Luminal kidney and intestine SLC6 amino acid transporters of B⁰AT-cluster and their tissue distribution in Mus musculus

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Submitted 12 July 2005; accepted in final form 12 September 2005


A major player of the luminal amino acid uptake machinery was identified in 2004 in mice, namely an Na⁺-dependent neutral amino acid transporter corresponding to system B⁰ (4). This transporter, called B⁰AT1, belongs to the mouse gene family Slc6 (SLC6 in human) of Na⁺ (and Cl⁻)-dependent (neurotransmitter) transporters. It was shown to be expressed most notably in kidney and small intestine and to localize to the luminal brush-border membrane (13). Its function as an Na⁺-dependent neutral amino acid cotransporter was demonstrated by flux and electrophysiological measurements of transporter expressed in Xenopus laevis oocytes (4). Unlike other members of the Slc6 family, its function does not depend on the presence of Cl⁻, as expected for system B⁰. The ortholog human gene Slc6A19 localizes to chromosome 5p15.33, and its defect was shown by us together with others to cause Hartnup disorder, an autosomal recessive condition characterized by an increase in the urinary excretion of neutral amino acids (15, 28). This amino aciduria is often asymptomatic but can be accompanied by pellagra-like skin rash, attacks of cerebellar ataxia, and other neurological or psychiatric symptoms (10, 11, 27, 30).

Two quite similar human transporters called XT2 (Xtrp2) and XT3 (Xtrp3) have been identified earlier and represent the products of the genes Slc6A18 and 20 (13, 20). Together with B⁰AT1, they form a separate cluster within the Slc6 family (15), as shown in Fig. 1. One of these genes, Slc6A18, is localized on chromosome 5p15.33, just next to Slc6A19, whereas Slc6A20 is on chromosome 3p21.3. In the mouse genome, there are two Slc6A20-related genes arranged sequentially on chromosome 9 that possibly have arisen from a duplication that took place after the evolutionary separation of rodents and primates. The first of these gene products identified in mice was named XT3, whereas the second one, XT3s1, is 90% identical to it but resembles, in terms of primary structure and tissue distribution, more human XT3. In human, the single XT3 gene gives rise by alternative splicing to two isoforms (13). One of these human XT3 isoforms and the rodent XT3s1 have been shown in 2005 to correspond to system imino, which transports L-proline and some other substrates such as pipecolate and N-methylated amino acids (e.g., MeAIB and sarcosine) with a high apparent affinity and have correspondingly been renamed SIT1 or Inimoβ (16, 32).

The original studies on the still orphan transporter XT2 and mouse XT3 showed that their mRNAs are expressed in kidney and possibly also in small intestine (20). Expression experi-

INGESTED DIETARY PROTEINS are cleaved into small oligopeptides and single amino acids that are then absorbed across small intestine enterocytes. Similarly, small oligopeptides and single amino acids are reabsorbed across kidney proximal tubule epithelial cells to prevent their loss in the urine. The first step of this transcellular transport is the influx across the luminal brush-border membrane that is mediated by symporters (co-transporters) and antiporters (exchangers; see Refs. 36). The basolateral efflux of amino acids from the epithelial cells into the extracellular space is mediated by facilitated diffusion pathway(s) and exchangers (36).
ments of XT2 and mouse XT3 in X. laevis oocytes and in other expression systems have not revealed their function (16, 20, 25). A recently published study on a XT2 knockout mouse showed that these mice loose some glycine in their urine and possibly little quantities of other neutral amino acids as well, suggesting that this transporter might similarly to B0AT1 transport amino acids (25).

To address in mice the question of the putative role of these four B0AT1-related gene products, we localized their expression by quantifying their mRNAs in different tissues by real-time RT-PCR. Furthermore, we tested their localization at the protein level in kidney and intestine using Western blotting and immunofluorescence.

