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Regulation of renal amino acid (AA) transport by hormones, drugs and xenobiotics – a review

Review Article

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Summary. Major advances have recently been made in our understanding of the mechanisms and functions of amino acid transport in mammalian cells: – from the whole organism to transporter molecular structure. In this article, we present a brief overview of current knowledge concerning amino acid transporters, followed by a detailed discussion of the relevance of this new information to our broader understanding of the physiological regulation of amino acid handling in the kidney. We focus especially on the influence of hormones and xenobiotics on renal amino acid transport systems. In a growing number of cases, it now seems possible to correlate the effects of hormones, drugs, and xenobiotics with the capacity of renal amino acid transporters. This topic is of clinical relevance for the treatment of many amino acid reabsorption disorders. For example, under suitable conditions glucocorticoids and thyroid hormones stimulate renal reabsorption of amino acids and might therefore be of benefit in the treatment of different kinds of aminoaciduria. Hormonal regulation also underlies the postnatal development of renal amino acid reabsorption capacity, which can be stimulated to mature earlier after exogenous administration of e.g. glucocorticoids. In contrast, many compounds (e.g. heavy metal complexes) selectively damage renal amino acid transporters resulting in urinary amino acid loss. These types of phenomena (stimulation or inhibition of amino acid transporters in the kidney) are reviewed from the perspectives of our new molecular understanding of transport processes and of clinical relevance.

Keywords: Amino acids – Transport – Kidney – Hormones – Xenobiotics – Stimulation – Inhibition

1. Introduction

The recent rapid increase in novel findings in the field of amino acid (AA) transport is impressive (Wagner et al., 2001; Zorzano et al., 2000). We want to contribute to this developing story, but by focusing mainly on the influence of hormones, drugs and xenobiotics on renal AA transport systems (see Gonska et al., 2000).

AA transport across cellular membranes is mediated by multiple transporters with overlapping specificities, such that transport of an individual AA across a given membrane may depend on the integrated activity of several parallel-functioning (co-)transporters and exchangers. For example, there is evidence for the presence of at least five different transport systems in animal cells which are involved in the cellular uptake of cationic AAs (Christensen, 1989; Van Winkle, 1993; Kilberg et al., 1993; McGivan and Pastor-Anglada, 1994). The "classical" System-based classification of AA transport mechanisms based on functional similarities is now being superseded by classification based on similarities in transporter protein structure, although in most cases the Systems can be readily identified with a particular transporter gene family. Since 1990, cDNAs have been isolated for over 30 mammalian AA transporters. Several recent reviews (e.g. Palacin et al., 1998a, b; Verrey et al., 2000; Zorzano et al., 2000; Bode, 2001) describe in detail the tissue expression, transport characteristics, structure-function relationships and the putative physiological roles of these transporters. Structurally related trans-

Table 1. The currently known families of plasma membrane AA transporters

Family	Acronym	Full name (other names)	Na^+-dependent	Transporter type
Glycoprotein-associated AA transporters (gpaAT)	LAT1, 2	System L transporter (4F2 heteromer)	–	L
	y + LAT1, 2	System y+L transporter (4F2 heteromer)	−/+†	y^+L
	xCT	Cystine-glutamate exchanger (4F2 heteromer)	–	x^-_C
	Asc-1	System asc transporter (4F2 heteromer)	–	asc
	b^{o+}AT	b^{o+} amino acid transporter (rBAT heteromer)	–	b^{o+}
Cationic AA transporters	CAT1–4	Cationic amino acid transporter 1, 2A/B, 3	−/+†	y^+
Excitatory AA transporters	EAAT1–5	Excitatory amino acid transporter 1–5(GLAST, GLT-1)	+(K^+ exchange)	X^-_{AG}
	ASCT1, 2	ASC transporter	+	ASC
	ATB^o	B^o amino acid transporter	+	B^o
Neutral AA transporters	SN1, 2	System N transporter	+(H^+ exchange)	N
	SAT1-3	System A transporter (ATA1-3; SA1-3)	+	A
Monocarboxylate transporters	TAT1	System T transporter	–	T
Neurotransmitter transporters	GAT1-3	GABA transporter	+(+Cl^-)	GABA
	GLYT1, 2	Glycine transporter	+(+Cl^-)	Gly
	PROT etc.	Proline/betaine transporters	+(+Cl^-)	Pro
	$ATB^{o,+}$	$B^{o,+}$ amino acid transporter	+(+Cl^-)	$B^{o,+}$

