardation was also reported in a more recent case (366). The response of the plasma levels of aspartate and glutamate to oral loading suggests that a different glutamate transporter may be expressed in the intestine than in the kidney. The defect appears to affect glutamate transport in skin fibroblasts (207). The glutamate transporter EAAT3 is a strong candidate for this disorder (see sect. IV C and Table 2).

B. Anionic Amino Acid Transport in Kidney and Intestine

1. Transport of anionic amino acids across the apical membrane of renal epithelial cells

There is widespread evidence for a common transport system specific for anionic amino acids. The interaction between aspartate and glutamate was noted in clearance studies and microperfusion experiments (27, 161, 343, 386, 400). Studies using renal BBMV further refined the properties of the renal carrier for anionic amino acids. Glutamate uptake into BBMV shows a large overshoot in the presence of a Na⁺ gradient (313, 401). The initial rate increases with increasing extracellular Na⁺ concentration. The transporter has a high affinity for its substrate in the range of 25 μM. The transporter is stereospecific for glutamate but not for aspartate and is not inhibited by neutral or cationic amino acids. Other inhibitors are L-cysteate and DL-threo-3-hydroxyaspartate. Although an
An electroneutral mechanism was proposed by Schneider et al. (313), later experiments convincingly demonstrated an electrogenic mechanism in BBMV (52) and also in the intact tubule (310). The stimulation of glutamate uptake by an inside-negative membrane potential is only observed in the presence of intravesicular K⁺, the efflux of which is coupled to glutamate uptake (52). A mechanism was proposed involving the cotransport of three Na⁺ and an antiport of one K⁺. The transporter may work in the absence of intravesicular K⁺ as a slow electroneutral transporter. A very slow uptake is observed in the absence of Na⁺, suggesting that diffusion does not play a significant role in glutamate transport (313). A low-affinity system for glutamate uptake has been reported by Weiss et al. (401). This may reflect system ASC-like activity or a low affinity of system B0 for glutamate at low pH (see sect. II B).

In contrast to most other amino acids, glutamate and aspartate are rapidly metabolized by the renal parenchyma. Both compounds are transaminated to form 2-oxoglutarate and oxaloacetate, respectively (403). The properties of the kidney glutamate transporter match those of the glutamate transporter characterized in brain (166); this was subsequently confirmed by the cloning of the kidney glutamate transporter EAAT3 (see sect. IV C and Fig. 3).

2. Transport of anionic amino acids across the apical membrane of intestinal epithelial cells

Glutamate uptake in the intestine appears to be mediated by a similar system as in kidney (191, 227). Glutamate uptake is strictly Na⁺ dependent. The small residual uptake in the absence of Na⁺ most likely reflects diffusion (227). Na⁺ activation of glutamate transport indicates a stoichiometry of 2:1 for glutamate uptake in rabbit intestine. At equal concentrations, glutamate uptake is about five times higher than uptake of D-aspartate, suggesting an additional glutamate-specific system that is not shared by D-aspartate. In agreement with this notion, only one transport activity was found for D-aspartate but two kinetic components were detected for glutamate (192, 227). The high-affinity component (Kₘ value 40–60 μM) is shared between the two amino acids; the low-affinity component (Kₘ = 7.0 mM) is only accessible to L-glutamate (192). At pH 6.0 and at a substrate concentration of 50 μM, only 30% of glutamate transport occurs via the high-affinity system. The remaining transport capacity is inhibited by a large variety of neutral amino acids including phenylalanine (192, 227, 232). As a result, it was proposed that the low-affinity glutamate transport was mediated by system B⁰ in intestine, the low-affinity system appears to make a significant contribution to glutamate uptake. The levels of glutamate in the urine of patients with dicarboxylic aminoaciduria suggest that system ASC does not play a similarly significant role in the kidney. It is thought that ~50% of the intestinal glutamate uptake is mediated by system ASC (compare, for example, Refs. 314 and 131; see also Ref. 233).

3. Transport of anionic amino acids across the basolateral membrane of epithelial cells

For both glutamate and aspartate, a Na⁺-dependent transporter has consistently been described in the basolateral membrane of renal and intestinal epithelial cells (40, 306, 310). The transporter is stimulated by intracellular K⁺ and as a result appears to be similar to the apical
system (40, 306). Evidence has been provided that EAAT2 (GLT-1) is expressed in the basolateral membrane of the kidney (403). It is important to note that glutamate is rapidly metabolized by both kidney and intestine. Particularly in the intestine, >98% of glutamate is converted into α-ketoglutarate (403) and is further metabolized. Thus almost all glutamate and aspartate in the nutrition does not enter the portal circulation (413). A specific glutamate efflux transporter in the basolateral membrane has not been reported.

C. Anionic Amino Acid Transporters

1. The system $X^{-}_{\text{AG}}$ anionic amino acid transporter EAAT3 (SLC1A1)

The kidney glutamate transporter was identified by expression cloning (164). It has all properties of the high-affinity glutamate transporter described in kidney and intestine and is the molecular correlate of system $X^{-}_{\text{AG}}$ together with other glutamate transporter isoforms in this family (163). EAAT3 cotransports three Na$^+$ together with other glutamate transporter isoforms in this family (163). EAAT3 cotransports three Na$^+$ together with each glutamate molecule; return of the carrier is facilitated by the binding of K$^+$ (165). Later studies also demonstrated the cotransport of one H$^+$ (420). It transports L-glutamate with a $K_m$ of 14 μM and shows a preference for D-aspartate over L-glutamate.