MATERIALS AND METHODS

Phylogenetic tree construction. The amino acid sequences of Slc6 family proteins were obtained from Ensembl database (www.ensembl.org). Sequence alignment was performed using ClustalW (http://www.ebi.ac.uk/clustalw; see Ref. 33). Subsequently, the phylogenetic tree was visualized using the program Treeview (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

RNA extraction and reverse transcription. Male C57BL/6J mice ~12 wk old were killed by an intraperitoneal injection of ketamine/xylazine and subsequent cervical dislocation; tissues were collected and rapidly frozen until further use. Animal handling was according to the Swiss Animal Welfare laws and approved by the Kantonales Veterinäramt Zürich. Stomach, duodenum, jejunum, ileum, and colon were harvested and rinsed several times with ice-cold PBS (pH 7.4), and the mucosa cell layers were scraped off on ice and rapidly frozen. The duodenum was proximal of the ligament of Treitz (first 2 cm), the jejunum as the upper part (upper 2/5) of the small intestine distal of the ligament of Treitz, and the ileum as the lower part (lower 2/5) of the small intestine ending before the cecum.

Between duodenum and jejunum, 5 cm tissue were removed as a safety margin as well as 10 cm between jejunum and ileum. Total colon was used for mucosa isolation. Total RNA was extracted from the different tissues using the RNeasy Mini Kit (Qiagen, Basel, Switzerland). For RNA extraction, tissues were thawed in RLT buffer (Qiagen) containing 10 μl ml β-mercaptoethanol (Sigma-Aldrich, Buchs, Switzerland) and homogenized on ice. RNA was bound to columns and treated with DNase for 15 min at 25°C to reduce genomic DNA contamination. Quantity and quality of total eluted RNA were assessed by spectrometry and agarose gel electrophoresis. Each RNA was diluted to 100 ng/μl, and 1 μl was used as template for reverse transcription with the TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, CA) in the presence of 2.5 μM random hexamer primers (Applied Biosystems).

Real-time PCR. Real-time PCR was performed using as template 1 μl cDNA synthesized by reverse transcription. The reaction was set up following the Applied Biosystems recommendations in the TaqMan Universal PCR master mix (Applied Biosystems). Primers and probes were designed using Primer Express software from Applied Biosystems. Primers were chosen to result in amplicons of 70–100 bp and were tested on cDNA derived from several tissues and resulted in a single product of the expected size (data not shown). Probes were labeled with reporter dye FAM at the 5’ end and the quencher dye TAMRA at the 3’ end (Microsynth, Balgach, Switzerland). Reactions were run in 96-well optical reaction plates using a Prism 7700 cycler (Applied Biosystems). Thermal cycles were set at 95°C (10 min) and then 40 cycles at 95°C (15 s) and 60°C (1 min) with auto ramp time. To analyze the data, the threshold was set to 0.06 (value in the linear range of amplification curves). All reactions were run in duplicate. The abundance of the target mRNAs was calculated relative to a reference mRNA. Assuming an efficiency value of two (degree of increase in input mRNA required to decrease the cycle number by 1),
relative expression ratios were calculated as \( R = 2^{\frac{1}{n}} C_i(\text{GAPDH}) - C_i(\text{test}) \), where \( C_i \) is the cycle number at the threshold, GAPDH is glyceraldehyde-3-phosphate dehydrogenase, and test stands for the tested mRNAs (7).

**Immunohistochemistry.** Male C57BL/6J mice (10–12 wk old) were anesthetized with ketamine and xylazine intraperitoneally and perfused through the left ventricle with PBS followed by a buffered paraformaldehyde solution (4%, pH 7). Kidneys and small intestine were removed, flushed with fixative solution, cut, and embedded in Tissue-Tek O.C.T Compound (Sakura Finetek Europe) just before freezing in liquid nitrogen. Frozen tissues were stored at −80°C. Serial sections of 5 μm were cut on a cryostat and collected on polylysine-coated slides (Kindler, Freiburg, Germany). Sections were incubated 5 min in 0.1% SDS, washed three times with PBS, and then incubated with the primary antibodies (diluted in PBS-0.04% Triton X-100) for 1 h at room temperature. Sections were then washed three times and incubated with the secondary antibody (diluted in PBS-0.04% Triton X-100) for 1 h at room temperature. After being washed five times, the sections were mounted using DAKO Mounting medium (DakoCytomation Denmark). Sections were viewed on a Leica SP1 UV CLSM confocal microscope (Leica Microsystems, Buffalo, New York). Images were further processed (merged) using Photoshop 7.