† Transports neutral amino acid substrates Na^+-dependently and cationic amino acids Na^+-independently

porters have been grouped into one of (currently) six distinct gene families, which are summarised alongside their functional correlates in Table 1. In this paper, we will first review the physiological properties of renal AA transport then attempt to correlate these with patterns of expression and function of cloned transporters where possible (noting areas where gaps in our knowledge are the greatest), before proceeding to examine regulatory properties in the light of recent advances.

2. Functional and physiological properties of renal AA transport systems

The pattern of renal AA reabsorption and excretion principally depends on the filtered load (i.e., on plasma AA concentration and glomerular filtration rate (GFR)) and also on the characteristics of tubular reabsorptive (or secretory) mechanisms. Sodium dependent AA transporters play the main role in renal AA reabsorption (Palacin, 1994). Na^+-dependent AA transport systems occur mainly at the apical (brush border or luminal) membrane of the renal epithelium, whereas passive facilitated transport and simple diffusion occur at both at apical and basolateral membranes. The integrated activity of these polarised

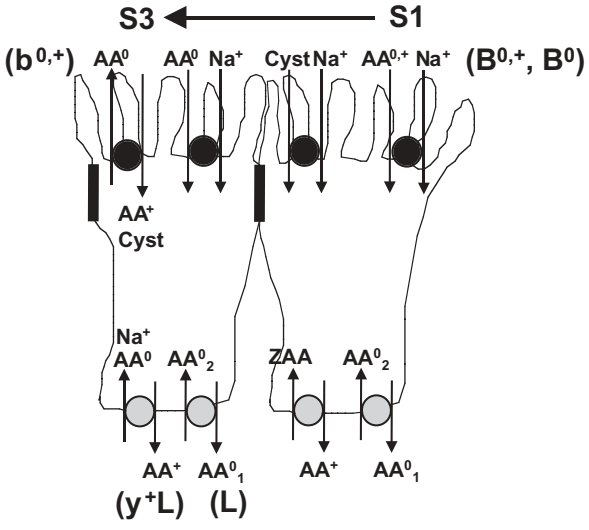

Fig. 1. Polarised localisation of AA transport systems in renal epithelium and mechanisms of transepithelial reabsorption. *ZAA*-Zwitterionic AA. *S1*, *S3* refer to renal proximal tubule regions

mechanisms facilitates transepithelial transport of AAs from renal lumen to blood (see e.g. Fig. 1). The apical uptake of most AAs occurs by Na^+-AA cotransport driven by both the chemical and voltage components of the electrochemical Na^+ gradient from ultrafiltrate into the proximal tubular cell (Sacktor and

Kinsella, 1985). In mammals no uphill transport of AAs from blood to lumen has been observed (Gekle and Silbernagl, 1991).

2.1. The proximal tubule

Most AAs filtered at the glomerulus are reabsorbed in the early proximal tubule; the endproximal fractional reabsorption amounts to more than 96% (Silbernagl, 1992; Gloy et al., 2000). We list below the battery of AA transport systems reported in tubular cells and link these where possible to expression of transporter genes described in Section 3 and summarised in Table 1.

2.1.1. System A

System A transport is Na^+-dependent with broad substrate selectivity for the smaller neutral AAs (Christensen and Liang, 1965; Wang et al., 2000; Freeman and Mailliard, 2000) and tolerates N-methylated AA substrates. System A properties are similar in a variety of cell types (McGivan and Pastor-Anglada, 1994; Ling et al., 2001). N-methylaminoisobutyric acid (MeAIB) is used extensively as a non-metabolisable System A-specific model substrate. System A emerges on AA deprivation (Felipe et al., 1992) and is also induced by hyperosmotic shock following AA deprivation (Soler et al., 1993); indeed System A has become the "classic" adaptive AA transport system (Woodlock et al., 1989; Kilberg et al., 1993; Shotwell et al., 1983, AvilaChavez et al., 1997; Alfieri et al., 2001). A survey of the AA transport systems in the renal cortex showed that the majority of System A-mediated AA transport was present in membranes of possibly apical origin (Hensley and Mircheff, 1994). System A is crucial for maintaining a high concentration of organic osmolytes inside the cell and is thus involved in cell volume control (Bode and Kilberg, 1991; Hediger, 1994; Hediger et al., 1995), conceivably in renal epithelial cells. System A transport is now known to be mediated through expression of the SAT genes (SAT1-3) as described in 3.4.