In situ hybridization and immunofluorescence data indicate an increased expression of EAAT3 from the S1 segment to the S3 segment (335) (Fig. 3). Most of the protein is found in the cortex, but significant amounts are also expressed in the outer medulla. Accordingly, the transporter is also found in the thick ascending limbs, thin descending limbs, and the distal convoluted tubule of juxtamedullary nephrons. This localization is in agreement with functional data, showing absorption in these parts of the nephron (87). More than 90% of glutamate is reabsorbed in the first one-third of the proximal tubule where expression of EAAT3 is relatively low, but the stereospecificity of the transport process matches that of EAAT3 (344). Expression of EAAT3 has also been observed in the small intestine (164). Hyperosmotic stress resulted in a large increase of EAAT3 mRNA followed by a threefold increase of transport protein (199). Glutamate transport is also induced by amino acid deprivation, resulting in a threefold increase of the transport activity, which was not accompanied by an increase of the EAAT3 mRNA or its protein (247). The upregulation of other known glutamate transporter isoforms was excluded. Interestingly, it has been reported that EAAT3 transports cysteine with a $K_m$ value of ~100 μM (139), but the physiological relevance of cystine transport by EAAT3 remains to be determined.

EAAT3 is a strong candidate for the molecule underlying dicarboxylic aminoaciduria (Table 2). The human EAAT3 gene is found on chromosome 9p24. Because of the limited number of cases reported in the literature, mapping data are not available. However, EAAT3-deficient mice do develop dicarboxylic aminoaciduria, but increased cystine levels in the urine have not been reported (267). Initially it was reported that EAAT3-deficient mice do not develop neurodegeneration, although EAAT3 resides in neurons. In contrast, a recent report shows development of neurodegeneration in these mice (8). Dicarboxylic aminoaciduria is generally considered to be a benign disorder, but cases with mental retardation have been reported as well (366, 372). Final clarification of these discrepancies will await genotypic analysis of patients with dicarboxylic aminoaciduria. Evidence has been provided that the molecular correlate of the basolateral glutamate transporter is EAAT2 (GLT-1) (403).

2. The anionic amino acid transporter AGT1 (SLC7A13)

The AGT1 transporter belongs to the family of light chains that are associated with the glycoproteins 4F2hc (sect. iiC) and rBAT (sect. iiC). However, coexpression of any of the two proteins together with AGT1 does not result in active transport, neither in *Xenopus* oocytes nor in COS-7 cells (30, 197). The mouse transporter is a protein of 478 amino acids. The transporter could be forced into the membrane by generating a fusion protein with 4F2hc or rBAT. As a result, it is assumed that it interacts with an unknown heavy chain. Studies of these fusion proteins demonstrated that AGT1 mediated Na$^+$-independent uptake of aspartate and glutamate with $K_m$ values of ~20 μM (197). Cysteine exerted significant inhibition of glutamate uptake but was not transported itself. The transporter showed a strong preference for L-aspartate over D-aspartate. The transport mechanism remains to be elucidated. Most light chains carry out obligatory antiport, which would result in an unproductive exchange of glutamate/aspartate versus glutamate/aspartate. However, other anions such as bicarbonate, sulfate, phosphate, and dicarboxylic acids were not tested. If AGT1 would function as a uniporter, glutamate and aspartate would be driven out of the cell. This could explain why the transporter is expressed in the kidney only, because intestinal cells metabolize glutamate derived from the lumen and from the blood plasma, whereas kidney epithelial cells continuously recruit amino acids from the urine. In the kidney, the transporter was found in the basolateral membrane of the proximal straight tubule, the outer medulla, and the distal tubule. This distribution is compatible with that of the apical glutamate transporter EAAT3. As a result, AGT1 could constitute a net efflux pathway for anionic amino acids in kidney (Fig. 3).
V. PROLINE, HYDROXYPROLINE, AND GLYCINE