**Antibodies.** The following antibodies were used: rabbit-anti-mB0AT1 antibody [NH2-terminal; antigen peptide NH2-MVR LVL PNP GLE ERIC-CONH2 (16 amino acids); affinity purified antibody, dilution 1:200], rabbit-anti-mXT2 [NH2-terminal; antigen peptide NH2-MAQ ASG MDP LVD IED ERC-CONH2 (18 amino acids); affinity purified, dilution 1:200], rabbit-anti-mXT3 [NH2-terminal; antigen peptide NH2-MES PSA HAV SLP EDE ELC-CONH2 (18 amino acids); affinity purified, dilution 1:200], rabbit-anti-mXT3s1 [COOH-terminal; antigen peptide NH2-CIRN RLK RGG SAP VA-COOH (15 amino acids); affinity purified antibody, dilution 1:200], and goat-anti-m4F2hc (1:400; Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies were Alexa Fluor 594 donkey anti-rabbit IgG (1:1,000; Molecular Probes, Portland, OR) and Alexa Fluor 488 donkey anti-goat IgG (1:500; Molecular Probes). The secondary antibodies alone and antibodies preincubated with the specific peptides (1 h at room temperature) did not produce any significant staining in all tissues tested (data not shown).

**Western blotting.** Male C57BL/6J mice (10–12 wk old) were anesthetized with ketamine and xylazine intraperitoneally followed by cervical dislocation, and organs were rapidly harvested. The segment from duodenum to ileum was harvested and rinsed several times with ice-cold PBS (pH 7.4), and the mucosa cell layers were scraped off on ice-cold homogenization buffer (300 mM mannitol, 5 mM EGTA, and 12 mM Tris-HCl, pH 7.1) with protease inhibitor cocktail (Sigma-Aldrich) containing 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin, and aprotinin. Kidneys were harvested too. The samples were homogenized with a tip sonicator, 10 mM MgCl2 was added, and samples were kept on ice for 30 min. Samples were then centrifuged for 15 min at 900 g. The supernatant was then centrifuged at 12,000 g for 30 min at 4°C, and the pellet was resuspended in membrane buffer (300 mM mannitol and 20 mM HEPES-Tris, pH 7.4) containing protease inhibitor cocktail. After measurement of the total protein concentration (Bio-Rad protein kit; Bio-Rad, Hercules, CA), 20 μg brush-border membrane vesicles (BBMVs) were used for the deglycosylation reaction, as described below. Samples were solubilized in Laemmli sample buffer and separated on a 10% polyacrylamide gel. For immunoblotting, proteins were transferred electrophoretically from unstained gels to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA). After being blocked with 2% Top BLOCK powder (Juro, Lucerne, Switzerland) in Tris-buffered saline/0.1% Triton X-100 for 60 min at room temperature or overnight at 4°C, the blots were incubated with the primary antibodies for 3 h at room temperature or overnight at 4°C. The primary antibodies were the same affinity-purified rabbit antibodies as described above, and all were used at a 1:1,000 dilution. After being washed and subsequently blocked for 1 h at room temperature, blots were incubated with secondary antibody goat anti-rabbit conjugated with horseradish peroxidase (1:10,000; BD Transduction Laboratories, Lexington, KY) for 1 h at room temperature. Antibody binding was detected with the SuperSignal West Pico Substrate (Pierce Sochocim, Lausanne, Switzerland). Chemiluminescence was detected with a DIANA III camera (Raytest Schweiz, Dietikon, Switzerland). The specificity of the antibodies was tested using preincubation (1 h at room temperature) with the immunizing peptides (final concentration peptide: 200 μg/ml XT2 and 400 μg/ml B0AT1, XT3, and XT3s1).