2.1.2. System ASC

System ASC mediates Na^+-dependent transport of small neutral AAs such as Ala, Ser and Cys (Christensen et al., 1994) but does not interact with MeAIB. In many types of mammalian cell, the basal Na^+-dependent uptake for small neutral AAs is mediated by System ASC. System ASC activity has been detected in basolateral membranes of the kidney, where its function is believed to be the transport of AAs from blood into epithelial cells to nourish these cells (Stevens et al., 1984). System asc has a comparable substrate specificity to ASC, but is Na^+ independent (Fukasawa et al., 2000: Verrey et al., 2000). System ASC transport is effected by expression of the ASCT genes (ASCT1-2) as described in 3.1. ASCT transporters are, perhaps surprisingly, members of a completely different gene families than either SAT transporters or the Asc-1 transporter producing System asc activity (see Section 3.2).

2.1.3. System B^o

Na^+-dependent System B^o appears to be the main neutral AA transporter of renal brush border membranes and is characterized by an unusually broad specificity for AA substrates including branched chain and aromatic AAs as well as small hydrophilic AAs such as alanine and glutamine (Doyle and McGivan, 1992; Lynch and McGivan, 1987; Stevens et al., 1984; Stevens, 1992). System B (formerly NBB) does not accept N-methyl AAs such as MeAIB (Stevens, 1992) and is not inhibited by N-ethylmaleimide (Lynch and McGivan, 1987). Examination of the transport characteristics of the alanine analogue AIB in cultures from the proximal tubule of human adults showed that Na^+-gradient dependent influx of AIB occurred by a single, saturable transport system. All neutral AAs reduced uptake of AIB but there was no effect of taurine, aspartic acid, and arginine (Jessen et al., 1996). Cleavage of aminopeptidase N (Kenny and Maroux, 1982) from the membranes of solubilized bovine renal brush-border membrane vesicles inhibited Na^+-dependent AA transport activity. Removal of aminopeptidase increased the K_m values for the Na^+-dependent AA transport without affecting V_{max} (Plakidou-Dymock et al., 1993). These results suggest that the AA-binding sites of aminopeptidase N and the transporter interact in some way. A System B^o transport activity is produced by expression of the ATB^o gene from the same superfamily as the ASCT genes.

2.1.4. System $B^{o,+}$

System $B^{o,+}$, described in blastocysts and epithelial membranes, transports cationic and neutral AAs Na^+-

dependently (Christensen, 1990). The system also transports certain β-AAs (Van Winkle, 1993). System $B^{o,+}$ is developmentally regulated in blastocysts (Van Winkle, 2001). A System $B^{o,+}$ transport activity is produced by expression of the $ATB^{o,+}$ gene, which turns out to derive from a completely different gene family to the functionally similar ATB^{o} (see Section 3).

2.1.5. System N

Classical System N mediates Na^+-dependent transport of AAs containing nitrogen atoms in the side-chain but which bear no net charge at the prevailing pH (gln, asn, his) (Christensen, 1990). AA transport activities expressed in the MDCK renal cell line include System N (Boerner and Saier, 1985). System N transport is now known to be mediated through expression of the SN1-2 genes from the same family as SAT1-3 (as described in 3.1).

2.1.6. System L

Na^+-independent System L with broad substrate selectivity for zwitterionic AA contributes to renal apical membrane neutral AA transport (Stevens et al., 1984; Stevens, 1992). System L shows preference for the bulky branched chain and aromatic AAs. Other special features of this system include trans-stimulation and H^+-dependent modulation (Segel et al., 1988; Christensen, 1990; Woodlock et al., 1997). Two populations of rabbit proximal tubular cells were identified as having a predominantly basolateral phenotype (Hensley and Mircheff, 1994) – 82% of the total alanine transport in these populations was mediated by a system similar to the classical System L. The functionally similar System T selects for benzenoid substrates – it is not expressed in rat but in human kidney (Kim et al., 2002). System L transport is now known to be mediated through expression of heteromeric transporters with catalytic subunits from the glycoprotein-associated AA transporter (gpaAT) family, termed LAT1 and LAT2 (as described in 3.2). A recently-identified System T transporter (TAT1) is from a gene family distinct to the gpaATs (see Table 1).

