"This is a non-final version of an article published in final form in Current Opinion in Nephrology and Hypertension 22.5 (2013): 539-544 ".

Epithelial neutral amino acid transporters, lessons from mouse models

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Abstract (200 words max.):

Purpose of review: Epithelial neutral amino acid transporters have been identified at the molecular level in recent years. Mouse models have now established the crucial role of these transporters for systemic amino acid homeostasis. The review summarises recent progress in this field.

Recent findings: Epithelial neutral amino acid transporters play an important role in the homeostasis of neutral amino acid levels in the body. They are important for the maintenance of body weight, muscle mass and serve as fuels. They also serve a role in providing nutrients to epithelial cells. Changes of plasma amino acid levels are not necessarily correlated to the amino acids appearing in the urine, changes to organ amino acid metabolism need to be taken into account.

Summary: Genetic deletion of neutral amino acid transporters provides insight into their role in protein nutrition and homestasis.

Keywords:
Protein nutrition, intestine, kidney

Abbreviations: dss, dextran-sulphate; hpd, high protein diet; nd, normal diet

Introduction

The majority of epithelial amino acid transporters have been identified in recent years [1-3]. Many of these are expressed in both intestinal and renal epithelia (Fig. 1,2). Following their molecular characterisation a more systemic analysis is now achieved through the generation of mouse models. This review will focus on insights provided by mouse models of epithelial transporters in recent years.

Molecular and cellular organisation of amino acid uptake in the kidney and intestine

The bulk of neutral amino acid uptake in both kidney and intestine is mediated by the amino acid transporter B^0AT1 (SLC6A19), which accepts a broad (B) variety of neutral (0) amino acids [4]. Mutations in B^0AT1 (SLC6A19) cause Hartnup disorder, a rare inherited condition that is characterised by highly elevated amounts of neutral amino acids in the urine [5-7].

B^0AT1 is mainly expressed in the kidney and intestine, lower expression levels are found in the skin and pancreas. B^0AT1 expression at the apical cell surface requires coexpression of the single-pass membrane proteins collectrin (TMEM27) in the kidney [8] or angiotensin-converting enzyme 2 (ACE2) in the intestine [9,10]. B^0AT1 is highly expressed in the early segments of the proximal tubule (mainly S1), while collectrin is found along the entire proximal tubule [11,12]. The accessory protein does not seem to influence the transport activity, but some B^0AT1 mutations appear to interact slightly differently with ACE2 and collectrin [9]. A differential interaction of B^0AT1 mutations with ACE2 and collectrin may explain rare cases of purely renal [13,14] or purely intestinal [15] Hartnup disorder. Transport activity of B^0AT1 is affected by the presence of Aminopeptidase N, which forms larger complexes with B^0AT1 and ACE2 generating transport metabolomes that optimise peptide digestion and transport [16].

Proline and glycine have additional transporters in both kidney and intestine (Fig. 1) and species differences have to be taken into account to avoid confusion (reviewed in [17]). In mice, renal glycine uptake is largely mediated by B^0AT3 (SLC6A18), which also contributes to the reabsorption of other neutral amino acids [12]. B^0AT3 is mainly expressed in the apical membrane of epithelial cells in the S3 segment of the proximal tubule. Like B^0AT1 it requires coexpression of ACE2 or collectrin to reach the cell surface [11]. The physiological partner is collectrin, which has overlapping expression with B^0AT3 [11,12]. The transporter prefers short neutral amino acids such as glycine and alanine. Renal proline uptake is mediated by SIT1 (SLC6A20) in mouse and humans [11]. B^0AT3 appears to be inactive in humans and is replaced by the proton amino acid transporter PAT2 (SLC36A2), which transports both glycine and proline [18]. Mutations in PAT2 cause iminoglycinuria, a benign condition, characterised by the spillover of glycine and proline into the urine [18]. SIT1 mutations have a modifying effect on the urinary profile. In the intestine proline and glycine uptake is mediated by the proton amino acid transporter PAT1 in both mice and humans [19]. Release of neutral amino acids through the basolateral membrane is not fully understood. The heteromeric amino acid transporter 4F2hc/LAT2 (SLC3A2/SLC7A8) is localised in the basolateral membrane and accepts all neutral amino acids [20-22]. However, the transporter is an obligatory antiporter and thus cannot mediate net efflux of bulk amounts of neutral amino acids. The transporter is comprised of two subunits, the 4F2 heavy chain and LAT2 light chain, which are linked by a disulphide bridge. For aromatic amino acids a specific transporter, TAT1 (SLC16A10), has been identified in the basolateral membrane of both kidney and intestine [23]. TAT1 is found in the early segments of the proximal tubule (S1 and S2) and is colocalised with LAT2 [24]. TAT1 can mediate net efflux of aromatic amino