**Endo-β-N-acetylglucosaminidase F assay.** Hydrolysis of Asn-oligosaccharides was carried out using endo-β-N-acetylglucosaminidase F enzyme (PNGase; New England Biolabs), Kidney or small intestine BBMVs (20 μg) from adult (10–14 wk old) C57BL/6J and NRM1 mice were the substrate for the enzyme. The samples were first heated at 65°C for 15 min in the provided buffer containing β-mercaptoethanol and SDS. The reaction mix was then incubated for 1 h at 37°C in the reaction buffer and 1% Nonidet P-40 in the presence or absence of the enzyme. The reaction mixture was run on a 10% SDS-polyacrylamide gel, and the Western blotting was performed as described above.

**RESULTS**

**Tissue mRNA distribution of B0AT-cluster transporters.** The relative abundance of mRNAs encoding the four related transporters B0AT1, XT2, XT3, and XT3s1/SIT1 was measured in several organs/tissues by real-time RT-PCR using GAPDH as internal standard. Figure 2 shows the relative abundances of these transcripts. A high expression level of all four mRNAs was found in kidney, relative to GAPDH ~10% for B0AT1, XT2, and XT3 and 1% for XT3s1. In small intestine mucosa, the level of B0AT1 mRNA was similar to that found in kidney, and XT3s1/SIT1 mRNA was abundant as well. In contrast, XT2 and XT3 mRNAs were 100- to 1,000-fold less abundant and displayed an axial gradient toward the ileum. In all tested tissues but muscle (and very low in heart), the transcript of at least one of these transporters was detected. Interestingly, the spleen that is not considered as an epithelial organ does express all four mRNAs to some extent. In the brain, XT3s1/SIT1 showed the strongest expression, whereas no XT2 mRNA was detected. In contrast to some of our negative data obtained by TaqMan real-time RT-PCR on tissues of two C57BL/6J mice, a recent publication showed qualitatively the expression of XT3 mRNA in mouse lung and of XT3s1/SIT1 mRNA in mouse muscle and heart (16). We have no explanation for this difference that could be of technical nature or due to the use of another mouse strain.

**Glycosylation of B0AT1 and related transporters.** B0AT1 displays five or six N-glycosylation consensus sites within its putative extracellular loops that are distant enough from the closest transmembrane segment to be potentially glycosylated (http://www.cbs.dtu.dk/services/NetOGlyc). In contrast, the three transporters XT2, XT3, and XT3s1/SIT1 display only two to three such potentially glycosylated consensus sites (2, 6, 19). Figure 3 shows the effect of the treatment of small intestine and kidney brush-border vesicle proteins with PNGase F, an enzyme that cleaves off high-mannose and terminally glycosylated N-glycans (8).
The unique strong band corresponding to B0AT1 migrates at the level of \( \sim 60 \) kDa, and deglycosylation shifts this band to \( \sim 48 \) kDa. This fast migration for a protein with a calculated molecular mass of 71 kDa could be because of its very hydrophobic nature. The shift in the relative migration induced by deglycosylation is compatible with the cleavage of four to six complex N-glycans. In the case of XT2, the fully glycosylated form migrates \( \sim 57 \) kDa and the deglycosylated one \( \sim 48 \) kDa (calculated mol mass 69 kDa), like that of B0AT1. Again, the apparent molecular mass of the deglycosylated form is compatible with the highly hydrophobic nature of the polypeptide, and that of the glycosylated form suggests, as expected, the presence of two or three complex N-glycans. The migration pattern of XT3 is more complex. There are two major bands, one migrating at \( \sim 62 \) kDa and another, of variable intensity between experiments, \( \sim 120 \) kDa. We do not know why deglycosylated XT3 migrates slower than B0AT1 and XT3s1/SIT (apparent mol mass of 62 vs. 48 kDa) and also apparently forms a dimer. A potential explanation for the slightly slower migration of XT3 could be a different posttranslational modification other than N-type glycosylation. Concerning the higher molecular mass band, the most likely hypothesis is that it corresponds to a homodimer that resists denaturation and reduction. Endo F treatment produced a shift of both bands, indicating the presence of N-glycans. In the case of XT3s1/SIT1, no such information could be obtained, since the antibody failed to recognize any specific band in the Western blot (data not shown).