2.1.7. System $b^{o,+}$

Originally described in mouse blastocysts (Van Winkle, 1988, 1993) for the transport of cationic and zwitterionic AAs, System $b^{o,+}$ prefers larger substrates that do not branch at the α or β positions and is now known to transport cystine with high-affinity. In the kidney, a high-affinity system for cystine uptake is located in proximal straight (S3) tubule (Völkl and Silbernagl, 1982; Schafer and Watkins, 1984). Schafer and Watkins (1984) showed that this $b^{o,+}$-like System in renal tubules is a tightly coupled exchanger with 1 : 1 stoichiometry. This AA brush border resorption mechanism shows high-affinity for cystine and cationic AAs. Defects in the System $b^{o,+}$ transport mechanism are responsible for classical cystinuria (Scriver, 1986). In contrast, the cystine reabsorption mechanism in the proximal convoluted tubule (S1, 2) was found to have a low affinity but high capacity and the fractional reabsorption rate of cystine was decreased only by methionine (other AAs had no effect). The overall fractional reabsorption rate of cysteine was reduced significantly by e.g. alanine, methionine, citrulline, AIB, phenylalanine, and cycloleucine. It was concluded that, at least in the convoluted tubule, cysteine shares a transport system with other neutral AAs which is not identical with the reabsorption mechanism for cystine. Reabsorption of cystine and cysteine occurs also in a tubular section between late proximal and early distal sites (Völkl and Silbernagl, 1982). The driving force for the reabsorption of cystine is provided by intracellular reduction to cysteine that exits the cell by a basolateral transport system (Silbernagl, 1988).

System $b^{o,+}$ transport is mediated through expression of a heteromeric transporter with a catalytic subunit from the glycoprotein-associated AA transporter (gpaAT) family, in this case termed $b^{o,+}AT$ (as described in 3.2). The low affinity cystine transporter in the S1-2 tubule has not been identified conclusively, but it is conceivable that the transport occurs at least partly through activity of System X_{ag}^- (see below), which in some circumstances may accept cystine as a substrate (see Section 3).

2.1.8. System y^+

Cationic AAs are transported by System y^+ (Christensen, 1984; Kim et al., 1991; Wang et al., 1991; Kavanaugh, 1993). System y^+ also binds some neutral AAs with lower affinity in the presence of Na^+. The discovery of other transporters for cationic AA (e.g. Systems $b^{o,+}$ and y^+L) leads to the suggestion that some properties, originally attributed to System y^+,

may relate to other transport systems. The specificity of System y^+ was shown to be clearly distinct from that of System y^+L, a cationic AA transporter that accepts neutral AAs with high affinity in the presence of Na^+ and which influenced the classical definition of System y^+ (Rojas and Deves, 1999). Both types of transport activity appear to be expressed in renal plasma membranes. System y^+ transport is mediated by the CAT family of cationic AA transporters (see Section 3.2).

2.1.9. System y^+L

System y^+L is a broad scope AA transporter which was first identified in human erythrocytes. It can bind cationic and neutral AAs and its specificity varies depending on the ionic composition (Deves et al., 1992, 1993; Eleno et al., 1994; Deves and Boyd, 1998; Boyd et al., 2000). System y^+L is a relatively recent addition to the group of transport systems involved in the cellular uptake of cationic AAs in mammalian cells. Leucine transport is predominantly Na^+-dependent, whereas arginine transport is Na^+-independent. Basolateral exchanges of zwitterionic and cationic AAs between epithelial cells and the blood occur through System y^+L (Stevens, 1992; Boyd et al., 2000), enabling net transfer of cationic AAs into the blood against the prevailing electrical gradient. Depolarization of the OK renal cell line in response to phenylalanine and arginine superfusion showed System y^+L-like properties, being stereoselective, dose-dependent, saturable and sodium-dependent for phenylalanine, but sodium-independent for arginine (Schwegler et al., 1989). Defects in System y^+L transport are responsible for lysinuric protein intolerance (LPI; Torrents et al., 1999). System y^+L transport is mediated through expression of heteromeric transporters with catalytic subunits from the glycoprotein-associated AA transporter (gpaAT) family, in this case termed y^+LAT1 or 2 (as described in 3.2).