A. Inherited Disorders of Imino Acid and Glycine Transport

1. Iminoglycinuria (OMIM 242600)

Iminoglycinuria (in earlier publications referred to as Joseph’s syndrome) is a benign condition, characterized by the excretion of proline, hydroxyproline, and glycine in the urine. The disorder is of autosomal recessive inheritance and occurs with an estimated frequency of 1:15,000 (67). Increased excretion of glycine, proline, and hydroxyproline is regularly observed in infants without the disorder. If the hyperexcretion persists beyond 6 mo, however, it is considered an abnormal aminoaciduria. Only very few cases of nonbenign iminoglycinuria (158, 354) have been reported. Renal clearance in iminoglycinuria is well below the GFR, suggesting the presence of additional transporters for proline and glycine. Proline clearance, for example, is \( \sim 7 \text{ ml/min} \cdot 1.73 \text{ m}^2 \), and hydroxyproline clearance is \( \sim 13 \text{ ml/min} \cdot 1.73 \text{ m}^2 \) in iminoglycinuria (67). For glycine, the clearance is around 20 \( \text{ml/min} \cdot 1.73 \text{ m}^2 \) (normal range \( <10 \text{ ml/min} \cdot 1.73 \text{ m}^2 \)) (318). Similar to cystinuria, evidence suggests that iminoglycinuria is a multigene disorder. In about half the pedigrees, heterozygote carriers of the disorder are normal; the mutant allele is completely recessive. In other pedigrees, glycinuria is observed in heterozygotes (318). Additionally, impaired intestinal transport is observed in some pedigrees (375) but not in others (67). As a result, a total of three mutant alleles have been proposed (67). All mutated alleles generate renal iminoglycinuria when homozygous, but heterozygotes differ. For allele 1, heterozygotes are normal, and the homozygotes have an intestinal transport defect. For allele 2, heterozygotes are normal, and homozygotes do not show an intestinal transport defect. For allele 3, heterozygotes show glycinuria, and homozygotes do not have an intestinal transport defect. In contrast to isolated glycinuria, isolated prolinuria has not been reported. In agreement with this observation, elevated plasma concentrations of proline are known to cause both prolinuria and glycinuria (321, 326). Amino acid infusion studies revealed the presence of three transport systems in proline, hydroxyproline, and glycine (328). In the iminoglycinuric individuals, a common system for all three compounds was missing, revealing the presence of a glycine-specific system and a system shared by proline and hydroxyproline. This pattern was confirmed in three pedigrees with iminoglycinuria, in which heterozygotes showed isolated glycinuria (318). In these cases an intestinal phenotype was not observed, suggesting that kidney and intestine have at least in part different transport systems for these amino acids. Furthermore, absorption of an oral load of L-proline in these patients was equivalent to that observed in normal subjects. In summary, there are likely to be four transporters for glycine and proline: 1) a common transporter in the intestine and 2) in kidney, 3) a glycine-specific transporter, and 4) a proline-specific transporter. The common system in the intestine has been identified as the proton amino acid transporter PAT1. In the kidney, PAT1 and PAT2 are expressed, but the relative contribution of these transporters is unknown (Table 2). The proline-specific system has been identified as the IMINO transporter (Table 2); a candidate for the glycine-specific system is XT2. These transporters are described in section vC.

2. Glycinuria (OMIM 138500)

Glycinuria is a fairly common observation, particularly in newborns, where glycine absorption is still developing. Glycinuria is also observed in hereditary hypophosphatemic rickets (322) and in some pedigrees with iminoglycinuria in heterozygotes. A specific renal glycinuria was observed by De Vries et al. (91) associated with renal stones. Interestingly, glycinuria may be associated with hypertension (276). A candidate for this transport activity is the transporter XT2 (sect. vC and Table 2).

B. Imino Acid and Glycine Transport in Kidney and Intestine

1. Transport of imino acids and glycine across the apical membrane of renal epithelial cells

Proline is completely absorbed in the proximal tubule of the kidney (343). Glycine absorption, in contrast, appears to be limited by transport capacity. In hyperprolinemia, prolinuria occurs at blood plasma concentrations exceeding 0.8 mM (normal \( <0.3 \text{ mM} \)) and is usually accompanied by glycinuria and hydroxyprolinuria, suggesting a common transport system for all three compounds (321, 326). This was confirmed by infusing increasing concentrations of proline and hydroxyproline, both resulting in increased secretion of all three amino acids (328). Other amino acids were not affected. Infusion of glycine, in contrast, resulted in little prolinuria or hydroxyprolinuria. In addition, elevated amounts of serine and threonine were detected in the urine. Further investigation of the phenomenon suggested the presence of a common low-affinity system for glycine and proline and two high-affinity systems, one proline specific and the other glycine specific (328). This notion is supported by developmental changes of glycine and proline transport in rat kidney. Renal tubular absorption of glycine, proline, and hydroxyproline is incomplete in newborn mammals compared with the adult as indicated by the excretion of significant amounts of glycine and proline (319). In rat,
proline excretion decreases sharply in the second week of life, whereas glycine excretion does not abate until the third week (17). A similar albeit more extended time course is observed in humans, namely, hyperiminoaciduria disappears after 3 mo, whereas hyperglycinuria vanishes after 6 mo (327). This initial deficiency of proline and glycine absorption is most likely caused by a lack of the two independent high-affinity transporters for proline and glycine, whereas the common low-affinity system appears to be active in newborns. In the rat, the high-affinity proline-specific system appears in the second week, whereas the high-affinity glycine system increases its activity in the third week (16). Moreover, the concentration dependence of proline and glycine uptake shows only one component in newborns, whereas two components were noticed in adult animals (16). Vesicle studies also suggest several systems for proline and glycine transport (143, 144, 200). A high-affinity Na\(^+\)-dependent proline transporter was detected in rat and rabbit BBMV (126, 135, 200). Decreasing the Na\(^+\) concentration increased the \(K_m\) value of proline. Proline uptake into BBMV was partially inhibited by most neutral amino acids, reflecting the activity of system B\(^0\), but was completely inhibited by proline and hydroxyproline. The inhibitory potential of glycine was similar to that of other neutral amino acids. Compatible results were reported for glycine transport (143). Glycine was transported by a system that is shared with alanine, most likely B\(^0\), and by a system shared with proline. Both systems appeared to be Na\(^+\) and energy dependent. In contrast to the high-affinity system for proline, changes of the Na\(^+\) concentration largely affected the \(V_{\text{max}}\) rather than the \(K_m\) of the glycine transporter (200). The pH dependence also indicates that glycine and proline are partially transported by separate systems (200). An additional high-affinity system for glycine that is shared by neutral amino acids has been detected in the proximal straight tubule (296). It is likely to be the high-affinity transporter for neutral amino acids detected in the proximal straight tubule (see sect. iiB).