acids across the basolateral membrane. The major net efflux transporter for neutral amino acids in the basolateral membrane of the kidney and intestine remains to be identified.
Cationic amino acids and cystine are taken up across the apical membrane by the heteromeric transporter rbat/$b^{0,+}$AT (SLC3A1/SLC7A9) (reviewed in [25])(Fig. 2). The transporter and its role in cystinuria have been reviewed extensively in the past [26,27]. Release of cationic amino acids across the basolateral membrane is mediated by the heteromeric amino acid transporter 4F2hc/y^+LAT1 (Slc3a2/Slc7a7) [25]. Mutations in y^+LAT1 cause lysinuric protein intolerance (LPI), a multisystemic disorder, affecting lung, liver and renal epithelial cells. The reader is referred to a recent review discussing the pathophysiological mechanisms of LPI [28]. Transport of anionic amino acids across the apical membrane in both kidney and intestine is mediated by the glutamate/aspartate transporter EAAT3 (SLC1A1) [29] (Fig. 2). Mutations in EAAT3 cause dicarboxylic aminoaciduria (DA) [30]. Glutamate is intensely metabolised in epithelial cells [31], but release of aspartate occurs through the basolateral membrane, the mediator of which remains unknown.

Mice lacking the neutral amino acid transporter B^0AT1 (SLC6A19). Three mouse models are relevant to this transporter. Collectrin-deficient mice lack B^0AT1 [8] in the kidney, ACE2-deficient mice lack B^0AT1 in the intestine [32] and B^0AT1-deficient mice lack the transporter in all tissues without affecting collectrin or ACE2 expression [33]. B^0AT1-deficient mice are a model of human Hartnup disorder. Hartnup disorder is characterised by highly elevated levels of all neutral amino acids in the urine and reduced absorption of neutral amino acids in the intestine [7]. Clinically it is a benign disorder in adults, children frequently develop a skin rash and in rare cases show cerebellar ataxia. In countries with high quality nutrition symptoms are rare. The symptoms of Hartnup disorder are related to pellagra which is caused by a lack of niacin (nicotinamide or nicotinic acid). The common cause appears to be the reduced synthesis of NAD(P)H for which both tryptophan and niacin are precursors. B^0AT1-deficient mice show reduced growth and body weight after weaning, particularly in males [33]. Histological analysis revealed no obvious abnormalities. High levels of all neutral amino acids were found in the urine. In the intestine Na^+-dependent uptake of leucine, glutamine, tryptophan and histidine was completely abolished. Surprisingly, glucose uptake was also reduced by about 50% in animals lacking B^0AT1. Peptide uptake was unaffected, suggesting that no compensation of the reduced amino acid uptake by way of peptide uptake occurred. In the kidney Na^+-dependent uptake of leucine and tryptophan was completely abolished, while residual transport activity was identified for glutamine, histidine, glycine and proline. The residual uptake of proline (40%) had characteristics consistent with the expression of SIT1 (Slc6a20a). The residual glycine uptake was most likely mediated by B^0AT3 (Slc6a18). B^0AT1-deficient mice had less body fat than wildtype mice and could not maintain body weight on diets with different protein contents. Blood glucose levels before and after feeding were normal, but the corresponding insulin release was significantly smaller pointing to improved insulin sensitivity. These results are similar to those reported for collectrin-deficient mice (see below and [34]). Enterocytes showed signs of amino acid starvation as indicated by reduced mTOR activation and upregulation of the GCN2 pathway [3].

Mice lacking angiotensin converting enzyme 2 (ACE2)