**Axial and subcellular localization of B0AT-cluster transporters in kidney.** Immunohistochemistry showed that B0AT1, XT2, XT3, and XT3s1/SIT1 localize to the apical brush-border membrane of proximal tubule cells. No specific staining was observed in other nephron segments or structures. The axial distribution of the four proteins along the proximal tubule differs, as shown in Fig. 4. The heavy chain of the heterodimeric amino acid transporter 4F2hc (CD98) is shown in green in Fig. 4 as a basolateral marker (7, 26). B0AT1 is expressed mainly in the early proximal tubules (S1 segment) starting at the glomerulus. The distribution of XT2 along the proximal tubule is essentially restricted to the later segments S2 and S3 and thus quite complementary to that of B0AT1. XT3 and XT3s1/SIT1 were detected in the brush-border membrane all along the proximal tubule, their levels decreasing slightly toward the end (Fig. 4G). The axial distribution of the transporters (red) is clearly visible in the low-magnification pictures of consecutive mouse kidney sections (Fig. 4, A-D), whereas the characteristic brush-border membrane staining is appreciable in the high-magnification pictures (Fig. 4, E-H).

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**Fig. 3. Endoglycosidase assay on kidney and small intestine brush-border membrane vesicles (BBMVs).** Kidney and total intestine BBMVs (20 µg) were separated on a 10% polyacrylamide gel after treatment with (+) or without (−) endo-ß-N-acetylglucosaminidase F (Endo F) enzyme and submitted to Western blotting. XT2 and XT3 were not detected in intestine, whereas the anti-XT3s1/SIT1 antibody does not function in Western blotting. As expected from N-glycosylation site predictions, a shift of the specific band toward lower molecular masses was observed for B0AT1, XT2, and XT3. The particular migration of deglycosylated XT3 in comparison with deglycosylated B0AT1 and XT2 could be because of differential posttranslational modifications not investigated in this study.

**Fig. 2. Relative mRNA abundance of mouse (m) B0AT1, mXT2, mXT3, and mXT3s1/SIT1.** The abundance of mRNAs was measured by real time RT-PCR (see MATERIALS AND METHODS), and the level of the tested transporter mRNAs relative to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is indicated for each tissue. A relative abundance of 1 corresponds to the copy number of GAPDH mRNA, the expression of which was relatively homogenous within RNA of epithelial tissues [cycle no. to reach threshold (Ct) from 23.23 for kidney to 26.59 for colon] and other tissues (Ct 22.74 for heart to 28.81 for spleen). Significant expression of all four B0AT-cluster mRNAs was found in kidney and small intestine where B0AT1 and XT3s1/SIT1 mRNAs were the highest. XT3s1/SIT1 mRNA is also abundant in brain, testis, spleen, stomach, and lung. The mean relative mRNA abundance tested independently in two mice is shown. Values are expressed as means ± SE.