Although proline transport has often been reported as entirely Na\(^+\) dependent, there is significant evidence for an H\(^+\)-dependent transporter of proline. BBMV prepared from rat kidney cortex at different ages showed very little Na\(^+\)-stimulated overshoot on day 7, but the overshoot increased until 28 days of age (204). McNamara et al. (200) showed that reducing the Na\(^+\) concentration from 100 to 10 mM had no effect on the low-affinity system (\(K_m = 0.78\) mM) for proline. A prominent overshoot in the presence of a Na\(^+\) gradient was observed for proline uptake into vesicles at low concentrations, but not at high concentrations, suggesting that the low-affinity system is Na\(^+\) independent. Unambiguous evidence for H\(^+\)-coupled proline and glycine transport was then presented by Roigaard-Petersen and co-workers (295, 296) and Rajendran et al. (285), who demonstrated that an overshoot occurred in the presence of a H\(^+\) gradient even when no Na\(^+\) gradient was imposed in BBMV from rabbit kidney cortex. The H\(^+\)-dependent transporter was electrogenic, had low affinity, did not discriminate between 1- and \(\alpha\)-proline, and was shared by glycine and betaine (414). The H\(^+\)-dependent proline/glycine transporter co-exists with at least one Na\(^+\)-dependent proline uptake system, the \(K_m\) of which is altered by the Na\(^+\) concentration. The H\(^+\)-coupled system was most likely confined to the proximal convoluted tubule, whereas transport in the proximal straight tubule was strictly Na\(^+\) coupled. Species differences may complicate the interpretation of results as exemplified by rat kidney cortex where proline uptake was largely mediated by a 2 Na\(^+\)/1 Cl\(^-\)-proline symporter (72).

Taken together, the functional data suggest at least four different transporters for proline and glycine (Fig. 4): 1) a Na\(^+\)-independent but proton-dependent transporter for glycine and proline (the common system, most likely corresponding to PAT1 and/or PAT2); 2) a transporter specific for proline and hydroxyproline, which appears to be the IMINO transporter first defined in the intestine;
3) a transporter for glycine and proline, which is shared by other neutral amino acids, which is most likely B⁰ (B⁰AT1, see sect. uC); and 4) a glycine-specific transporter, most likely corresponding to XT2. The developmental changes of iminoglycine transport can be explained by the presence of significant amounts of PAT1/2 in newborn animals, followed by the appearance of the proline-specific IMINO and glycine-specific XT2 after 2 and 3 wk, respectively. The transporters are described in section vC.

2. Transport of imino acids and glycine across the apical membrane of intestinal epithelial cells

Wiseman (410) suggested three different mechanisms for proline and glycine transport in the intestine: 1) the general neutral amino acid transporter B⁰ (designated system 1); 2) a transporter for proline, hydroxyproline, sarcosine, N-dimethylglycine, and betaine (system 3, in this classification system 2 is for basic amino acids); and 3) a mediator of glycine and proline uptake (system 4) (410). In the rat intestine, Munck (222) found only two carriers for imino acids. One was defined as the imino acid carrier, which accepts amino acids with substituted amines, such as proline, sarcosine (methylglycine), betaine, MeAIB, and glycine. It corresponds to system 4 as defined by Wiseman (410). The second carrier is the neutral amino acid transporter B⁰. The order of amino acid preference for the imino acid carrier is proline > alanine > betaine > glycine (222). The transporter also accepts taurine and GABA as substrates. All substrates bind with low affinity (Kₐ values 12–40 mM). The transporter shows little discrimination between amino acids in the D- or L-configuration. In fact, the D-configuration of hydroxyproline binds with higher affinity than the L-configuration (224). A transporter of similar substrate specificity was described by Thwaites et al. (378) as a H⁺-dependent amino acid transporter of the intestinal cell line Caco-2. While initial studies suggested that imino acid transport in rat intestinal epithelium is at least partially Na⁺ dependent (235), recent studies confirmed the presence of a H⁺-amino acid cotransporter in this tissue (7, 150). The characteristics of the rat intestinal imino acid transporter are significantly different from the major intestinal proline transporter of the rabbit jejunum. This transporter is not shared by glycine and has been termed system IMINO (359). In BBMV of the rabbit small intestine, also only two transporters contribute to proline uptake, namely, system B⁰ and system IMINO (356). The IMINO system accepts proline, hydroxyproline, pипеоксил, proline methyl ester, betaine, and MeAIB; phenylalanine is a low-affinity inhibitor of the transporter (359). It corresponds to system 3 as defined by Wiseman (410). The transport system is much more stereospecific than the imino acid carrier, restricting α-proline by a factor of 30. The transport mechanism of the IMINO carrier has been studied in some detail (358). The transporter is Na⁺ dependent, most likely cotransporting two Na⁺ together with the substrate. The kinetic data are consistent with Na⁺ binding to the carrier before the substrate. The transporter is inhibited by high intracellular Na⁺ concentrations; inhibition is relieved when substrate is present on the trans-side as well. The major effect of increasing extracellular Na⁺ concentration is to affect the Kₘ of the substrate, whereas Vₘₐₓ remains constant. The transporter was later found to be chloride dependent (235).

In summary, it appears that proline uptake in rabbit intestine is mediated by the IMINO system and system B⁰, whereas in rat intestine the two principal components are the imino acid carrier and system B⁰ (Fig. 4). It has been suggested that nutritional differences may account for the prevalence of two different transporters in these species. The species differences are inverse in kidney. In BBMV from rat kidney cortex, the IMINO systems appears to be dominant (72), whereas in BBMV from rabbit kidney cortex, the imino acid carrier prevails (295).

