Nephrol Dial Transplant (2007) 22: 2449–2451
doi:10.1093/ndt/gfm214
Advance Access publication 3 May 2007

Translational Nephrology

NDT
Nephrology Dialysis Transplantation

Does kidney amino acid transport have something to do with blood pressure?*

François Verrey

Institute of Physiology and Centre for Integrative Human Physiology (ZIHP), University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Keywords: Amino acid transport; Angiotensin-converting enzyme; B^0AT1; Blood pressure; cotransporter; kidney proximal tubule; SLC6A19; TMEM27

A paper recently published in *Nature* by Danilczyk *et al*. shows that collectrin (Tmem27), a homologue of angiotensin-converting enzyme 2 (ACE2), surprisingly plays a central role in kidney amino acid transport [1]. This was discovered when the phenotype of a collectrin knock out mouse, generated in the laboratory of Josef Penninger, was analysed. The deficiency of this relatively short type I transmembrane protein causes a massive urinary loss of neutral amino acids (NAA) that even leads to the formation of urinary amino acid crystals. This was shown to be the consequence of a major impairment of amino acid reabsorption, due to the fact that collectrin is necessary for the functional expression of major Na^+–amino acid cotransporters of kidney proximal tubule. The analysis of another collectrin knock out mouse, published in the *American Journal of Physiology*: Renal Physiology by Malakauskas *et al*. [2], confirmed that the lack of collectrin leads to a massive urinary amino acids loss. A central question remains open, namely whether there is a functionally understandable rationale for the fact that collectrin, a protein that shares ~50% identical amino acid residues with the membrane anchor region of ACE2, controls kidney amino acid transport [3].

Amino acid reabsorption in proximal kidney tubule

Similar to glucose, plasma free amino acids are reabsorbed in the proximal kidney tubule in such a way that urinary loss of amino acids is prevented and thereby also osmotic diuresis, as demonstrated in both collectrin knock out papers [1,2]. Compared with glucose reabsorption, the different side chains of amino acids represent a complication, making the presence of several transporters necessary.

Transepithelial reabsorption of NAA involves their active import from the primary urine into the proximal tubule epithelial cells via secondary active transporters (Fig. 1) and then their passive basolateral efflux via facilitated diffusion and/or obligatory exchange [4]. The active luminal import of NAA is mostly driven by the cotransport of Na^+ with the exception of a part of the imino acid and small L- and D-amino acid import that is driven by H^+-cotransport via PAT1 (SLC36A1, imino acid carrier).

The major broad-selectivity NAA-sodium cotransporter (symporter) is called B^0AT1 (SLC6A19) and was identified in 2004 in mouse kidney by Broer *et al*. [5]. The mutation of its human orthologue was shown to cause a broad neutral aminoaciduria condition called Hartnup disorder, which is sometimes accompanied by pellagra-like symptoms [6,7]. This transporter has been shown to localize mainly to the early segment (S1) of the proximal kidney tubule, similar to the luminal cystine and cationic amino acid transporter $b^{0,+}AT1$-rBAT and to the basolateral efflux transporters of the so-called transepithelial amino acid transport machinery [4,8,9].

The SLC6 Na^+–amino acid cotransporters of the kidney

The Hartnup transporter B^0AT1 belongs to the gene family SLC6, to which numerous other transporters belong, in particular the neurotransmitter re-uptake

Correspondence and offprint requests to: François Verrey, Institute of Physiology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. Email: verrey@access.uzh.ch
*Comment on Danilczyk U, Sarao R, Remy C *et al*. Essential role for collectrin in renal amino acid transport. *Nature* 2006; 444: 1088–1091.

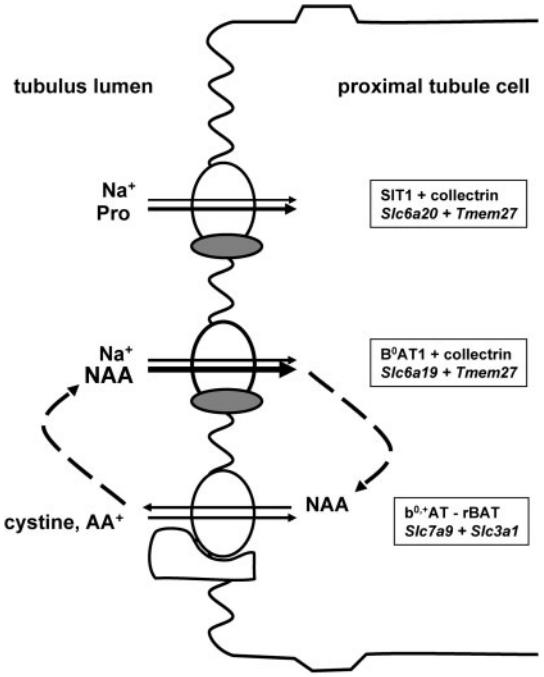

Fig. 1. The ACE2 homologue collectrin (grey oval structures) is associated with luminal SLC6 Na^+–amino cotransporters of kidney proximal tubule (B^0AT1 and SIT1). Recycling of NAA allowing the influx of cystine and cationic amino acids (AA^+) via the heteromeric exchanger $b^{0,+}AT$-rBAT is indicated with dashed arrows.