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**Fig. 4. Axial and subcellular localization of SLC6 transporters in kidney.** Immunohistochemistry showed that B0AT1, XT2, XT3, and XT3s1/SIT1 localize to the apical brush-border membrane of proximal tubule cells. No specific staining was observed in other nephron segments or structures. The axial distribution of the four proteins along the proximal tubule differs, as shown in Fig. 4. The heavy chain of the heterodimeric amino acid transporter 4F2hc (CD98) is shown in green in Fig. 4 as a basolateral marker (7, 26). B0AT1 is expressed mainly in the early proximal tubules (S1 segment) starting at the glomerulus. The distribution of XT2 along the proximal tubule is essentially restricted to the later segments S2 and S3 and thus quite complementary to that of B0AT1. XT3 and XT3s1/SIT1 were detected in the brush-border membrane all along the proximal tubule, their levels decreasing slightly toward the end (Fig. 4G). The axial distribution of the transporters (red) is clearly visible in the low-magnification pictures of consecutive mouse kidney sections (Fig. 4, A-D), whereas the characteristic brush-border membrane staining is appreciable in the high-magnification pictures (Fig. 4, E-H).
Segmental and subcellular localization of B₀AT-cluster transporters in small intestine. All along the mouse small intestine (duodenum, jejunum, and ileum), the affinity-purified anti-B₀AT1, anti-XT2, and anti-XT3s1/SIT1 antibodies showed a strong apical staining of the enterocytes lining the intestinal villi, whereas no signal was detected with the anti-XT3 antibody. The staining is shown in Fig. 5 for jejunum with the four B₀-cluster transporter antibodies (red channel) and anti-4F2hc (green channel) as the basolateral marker (7, 26). Mucus-secreting goblet cells did not show any basolateral or apical staining, indicating that these transporters are expressed only by the transporting enterocytes. The fact that staining was observed for XT2, whereas no specific staining was seen with the anti-XT3 antibody, is probably antibody specific. Indeed, the mRNA of these two gene products was of similar low abundance in small intestine, and for both proteins we did not obtain any signal on Western blots of small intestine brush-border membrane, unlike with kidney brush-border membranes where both were detected. As expected for proteins involved in the function of differentiated enterocytes, the signal showed a clear gradient along the villi, being low in the crypts and stronger toward the tips.

DISCUSSION

Cluster of three B₀AT-related transporters in human and of four in mice. XT2 and B₀AT1 genes are organized in tandem on chromosome 5p15 in human, the locus where the Hartnup disorder has been mapped (15, 28). In mouse, these genes are
located on chromosome 13 in a region that is syntenic to human 5p15. The degree of identity of these two gene products is 50–52%.

In human, there is a single XT3 gene (SLC6A20) located on chromosome 3p21.3 for which two splice variants have been detected. Surprisingly, the brain-specific isoform is predicted to lack one of the 12 putative transmembrane domains, whereas the isoform expressed in kidney has an overall domain structure similar to B0AT1 (13). In mouse and in rat, there are two corresponding genes organized in tandem on chromosome 9, XT3 and XT3s1, the latter encoding XT3s1/SIT1 (32). The identity between XT3 and XT3s1/SIT1 proteins is 90%, with complete identity over long stretches and large differences only in the NH₂ and COOH termini. XT3s1/SIT1 shares a higher degree of identity (91%) with the two isoforms of human XT3 compared with mouse XT3, which is only 86% identical to human XT3. It appears thus that the mouse XT3 gene is the product of a relatively recent duplication and that mouse XT3s1/SIT1 more closely resembles human XT3 (14).

Localization data presented in this paper suggest that mouse XT3s1/SIT1 has a broader expression pattern than mouse XT3. Similarly, expressed sequence tag (EST) databases reveal that
rat SIT1 is also widely distributed, as pointed out in the publication on rat SIT1 (32). This rat gene, previously referred to as XT3 or rB21a, is in reality the ortholog of mouse XT3s1/SIT1 (32). In situ hybridization in mouse and in rat did not distinguish between the two gene products and revealed their localization in brain, kidney, and small intestine (16, 32). Their actual differential localization can be appreciated in Fig. 2.

**Tissue localization of the B′AT-cluster transporters.** The B′AT-cluster transporters are mainly expressed in tissues that contain epithelia. They are all abundantly expressed in kidney, and they are also present in small intestine where B′AT1 and XT3s1/SIT1 mRNAs are 100- to 1,000-fold more abundant than those of XT2 and XT3. Immunofluorescence experiments in kidney and small intestine show that all four transporters localize to the brush-border membrane of epithelial cells, a localization that is compatible with a role in apical uptake of solutes, for instance, amino acids in the case of B′AT1 and imino acids in the case of XT3s1/SIT1. This localization agrees with the known function of B′AT1 that is defective in Hartnup disorder (4, 15, 28) and with that of XT3s1/SIT1 that was shown in *Xenopus* oocytes to mediate a transport corresponding to that of a brush-border imino system (16, 32).