3. Transport of imino acids and glycine across the basolateral membrane of epithelial cells

Glycine transport across the basolateral membrane was found to be Na⁺ independent in the proximal convoluted tubule but to be Na⁺ dependent in the proximal straight tubule (20). Because there are few amino acids remaining in the lumen of the proximal straight tubule, epithelial cells may have to rely on supply of amino acids from the blood for metabolic purposes. The Na⁺-dependent glycine transporter appears to be similar to the apical system (Fig. 4). Proline transport was found to be entirely Na⁺ independent in BLTV from rat kidney (292, 347). A Na⁺-independent transporter for proline was also described in rat small intestine BLTV (90). Proline uptake was inhibited by L-proline, leucine, alanine, sarcosine, glycine, lysine, OH-proline, taurine, β-alanine, serine, and phenylalanine. Some amino acids such as threonine or methionine had very little effect on the uptake of proline. Currently, there is no molecular correlate of this system (Fig. 4), which may reflect a mixture of several systems. Glycine transport may be mediated by the basolateral transporter for neutral amino acids (see sect. uB).

C. Imino Acid and Glycine Transporters

1. The proton amino acid transporter

**PAT1 (SLC36A1)**

PAT1 is the molecular correlate of the rat intestinal imino acid carrier (7). PAT1 was initially identified as a lysosomal amino acid transporter (LYAAT1) (307). Accordingly, the transporter is most active at low pH. Ex-
pression in oocytes or HeLa cells, however, suggests that PAT1 is electrogenic and cotransports $\text{H}^+$ together with each amino acid, resulting in an acidification of the cytosol during substrate uptake. PAT1 has an affinity for protons of $\sim 100 \text{nM}$ ($pK = 7$). The maximum substrate-induced current appears to be limited by the substrate concentration but not by the proton concentration (115). As a result, a binding order was suggested in which protons bind first to the transporter. The substrate specificity of PAT1 matches that of the imino acid carrier as described in the apical membrane of intestinal cells and in Caco-2 cells (see sect. $\nu B$). In oocytes, PAT1 transports proline, glycine, alanine, $\beta$-alanine, betaine, sarcosine ($N$-methylglycine), MeAIB, and GABA. The transporter shows low affinity for all of its substrates, with $K_m$ values ranging from 3 to 7 mM (35). Proline has a significantly higher affinity than glycine. The PAT1 binding site allows only one carbon atom in the side chain (i.e., alanine or serine). Serine already has a very low affinity for the transporter. It tolerates an extension of the distance between the amino group and the carboxyl group of up to three carbon atoms (i.e., GABA) (33). PAT1 shows relatively little discrimination between optical isomers. $\beta$-Proline and $\beta$-alanine are transported with similar $K_m$ values as the $L$-isofoms and even induce larger currents in oocytes (33). It also tolerates secondary, tertiary, and quaternary amines instead of the free amino group. In fact, an amino group is not an essential requirement for PAT1 substrates, since it also accepts short-chain fatty acids (114). Mouse PAT1 shows the highest expression in the small intestine followed by brain, kidney, and colon. Cloning of the human PAT1 from Caco-2 cells further confirmed that it corresponds to the $\text{H}^+$-amino acid co-transporter in this cell type (65). Immunostaining demonstrated an apical localization in the rat intestine (7) (Fig. 4). This transporter is different from system IMINO, which has been extensively characterized in the BBMV from rabbit jejunum. Functional studies (see sect. $\nu B$) suggest that in rat intestine PAT1 is the major proline transporter, whereas in rabbit intestine IMINO is the dominant activity. In the kidney, the situation is reversed, with IMINO prevailing in rat, whereas a proton-dependent activity is dominant in rabbit. These proposed species differences require further verification by in situ hybridization or immunohistochemistry. The amino acid carrier has been characterized as partially Na$^+$ dependent in tissue sections of rat intestine (235). The discrepancy between the apparent Na$^+$ dependence in the intestine and the H$^+$ dependence of the cloned PAT1 could be resolved by the demonstration that a tight cooperation exists between the Na$^+$/H$^+$ exchanger and PAT1 as demonstrated in Caco-2 cells (7, 377).

As a result of its substrate specificity, PAT1 has been suggested as a candidate for the transporter mutated in iminoglycinuria (7, 44). The transporter would be more likely involved in those cases of iminoglycinuria where an intestinal transport defect has been demonstrated. As pointed out above, there is evidence for iminoglycinuria being a multigene disorder. As a result, PAT1 is only one of several candidates for the disorder (Table 2). The role of PAT1 in the kidney is less clear because localization studies have not yet been reported. The cloned PAT1 shows relatively little activity at neutral pH and might not be an efficient mediator of reabsorption in the apical membrane of the proximal tubule. The proton-dependent transport of glycine and proline that has been identified in kidney BBMV may thus reflect both PAT1 and PAT2 activity. In agreement with this notion, proline uptake into rabbit kidney BBMV was only partially inhibited by the PAT1 substrate GABA (217).