transporters for norepinephrine, serotonin, dopamine and GABA [10]. Three members of this family (four in mice) are expressed at the luminal brush border membrane of kidney proximal tubule: the earlier mentioned broad NAA transporter B^0AT1 (SLC6A19), the 'system IMINO' L-proline transporter SIT1 (XT3s1, SLC6A20) and the putative amino acid transporter XT2 (SLC6A18) [6,8,11,12]. Functionally, B^0AT1 and SIT1 have been characterized in the Xenopus laevis expression system [4,12–14]. Interestingly, whereas many transporters of the SLC6 family have been shown to cotransport Na^+ and Cl^- with their organic substrate, the reason for which this SLC6 family was first called the Na^+ and Cl^--dependent neurotransmitter transporter family, both B^0AT1 and SIT1 were shown to cotransport only Na^+, such that for each transported amino acid, one positive charge is translocated. The absence of Cl^- was shown nonetheless to decrease the efficiency of these transporters, suggesting that Cl^- might bind to the transporter but is not translocated. The transport function of XT2 has not yet been formally demonstrated, but based on observations made in a knock out mouse, it appears that XT2 functions as an amino acid transporter as well [11]. The axial distribution of the three luminal SLC6 transporters along the proximal kidney tubule is differential. Whereas B^0AT1 localizes essentially to the early S1 segment, SIT1 is expressed all along the proximal tubule and XT2 shows a localization that is complementary to that of B^0AT1, namely along the later proximal tubule segments S2 and S3.

Interestingly, it appears that it is mainly the expression of the three SLC6 Na^+–cotransporters B^0AT1, SIT1 and XT2 that is decreased in the proximal tubule of collectrin-deficient mice, whereas the expression of the cystinuria tranporter $b^{0,+}AT$-rBAT and of the anionic amino acid transporter EAAT3 (EAAC1) is not much affected [1]. The presented data also suggests that the three transporters are physically associated with collectrin, since this latter protein was co-immunoprecipitated with the three of them. Furthermore, immunofluorescence images confirmed that the localization of collectrin in the proximal tubule brush border corresponds to the localization of these three transporters [1,8]. It will thus be of interest to investigate when and where the interaction of collectrin with these transporters takes place during their biosynthesis and maturation. Is this interaction necessary for their exit from the endoplasmatic reticulum? Does their maturation in the Golgi apparatus and their targeting to the luminal surface require this association?

Comparison with heteromeric amino acid transporters and open questions

The interaction of an amino acid transporter with an 'accessory' protein subunit, functioning as a chaperone has been described previously in the case of heteromeric amino acid transporters, some of which are also part of the proximal kidney tubule amino acid transport machinery [4]. For instance, the luminal cystine and cationic amino acid transporter, the defect of which leads to cystinuria, is a heteromer composed of $b^{0,+}AT$ (SLC7A9) and rBAT (SLC3A1) (Fig. 1) [15]. The two basolateral amino acid exchangers LAT2 (SLC7A8) and y^+LAT1 (SLC7A7) also belong to this heteromeric transporter family, but are associated with the other SLC3 glycoprotein 4F2hc (SLC3A2, CD98) [4,15]. Unlike the newly described association of the luminal SLC6 Na^+–amino acid cotransporters with collectrin, heteromeric amino acid transporters are bound covalently to their associated glycoprotein subunit via a disulfide bridge. In this latter case, it has been demonstrated that the association of the subunits takes place before their terminal glycosylation, presumably in the endoplasmatic reticulum, and that this association is a prerequisite for the surface expression of the catalytic subunits [16]. In the case of the glycoprotein subunit rBAT (SLC3A1), its association to the catalytic subunit appears to be a prerequisite for its maturation and surface expression as well [17].

An intriguing observation is that collectrin is not expressed at a substantial level in small intestine, the other tubular organ in which the same SLC6 Na^+–amino acid cotransporters are expressed [1]. Based on the observation that collectrin is required for their

efficient expression in kidney proximal tubule brush border membrane, it can be anticipated that yet another protein may replace collectrin as associated protein for the luminal SLC6A18-20 amino acid transporters in small intestine. Actually, the fact that these three SLC6 amino acid transporters are not completely absent from the kidney proximal tubule of collectrin-deficient mice may suggest that this putative other associated protein is expressed to some extent in the kidney proximal tubule as well.