2.1.10. System X_{ag}^-

In 1998 Silbernagl et al. reported about the compartmentation of AAs in the rat kidney. They concluded that L- and D-aspartate, glutamate, cysteate and, to a much smaller extent, γ-carboxyglutamate, are accepted by a tubular reabsorption mechanism highly specific for "acidic" AAs. N-methylation, amidation of the β- or γ-carboxyl group or the removal of the α-amino moiety almost completely abolish the ability of such compounds to be reabsorbed via this carrier. The resorption of glutamate, but not of aspartate, is highly stereospecific. Studies originally on human fibroblasts described such a type of high-affinity, sodium dependent uptake system which was named X_{ag}^- (Dall'Asta et al., 2000). System X_{ag}^- is mainly found in brain and epithelial tissues (Lerner, 1987; Castagna et al., 1997). Systems ASC, L or A may also be involved in uptake of glutamate (Igo and Ash, 1995). Cystine is a low-affinity substrate for some glutamate transporters, so system X_{ag}^- is perhaps involved in cystine reabsorption in early renal proximal tubule. Glutamate and aspartate are quickly reabsorbed in early parts of the proximal convoluted tubule and saturation already occurs when there is a small increase in the filtered load. The nephron section between the late proximal and early distal nephron sites also reabsorbs "acidic" amino acids. Normally, however, the back leak cancels this out, and net flux becomes zero (Silbernagl, 1983). System X_{ag}^- transport is now well recognised to be mediated through expression of members of the excitatory amino acid transporter (EAAT) gene superfamily, which also includes the ASCT and ATB^o transporters (as described in 3.1).

2.1.11. System x_c^-

In addition to System $b^{o,+}$, cell types such as hepatocytes transport the anionic form of cystine through the plasma membrane via System x_c^-, an antiporter for cystine and glutamic acid. System x_c^- transport is mediated through expression of a heteromeric transporter with a catalytic subunit from the glycoprotein-associated AA transporter (gpaAT) family, in this case termed xCT (as described in 3.2).

2.1.12. Proline, glycine and β-AA system(s)

An electrogenic and Na^+-requiring system exists for transport of proline with a high affinity and low capacity in proximal straight tubules of rabbit kidney (Roigaard Petersen et al., 1988). This system seems to be a specific reabsorption mechanism for imino acids like proline, hydroxyproline, sarcosine, and N-methylalanine. Common neutral AAs are not accepted (Völkl and Silbernagl, 1980). An additional high-capacity proline reabsorption system is apparently a broad-specificity system for neutral AAs (possibly System A).