2. The proton amino acid transporter

PAT2 (SLC36A2)

PAT2 was identified by virtue of its homology to PAT1 (35, 66). PAT2 has a similar substrate specificity to PAT1 but considerably higher affinity for its substrates, with $K_m$ values ranging from 0.1 to 0.6 mM (116, 171). Proline binds with higher affinity than glycine. A notable difference in the substrate specificity between PAT2 and PAT1 is the very low affinity for GABA, $\beta$-alanine, betaine, and $\beta$-alanine in the case of the former (116). This is caused by the transporter being less tolerant to an increase of the distance between the amino and the carboxyl groups. Furthermore, PAT2 tolerates only a single aminomethylation. As a result, it transports secondary amines such as MeAIB and sarcosine, but not $N,N'$-dimethylglycine or betaine. Similarly to PAT1, it can also transport short-chain fatty acids (114). Modification of the carboxyl group is not tolerated, although some transport activity has been reported for $O$-methyl-alanine (171). PAT2 also shows a number of mechanistic differences to PAT1 as analyzed in Xenopus laevis oocytes. In contrast to PAT1, PAT2 shows higher activity at neutral pH. This is the result of an extremely high affinity for $\text{H}^+$ of 1 mM ($pK 9.0$) (116). The substrate $K_m$ values are strongly affected by the pH, when tested close to the $K_m$ value for protons. The maximal transport velocity is only moderately affected by the membrane potential, also discriminating it from PAT1. The reversal potential is shifted by the extracellular pH, and the oocyte cytosol acidifies during substrate transport. A 1:1 cotransport stoichiometry was demonstrated for PAT2. In summary, both PAT1 and PAT2 have the same general mechanism, but show subtle differences. The physiologically most relevant difference is the pH dependence of PAT2. It renders the transporter more active in the kidney proximal tubule at pH 6.8, compared with PAT1. The localization of PAT1 and PAT2 in the kidney has not been established, and the relative
contribution to renal reabsorption is not known (Fig. 4). Because PAT2 is not found in the intestine, it might be a candidate gene for iminoglycinuria without intestinal involvement (Table 2). Mouse PAT2 expression is particularly strong in the heart and lung, with lower amounts reported in kidney, muscle, and spleen (35). A slightly different expression pattern was reported for rat PAT2 (66). Highest expression was detected in spleen, whereas smaller amounts of rat PAT2 were detected in lung, muscle, and kidney. Human PAT2 mRNA was detected in lung, heart, testis, kidney, spleen, colon, and brain (34).

3. The system IMINO transporter (SLC6A20)

The molecular correlate of the system IMINO transporter is SLC6A20, referred to as SIT1 or IMINO (177, 368). IMINO is a member of the “orphan transporter” group of the SLC6 family (50). This subfamily was discovered by virtue of their homology to the neurotransmitter transporters for GABA, norepinephrine, serotonin, and dopamine (245). Because no substrates were identified for these transporters, they were referred to as NTTs (for neurotransmitter transporters), XTs, or XTRPs (transporters with unknown substrate). Following the discovery of SLC6A19 as a neutral amino acid transporter (46), XT3 was identified as the long-sought IMINO transporter (177, 368). Humans have only one version of the IMINO gene, whereas mouse and rat have two genes at the syntenic chromosomal region. These have been referred to as XT3 and XT3s1 in mouse or XT3s1 and rB21A in rat. Unfortunately, the orthologs have been assigned in opposite order in both species (i.e., rB21A in rat corresponds to XT3s1 in mouse, and XT3 in mouse corresponds to XT3s1 in the rat). In an attempt to solve this confusion, it was suggested to call the mouse XT3s1 (rat rB21A) IMINOB because it is highly expressed in the brain, whereas mouse XT3 and rat XT3s1 should be called IMINOB because they are highly expressed in kidney. In this nomenclature, IMINOB corresponds to the human SLC6A20. In all species, IMINOB is the active transporter, whereas IMINOK does not show any transport activity (177).

IMINO accepts only amino acids with secondary, tertiary, or quaternary amines, such as proline, sarcosine, betaine, and MeAIB (177, 368). Interestingly, the transporter has also weak affinity for neutral amino acids such as phenylalanine. IMINO is Na+- and Cl- dependent. Proline transport is accompanied by inward currents, which are equivalent to the cotransport of one positive charge per substrate. Replacement of Na+ by Li+ abolishes uptake of radiolabeled proline (177), but still permits small proline-induced currents (368). High-affinity substrates of IMINO are proline, betaine, hydroxyproline ($K_m$ values of 0.1–0.2 mM), whereas low-affinity substrates such as phenylalanine are transported with a $K_m$ of 3.0 mM (177). IMINO can be distinguished from PAT1 (imino acid transporter) by its inability to transport glycine, by its stereoselectivity for r-amino acids, and by its coupling to the Na+-electrochemical gradient. IMINO is expressed in all segments of the proximal tubule and of the intestine, showing the highest expression in the ileum (297, 373) (Fig. 4).

IMINO has been suggested as a candidate for iminoglycinuria (44) (Table 2). Although IMINO cannot transport glycine, it has been reasoned that overflow of proline into the proximal tubule causes iminoglycinuria because of a competition between proline and glycine for PAT1/2 (Fig. 4). Overflow of glycine into the urine is observed above plasma proline concentration of 0.8 mM.