The question is open as to whether some of the Hartnup-type broad neutral aminoacidurias that are not due to a defect in SLC6A19 could be caused by mutations of collectrin. Such a possibility is compatible with the fact that collectrin knock out mice have no obvious other pathological phenotype. However, whether a collectrin defect has the same effect on urinary amino acid transport in humans is yet to be determined. Furthermore, other potentially important functions of this intriguing protein also need to be investigated in more detail, in particular in view of its potential role in pancreatic β-cells [18,19].

Taken together, the defect of the ACE2 homologous protein collectrin induces a massive neutral aminoaciduria in mice. The qualitative similarity of this aminoaciduria with that of Hartnup patients is not surprising, in view of the observation that the expression of the Hartnup transporter B^0AT1 (SLC6A19) and of the two other SLC6 kidney proximal tubule Na^+–amino acid cotransporters SLC6A18 and 20 is strongly decreased in collectrin knock out mice. However, despite the homology of collectrin with ACE2 and its localization to kidney tubule, as yet no role of this membrane protein in blood pressure control has been detected (Singer and Verrey, unpublished results) [1,2].

Conflict of interest statement. None declared.

References

1. Danilczyk U, Sarao R, Remy C *et al*. Essential role for collectrin in renal amino acid transport. *Nature* 2006; 444: 1088–1091
2. Malakauskas SM, Quan H, Fields TA *et al*. Aminoaciduria and altered renal expression of luminal amino acid transporters in mice lacking novel gene collectrin. *Am J Physiol Renal Physiol* 2007; 292: F533–F544
3. Zhang H, Wada J, Hida K *et al*. Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. *J Biol Chem* 2001; 276: 17132–17139
4. Verrey F, Ristic Z, Romeo E *et al*. Novel renal amino acid transporters. *Ann Rev Physiol* 2005; 67: 557–572
5. Broer A, Klingel K, Kowalczuk S *et al*. Molecular cloning of mouse amino acid transport system B^0, a neutral amino acid transporter related to Hartnup disorder. *J Biol Chem* 2004; 279: 24467–24476
6. Kleta R, Romeo E, Ristic Z *et al*. Mutations in SLC6A19, encoding B^0AT1, cause Hartnup disorder. *Nat Genet* 2004; 36: 999–1002
7. Seow HF, Broer S, Broer A *et al*. Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. *Nat Genet* 2004; 36: 1003–1007
8. Romeo E, Dave MH, Bacic D *et al*. Luminal kidney and intestine SLC6 amino acid transporters of B^0AT-cluster and their tissue distribution in Mus musculus. *Am J Physiol Renal Physiol* 2006; 290: F376–F383
9. Bauch C, Forster N, Loffing-Cueni D *et al*. Functional cooperation of epithelial heteromeric amino Acid transporters expressed in madin-darby canine kidney cells. *J Biol Chem* 2003; 278: 1316–1322
10. Chen NH, Reith ME, Quick MW. Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. *Pflugers Arch* 2004; 447: 519–531
11. Quan H, Athirakul K, Wetsel WC *et al*. Hypertension and impaired glycine handling in mice lacking the orphan transporter XT2. *Mol Cell Biol* 2004; 24: 4166–4173
12. Takanaga H, Mackenzie B, Suzuki Y *et al*. Identification of mammalian proline transporter SIT1 (SLC6A20) with characteristics of classical system imino. *J Biol Chem* 2005; 280: 8974–8984
13. Bohmer C, Broer A, Munzinger M *et al*. Characterization of mouse amino acid transporter B^0AT1 (slc6a19). *Biochem J* 2005; 389: 745–751
14. Kowalczuk S, Broer A, Munzinger M *et al*. Molecular cloning of the mouse IMINO system: an Na^+- and Cl^--dependent proline transporter. *Biochem J* 2005; 386: 417–422
15. Palacin M, Borsani G, Sebastio G. The molecular bases of cystinuria and lysinuric protein intolerance. *Curr Opin Genet Dev* 2001; 11: 328–335
16. Mastroberardino L, Spindler B, Pfeiffer R *et al*. Amino acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature* 1998; 395: 288–291
17. Bauch C, Verrey F. Apical heterodimeric cystine and cationic amino acid transporter expressed in MDCK cells. *Am J Physiol Renal Physiol* 2002; 283: F181–F189
18. Fukui K, Yang Q, Cao Y *et al*. The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. *Cell Metab* 2005; 2: 373–384
19. Akpinar P, Kuwajima S, Krutzfeldt J *et al*. Tmem27: a cleaved and shed plasma membrane protein that stimulates pancreatic beta cell proliferation. *Cell Metab* 2005; 2: 385–397

Received for publication: 23.2.07
Accepted in revised form: 19.3.07