Mice lacking ACE2 have a variety of pathologies, such as impaired cardiac contractility, late-onset nephrotic glomerulosclerosis, enhanced susceptibility to diabetic kidney injury and acute respiratory stress syndrome. These are unrelated to its interaction with amino acid transporters and the reader is referred to a recent review for further information [35]. Lack of ACE2 also generates a physiological phenotype due to the lack of B^0AT1 expression in the intestine, while reabsorption in the kidney is almost unaffected consistent with the dominant expression of collectrin in the proximal tubule [12]. Lack of neutral amino acid uptake in the intestine results in reduced plasma concentration of neutral amino acid (10-25%), particularly of tryptophan (60% reduced) [32]. In the intestine absorption was delayed, resulting in elevated amino acid concentrations in the terminal ileum. Urine output was increased in aged ACE2-deficient animals, resulting in reduced urine osmolality. The urine pH was more acidic. Muscle mass was not reduced in the animals, but may have a higher turnover, as observed in collectrin-deficient mice [34]. Similar to B^0AT1-deficient animals, significant weight loss was observed on a low protein diet, but no pellagra-like symptoms were observed. Reduced amino acid uptake in the intestine causes amino acid starvation in enterocytes [33]. This results in reduced secretion of antimicrobial peptides (defensins), which in turn causes changes to the intestinal microflora and causes intestinal inflammation [36]. Infusion of recombinant soluble ACE2 or deletion of the angiotensin receptor 1a did not alleviate any of these symptoms, demonstrating that this phenotype of ACE2 deficient mice is not related to its role in the local renin-angiotensin system. When challenged with dextran-sulphate (DSS), an irritant that disrupts the intestinal epithelial barrier and results in colitis, a profoundly increased inflammatory reaction was observed in ACE2-deficient mice compared to wildtype mice. Transplantation of bone marrow from ACE2-deficient mice to wildtype mice and vice versa did not change the DSS-induced colitis, suggesting that the response was not caused by an altered immune cell response due to lack of ACE2. Consistent with the role of ACE2, a protein-free diet also rendered the intestinal epithelium highly sensitive to DSS-induced colitis. As pointed out above tryptophan together with niacin are the main precursors for the synthesis of NAD(P)H. Pellagra and Hartnup disorder are both known to be associated with colitis. Indeed, provision of a tryptophan dipeptide, which is transported by the peptide transporter PepT1, thus circumnavigating the lack of B^0AT1, restored serum tryptophan levels and rescued enhanced DSS susceptibility. Cell death of epithelial cells was similar in both ACE2-deficient and wildtype animals. However, production of multiple antimicrobial peptides was reduced in ACE2-deficient animals, such as alpha-defensins 1,4 and 5. Both B^0AT1 and ACE2-deficient animals show reduced mTOR activation in the intestine, which in turn could regulate production of defensins. Consistent with this notion production of antimicrobial peptides was similarly reduced after administration of rapamycin and is also reduced in B^0AT1-deficient animals (unpublished data). Supplementation of tryptophan dipeptide activated mTOR and upregulated production of antimicrobial peptides. These alterations of the intestinal homeostasis caused a change of the intestinal microbiome, which also could be reversed by administration of tryptophan dipeptide. Transplantation of the ileocaecal gut microbiota from ACE2-deficient mice to wild-type mice transmitted the inflammatory phenotype.

Mice lacking collectrin (TMEM27)

Collectrin-deficient mice are healthy and do not have obvious histological anomalies. The initial characterisation showed significant aminoaciduria, similar to Hartnup disorder, but there is no intestinal phenotype [8,37]. Collectrin has been shown to be expressed in the

pancreas where it is localised on insulin-containing vesicles in β-cells [38]. A thorough morphometric analysis of pancreas function, however, failed to reveal any changes of β-cell numbers, proliferation and islet area and numbers [34]. Insulin secretion was similar in wildtype and collectrin-deficient mice in vivo and from isolated islets in vitro. Despite normal pancreas morphology and function, collectrin-deficient mice showed improved insulin sensitivity as assessed by insulin tolerance tests [34]. Glucose tolerance was similar, but required less insulin. Collectrin-deficient animals were slightly smaller. Investigation of amino acid metabolism showed a reduction of short-chain acyl-carnitines, which are the breakdown products of branched-chain amino acids. Also free carnitine was significantly reduced. The study concluded that an increased amount of energy utilisation occurred to compensate for the loss of amino acids. Muscle proteolysis appeared to be increased, but was balanced by increased protein synthesis, as no loss of muscle mass was observed.

Mice lacking the neutral amino acid transporter B^0AT3 (SLC6A18)

B^0AT3-deficient mice are healthy and breed normally. An initial characterisation revealed increased urinary excretion of glycine and hypertension [39]. Using mice that were back-crossed ten generations against the C57BL/6 background confirmed the glycinuria, but also found significantly increased fractional excretion of methionine [12]. Other amino acids were slightly elevated in the urine increasing the fractional excretion by 5-10%. No difference was found in blood pressure between wildtype and B^0AT3-deficient mice under resting conditions. However, under stress a slightly elevated blood pressure was observed in the nullizygous mice. In the human population a stop codon occurs at high frequency in SLC6A18, which is not associated with elevated blood pressure [40]. As pointed out above PAT2 has replaced B^0AT3 in the human kidney and therefore blood pressure changes would rather be associated with mutations in PAT2 [18].