4. The putative glycine transporter XT2 (SLC6A18)

The gene for the human XT2 is located next to the gene for B+AT1 on chromosome 5. XT2 and B+AT1 are most likely the result of a gene duplication event. The transporter cDNA was isolated from kidney (245, 399) and is expressed in several splice variants, the physiological role of which remains unknown. The transporter has been expressed in several heterologous systems and tested exhaustively for uptake of amino acids, osmolytes, neurotransmitters, and other substrates. Although it is found in the plasma membrane, it has so far failed to reveal its activity. An XT2-deficient mouse shows increased amounts of glycine in the urine, suggesting its involvement in glycine absorption. The absorption efficiency for glycine was reduced to 75%, whereas all other amino acids were resorbed with $>97$% efficiency (281). Further analysis of the XT2-deficient mice showed a lack of an autoradiographic signal of glycine accumulation in the outer medulla. This localization is in agreement with the expression of the transporter in the kidney (249, 297, 399). BBMV prepared from the XT2-deficient mice showed normal leucine uptake but lacked a high-affinity uptake system for glycine. In contrast, low-affinity uptake of glycine was still observed. The low-affinity uptake most likely represents the system shared with proline (PAT1/2), whereas XT2 would correspond to the high-affinity glycine-specific system. XT2-deficient mice also showed elevated levels of other amino acids in the urine (281). As a result, it has been suggested that XT2 could also correspond to the high-affinity neutral amino acid transporter of the proximal straight tubule (see sect. uH) (297).

Expression of XT2 is intense in the proximal straight tubule of kidney and much less abundant in the intestine (249, 297, 399) (Fig. 4). The transporter is upregulated under osmotic stress, indicating a role in balancing the high extracellular osmolarity in the kidney medulla. Although hypertension was reported in the XT2-deficient mouse, a nonsense polymorphism Y319X in the Japanese population was not associated with hypertension. In this
study, 1,004 individuals were analyzed, 157 of whom were homozygous for the premature stop codon (104). Unfortunately, glycine levels in the urine were not reported. As a result, it has not been clarified whether XT2 is a candidate for glycinuria (Table 2). This study demonstrates that a nonfunctional XT2 does not result in a severe disease phenotype. It is tempting to speculate that the variable amounts of glycine in the urine of the normal population may reflect frequently occurring mutations in glycine transporters. The glycine transporters GLYT1 and GLYT2, which also belong to the SLC6 family, appear not to be expressed in kidney (64).

VI. TRANSPORT OF TAURINE AND OTHER β-AMINO ACIDS

A. Inherited Disorders of β-Amino Acid Transport

1. β-Aminoaciduria (OMIM 210100)

Increased excretion of β-aminoisobutyric acid is a frequently observed benign anomaly. The “defect” is an impairment of β-aminoisobutyric acid catabolism due to deficient activity of β-aminoisobutyrate:pyruvate aminotransferase (159) and is probably the most frequently inherited malfunction of amino acid metabolism. Although this is not an amino acid transport disorder, the condition sheds a light on a transport system for β-amino acids in the kidney. Scriver et al. (325) initially described a case of hyper-β-alaninemia that resulted in hyperexcretion of β-aminoisobutyric acid, β-alanine, taurine, and GABA, suggesting a common transport system for β-amino acids and GABA. This common transport system has been identified as the taurine transporter TauT (sect. VI C and Table 2).

B. β-Amino Acid Transport in Kidney and Intestine

1. Transport of β-amino acids and taurine across the apical membrane of renal epithelial cells

A common system for taurine, β-alanine, and GABA has been described in the kidney proximal tubule (70, 71, 88, 123, 128). Uptake of β-alanine in kidney BBMV has all hallmarks of a Na⁺-coupled transporter (68, 133, 303). It is inhibited 95% when Na⁺ is replaced by any other cation. In the presence of Na⁺, an overshoot is observed in BBMV. An inside-negative membrane potential strongly stimulates uptake. The transport is also sensitive to changes of the anion. Replacement of Cl⁻ by other anions suppresses β-alanine transport (68). Concentrative uptake can be driven by Cl⁻ in the absence of any other driving force, suggesting that Cl⁻ is actively cotransported. A stoichiometry of >2 Na⁺:1 Cl⁻:β-alanine was proposed by Turner (383). A single high-affinity system with a \( K_m \) value of 35 \( \mu \)M was described in dog BBMV (383). Uptake of β-alanine is strongly inhibited by taurine and hypotaurine and more weakly by GABA, but not by any other amino acid. In addition, a low-affinity taurine transporter has been identified in the proximal convoluted tubule that appears to be H⁺ coupled (70, 71, 153, 154). Also another low-affinity Na⁺-dependent taurine transporter that is not chloride dependent has been reported in both proximal convoluted tubule and proximal straight tubule (153). The H⁺-dependent transport was inhibited by β-alanine, proline, glycine, and alanine and hence appears to be similar to the imino acid carrier (see sect. vB). A diet low in sulfur containing amino acids or low in taurine content results in upregulation of the high-affinity taurine transporter (69, 302).

In summary, there is strong evidence for a high-affinity Na⁺- and Cl⁻-dependent transporter for taurine and β-alanine and a low-affinity H⁺-dependent transporter that has the properties of the imino acid carrier (Fig. 5). The molecular correlate of the high-affinity Na⁺- and Cl⁻-dependent taurine transporter is the TauT trans-
porter (see sect. vC). It appears that the Na\(^+\) - and Cl\(^-\)-
dependent transporter carries the main load of taurine
absorption.