It is noteworthy that the two other luminal transporters did not produce, in the same expression conditions, any reproducible amino acid transport. Interestingly, these transporters (XT2 and XT3) appeared on immunofluorescence images not to localize to the surface of *Xenopus* oocytes (data not shown). From their high level of similarity with B′AT1 and XT3s1/SIT1, respectively, one can suggest the hypothesis that XT2 and XT3 might exert similar but complementary functions.

**Differential localization of the B′AT-cluster transporters along the kidney proximal tubule.** B′AT1 localizes to the early part of the proximal tubule (S1 and S2 segments), very similarly to B0−AT1, the catalytic subunit of the apical cystinuria transporter, and to the basolateral heterodimeric transporters y′LAT1- and LAT2-4F2hc that are known to play an important role in the basolateral export of amino acids (1, 3, 7, 9, 22, 34, 35). The common S1−S2− localization of these transporters suggests that they are part of a high-capacity amino acid transport machinery. In contrast, XT2, the “tandem” transporter of B′AT1, localizes also to the brush-border membrane of the proximal tubule, but with a complementary axial distribution, namely mainly in S1, the late segment of the proximal tubule. This differential and complementary axial localization of related transporters along the proximal tubule resembles that of the two Na +−glucose cotransporters (SGLT1 and -2) and of the two proton-driven peptide transporters (PEPT1 and -2) where in both cases the low affinity form localizes to the early part and the high affinity form to the late part of the proximal tubule (12, 17, 18, 21, 23, 29, 31, 37). As in these cases, B′AT1 that localizes to the early part of the proximal tubule displays a relatively low apparent affinity with a K0.5 of ~1 mM for its best amino acid substrates (5). However, the function of its potentially “complementary” transporter XT2 is not yet clear, although it has been suggested based on the characterization of a knockout mouse that it is a high-affinity glycine transporter (25). Interestingly, from these two transporters, it is the low-affinity B0−AT1 that is more abundant in small intestine, similar to the situation of PEPT1 (17).

In contrast, the axial distribution of the tandem transporters XT3s1/SIT1 and XT3 overlaps entirely along the kidney proximal tubule, whereas, in small intestine, only XT3s1/SIT1 is strongly expressed, both at the mRNA and protein levels. The localization of the XT3s1/SIT1 mRNA in the pia mater and plexus choroideus suggests that this transporter is also involved in t-proline transport in and out of the brain (16).

In conclusion, the transporters B′AT1, XT2, XT3, and XT3s1/SIT1 form a phylogenetic cluster within the SLC6 family. Besides their protein sequence similarity, they share brush-border localization in the proximal kidney tubule and are, as shown for B′AT1 and XT3s1/SIT1, Na +−dependent transporters of amino/imino acids. From this group of transporters, the two functionally characterized ones are also the ones that are highly expressed in small intestine. All tissues expressing B′AT-cluster members, with the exception of spleen, serve trans-epithelial/endothelial transport functions. Thus the localization and the structural similarity of the pairs B′AT-XT2 and XT3s1/SIT1-XT3 together suggest the hypothesis that the orphan transporters XT2 and mouse XT3 possibly exert epithelial amino- and imino(amon amino) acid transports that could be complementary to the function of B′AT1 and XT3s1/SIT1, respectively.

**ACKNOWLEDGMENTS**

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**GRANTS**

This project was supported by Swiss National Science Foundation Grants 31–95141.99/02 and 31–108021/1 to F. Verrey and the European Sixth Framework Programme (FP6) project EUGINDAT to F. Verrey and C. A. Wagner.

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