Mice lacking the neutral amino acid transporter LAT2 (SLC7A8). LAT2 is mainly expressed in the kidney, intestine, brain and testis. No abnormalities were observed in the histology of these organs [41]. LAT2-deficient mice have a normal body weight and size. Litter size and frequency are normal. Urine amino acid analysis revealed less than two-fold increases for serine, threonine, glutamine, leucine, valine and glycine. In vitro experiments in MDCK cells suggested a role of LAT2 in cysteine export [42]. Cysteine concentration, however, was reduced in the urine. Overall the results suggest a modulatory role of LAT2 in amino acid reabsorption. The results confirm in vitro experiments showing LAT2 to be an obligatory exchanger, not capable of mediating net flux of amino acids across the basolateral membrane. Notably, there was no increase of aromatic amino acids in the urine suggesting that these are transported by a separate transporter (TAT1, see below). In the blood plasma increased concentrations of the neutral amino acids glycine, alanine, serine, threonine, glutamine and valine were observed. Since the same amino acids showed an increased loss in the urine, the results suggest some changes to the absorption of these amino acids in the intestine or altered metabolism due to the lack of LAT2. In addition levels of aspartate and lysine were elevated, also pointing to altered absorption in the intestine.

Mice lacking the aromatic amino acid transporter TAT1 (SLC16A10)

TAT1 has an overlapping distribution with LAT2 in the intestine and kidney. In addition it is found in perivenous hepatocytes, muscle and brain [43]. In epithelial cells TAT1 is expressed in the basolateral membrane where it is thought to mediate the net export of aromatic

amino acids after uptake across the apical membrane in both kidney and intestine. TAT1-deficient mice grow and breed normally and do not have histological changes in organs expressing TAT1 [44]. There was no compensatory upregulation of other transporters in various organs. Plasma concentration of aromatic amino acids were elevated (Phe, ~2x, Trp ~3x, Phe ~8x) in TAT1-deficient animals compared to wildtype animals. Most others were slightly reduced (gly, ala, met, ser, thr, pro, asn, gln,lys, arg). Feeding a high protein diet increased the concentration of branched-chain amino acids in the plasma of wildtype and TAT1-deficient mice, but had little effect on other amino acids. Urine volume was increased in TAT1-deficient mice on normal and high-protein diet. With the exception of glycine, proline and glutamine urine amino acid excretion was minimal in wildtype mice (<500 µmol/24h) on both normal diet (nd) and high-protein diet (hpd). TAT1-deficient mice, by contrast, showed a dramatic increase of tyrosine excretion (50x nd; 64x hpd) and of tryptophan (6x nd; 95x hpd). Phenylalanine excretion was less pronounced (2x nd; 9x hpd). On the high-protein diet amino acid excretion of LAT2 substrates val, leu, ile, ser, thr, gln and his was also increased 3-7 fold, suggesting that recycling of aromatic amino acids could contribute to neutral amino acid reabsorption at a high load by being exchange substrates for LAT2.

Conclusion:
Mouse models of epithelial neutral amino acid transporters reveal a significant impact on amino acid homeostasis. These are caused both by reduced intestinal absorption and loss in the kidneys. Purely renal phenotypes are usually less severe than the phenotype caused by lack of the intestinal transport activity. A consistent picture has emerged how epithelial transport of neutral amino acids is mediated. The impact on whole body amino acid homeostasis is still only partially understood.

Key points:

- B^0AT1(SLC6A19) plays an important role for whole body amino acid homeostasis and also in the control of the intestinal microflora, a key metabolite for many of the observed symptoms due to lack of B^0AT1 appears to be tryptophan.
- Renal and intestinal phenotypes due to lack of B^0AT1 can be separated using collectrin- or ACE2-deficient mice.
- The main efflux protein in the basolateral membrane remains elusive, while the 4F2hc/LAT2 (SLC3A2/SLC7A8) heteromeric transporter has a modulatory role in amino acid absorption.
- B^0AT3(SLC6A18) mediates glycine absorption in mice but not in humans, its involvement in blood pressure regulation remains unclear.
- TAT1 (SLC6A18) serves to mediate epithelial transport of aromatic amino acids.

Acknowledgements: Research in the laboratory of the author has been supported by grants from the National Health and Medical Research Council and the Australian Research Council. The author has no conflicts of interest to declare.