2. Transport of \(\beta\)-amino acids and taurine across the
apical membrane of intestinal epithelial cells

Similar to the kidney, two major transport activities
for \(\beta\)-alanine and taurine have been reported in the inte-
testine (233) (Fig. 5): 1) a high-affinity low-capacity trans-
porter that is Na\(^+\) and Cl\(^-\) dependent and similar to the
kidney cortex transporter (21, 215, 216, 220); and 2) a
low-affinity high-capacity transporter, which is H\(^+\) depen-
dent and is shared by \(\beta\)-alanine, taurine, GABA, proline,
glycine, MeAIB, and betaine. The high-affinity system has
been detected in rat and rabbit intestine. Some species
differences appear to exist. In the rat, taurine is trans-
ported by a Na\(^+\) - and Cl\(^-\)-dependent transporter that is
not shared by \(\beta\)-alanine. In rabbit, in contrast, \(\beta\)-alanine
and taurine share a high-affinity Na\(^+\) and Cl\(^-\)-dependent
transporter (215, 216). In the rabbit distal ileum, \(\beta\)-alanine
is mainly transported by the general amino acid transport
system B\(\text{0}^{+}\), which is H\(^+\) dependent and is encoded by the ATB0,
porter that is Na\(^+\) and Cl\(^-\)-dependent and is able to accumulate taurine
several thousandfold. The mRNA for TauT is strongly
upregulated when MDCK cells are incubated in hyper-
tonic medium, which is accompanied by an upregulation
of taurine transport. Taurine transporter-deficient mice
showed excretion of taurine that reaches the GFR. This
suggests that TauT is the major mediator of taurine ab-
sorption in the kidney (149) (Fig. 5).

2. Other transporters for \(\beta\)-amino acids

The GABA/betaine transporter BGT1 transports
\(\beta\)-alanine and to a small extent taurine (196). It cotrans-
ports three Na\(^+\) and two Cl\(^-\) together with betaine.
The transporter is expressed in the basolateral membrane
of polarized epithelial cells and therefore does not contrib-
ute to the absorption of \(\beta\)-amino acids (272). The proton
amino acid transporter PAT1 most likely is the low-affin-
ity transporter of taurine in kidney and intestine. It is
described in section vC.

VII. SIGNIFICANCE OF
PARACELLULAR TRANSPORT

Munck and Schultz (231) studied the net mucosa to
erserosa flux of lysine in the presence and absence of a
transepithelial potential of 35 mV (mucosa negative). Net
lysine transport remained the same under both condi-
tions, causing the authors to conclude that paracellular
transport of lysine is insignificant. Similarly, it appears
that paracellular transport contributes very little to renal
absorption of arginine and tryptophan (61, 341). In con-
trast, it has been estimated that \(-50\%\) of glycine reabsorp-
tion in the kidney occurs via paracellular transport (343).
Glycine reabsorption does not appear to reach a transport
maximum and can be divided into two components (340).
One of these components appears to be nonsaturable and
is not energy dependent, most likely reflecting paracellu-
lar transport. A much more significant role for paracellu-
lar transport was proposed by Pappenheimer (263). It was
argued that paracellular transport becomes highly signif-
ificant at high substrate concentration (e.g., \(>100\) mM in
the case of glucose) and that these concentrations are
reached at the mucosal surface due to local enzymatic
degradation of oligosaccharides. The total concentration
of free amino acids in the human intestine can reach \(\sim 20\)
mM (2); however, as much as the equivalent of 120 mM
free amino acids is available from peptides. The brush-
border membrane is endowed with a variety of peptidases
(18), which could create high local concentrations of free
amino acids, but analytical data have not been reported.
Amino acid transport has also been studied in monolayers
of Caco-2 cells. The results indicate that carrier-mediated
transport of amino acids is significantly faster than their
paracellular transport (141, 148) or the transport of D-mannitol, which occurs entirely by the paracellular route (25, 26). These studies used low concentrations of radiolabeled amino acids and therefore may underestimate paracellular transport at higher concentrations. Paracellular transport driven by solvent drag would also explain why amino acid reabsorption in the kidney is still significant in cases of inherited aminoacidurias where the mutated transport system carries the large majority of the transport load (e.g., the basolateral transporter for cationic amino acids in lysinuric protein intolerance, see sect. IIIA). In contrast, it has been reported that an oral load of tryptophan does not increase plasma tryptophan levels in patients with Hartnup disorder (155, 336), suggesting that paracellular transport may be less important in the intestine than it appears to be in the kidney.

VIII. CONCLUSION

The past 15 years saw the molecular cloning and identification of almost all physiologically described amino acid transport activities in kidney and intestine (Table 1). Only a few transport activities remain to be identified, such as the Na\(^+\)-stimulated cationic amino acid transport, which might be associated with rBAT in the proximal straight tubule, and the mediator of net influx of neutral amino acids across the basolateral membrane. It has also not been fully resolved how intracellular neutral amino acids stimulate efflux of cationic amino acids, although it is likely that this function is associated with the 4F2hc/\(\gamma^+\)LAT1 protein. It further remains to be clarified how glycine, proline, and the \(\beta\)-amino acids cross the basolateral membrane.

It appears likely that the cell biology of epithelial transport will become the next phase of research in this area. This includes the following questions: Do epithelial transporters form complexes in the membrane? Are they held in place by scaffolding proteins? What signals change the expression of transporters, and what are the physiologic stimuli? How is tissue-specific, cell-specific, and developmental stage-specific expression of epithelial transporters achieved? Now that the mediators of epithelial transport are known, these questions can be readily addressed.

ACKNOWLEDGMENTS

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