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Figure 1: Organisation of neutral amino acid transport in epithelial cells.
Transporters are indicated by the name of the cloned transporter and also the corresponding transport activity (within circles). Sections of the proximal tubule are indicated on the right. Abbreviations: AA^0, neutral amino acids; P, proline; G, glycine.

Figure 2: Organisation of cationic and anionic amino acid transport in epithelial cells.
Transporters are indicated by the name of the cloned transporter and also the corresponding transport activity (within circles). Sections of the proximal tubule are indicated on the right. Abbreviations: AA^0, neutral amino acids; AA^+, cationic amino acids; AA^-, anionic amino acids; C^-, cysteine; G, glycine.

Fig. 1 **Neutral amino acids**

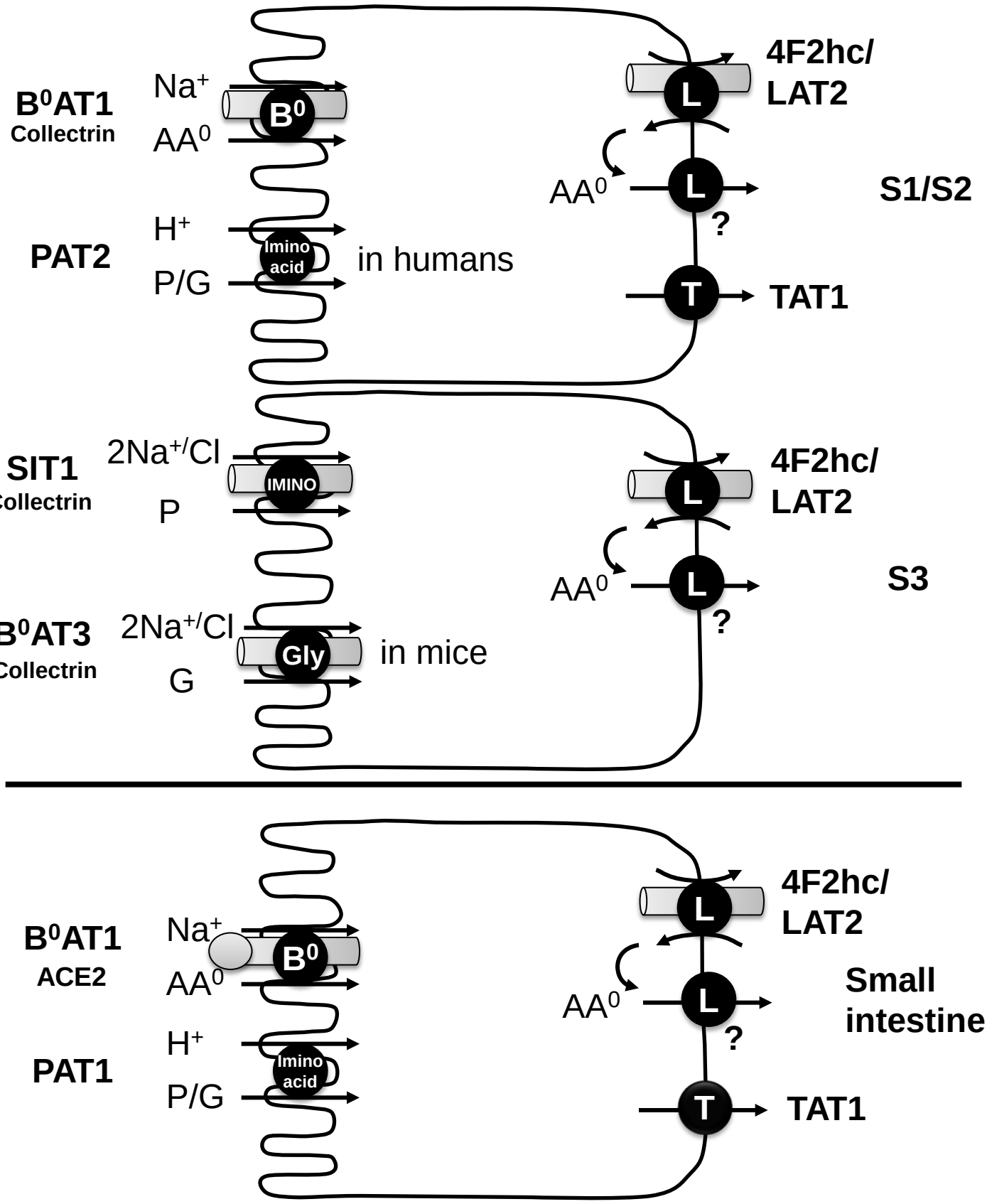

Fig. 2 Cationic and anionic amino acids