Taurine, β-alanine and GABA are reabsorbed slowly from the proximal tubule by a common transport system. This system appears to have a high affinity but low capacity (Dantzler and Silbernagl, 1976). A β-specific Na^+-dependent taurine transporter has been detected in renal basolateral membranes from mice (Mandla et al., 1988). In human embryonic kidney cell line the uptake of β-AAs was characterized by two Na^+-dependent transport components. Competition experiments revealed that taurine and β-alanine distinctly reduced the uptake of each other by the high-affinity Na^+-dependent transport system (Jessen, 1994). In addition, a low affinity, sodium-dependent and proton-gradient-dependent transport system has also been described in segment-specific brush border membrane vesicles from proximal tubule of rabbit kidney (Jessen and Sheikh, 1991). The uptake of taurine by luminal membrane vesicles from rabbit proximal tubule showed that in the pars convoluta, the transport of taurine was mediated by two Na^+-dependent systems and one Na^+-independent system, which were able to drive the transport of taurine into these vesicles (Uchida et al., 1992; Yamauchi et al., 1997). In contrast, in the pars recta, the transport of taurine occurred via a dual transport system, which was strictly dependent on Na^+. The investigation of anionic requirements as well as voltage- and pH-dependence of Na^+-AA symport into rat proximal tubule brush border membrane vesicles showed that the Cl^--gradient was a specific mediator of Na^+-coupled transport of proline, taurine and glycine: transport was electrogenic and driven by both Cl^- and Na^+ gradient (Zelikovic and Chesney, 1989a, c). Cl^--gradient alone under Na^+-equilibrated conditions could energize an overshoot of taurine accumulation by vesicles providing evidence that taurine is energetically activated by and coupled to Cl^--transport (Zelikovic and Budreau-Patters, 1999). Taurine can be transported beyond the end of the proximal convoluted tubule in a Na^+ and Cl^- dependent manner by a carrier interacting with furosemide. Thus proximal and distal taurine carriers seem to be different (Silbernagl et al., 1997). Only the Na^+-dependent high-affinity transport systems in both segments specifically required Cl^- (Jessen and Sheikh, 1991). It is speculated that the function of the β-AA transporter will differ depending on the origin of the cell: in the proximal cell, net transepithelial reabsorption will permit addition of filtered taurine to its body pool. In contrast, taurine accumulation by distal cells would regulate cell volume in response to osmotic stress (Kinne et al., 1998).

Na^+ and Cl^- dependent transport of AAs and analogues including proline, taurine and GABA is produced by expression of the members of a distinct neurotransmitter transporter gene superfamily (see Section 3.3) which includes proline (PROT), glycine (GLYT1,2), taurine (TAUT) and GABA (GAT1-3) transporters, as well as the $ATB^{o,+}$ transporter described above.

2.2. The loop of Henle and distal tubule

The AA transport mechanisms in tubule regions beyond the proximal tubule have been little studied and their molecular basis is uncertain. AA reabsorption in the loop of Henle is mediated by specific transport processes (Scriver and Bergeron, 1974; Silbernagl et al., 1975; Silbernagl, 1988). Reabsorption from Henle's loops is not stereospecific, not different for neutral and acidic AAs, and not inhibited by competitive inhibitors of proximal tubule AA transport (Dantzler and Silbernagl, 1991). There is an inter-nephron heterogeneity between deep and superficial nephrons (Silbernagl et al., 1994). The marginal net AA reabsorption in rat short loops of Henle is the result of high unidirectional reabsorptive and secretory AA fluxes of similar magnitude (Silbernagl et al., 1998). Cationic AAs can enter and leave the lumen of short loops of Henle through specific carrier(s) at high rates, but net transport is small or absent. After microinfusion into the tips of loops of Henle, 30% of lysine and 45% of arginine were reabsorbed (Dantzler and Silbernagl, 1993). Fractional reabsorption of taurine microinfused into early and late distal tubules was small but significant and could be saturated (Silbernagl et al., 1997). The AA reabsorption before loop tips appears to be more effective in juxtamedullary than in superficial nephrons. The distal site of reabsorption is unknown, but AAs may move passively out of the thin ascending limb and be recycled into vasa recta and descending limb (Dantzler and Silbernagl, 1988). Some AAs can move directly from vasa recta into tubules (probably descending thin limbs of Henle's loop) by a process showing significant specificity (Dantzler and Silbernagl, 1991). Approximately 25% of AAs also can be reabsorbed distal to the tips of the loops in juxtamedullary nephrons, and thus probably can be recycled among the loops and vasa recta in the papilla (Dantzler and Silbernagl, 1988; 1990). Brokl and Dantzler (1999) suggested that regulation of AA movement *in vivo* may involve the vasa recta, not the

descending thin limbs of Henle's loops. Cycloleucine microinfused into ascending vasa recta *in vivo* was transferred directly into ipsilateral tubular structures by a saturable and inhibitable process. These observations are like those made for the naturally occurring neutral AA alanine. The lack of saturation and inhibition suggest that transepithelial movement of AAs in thin limbs of Henle's loop may occur via a paracellular route (Pannabecker et al., 2000). Distal reabsorption was only confirmed by microinfusion for glycine and glutamine (Dantzler and Silbernagl, 1988) but a transstimulation of AA transport systems for glycine and lysine has been reported (Hammerman and Sacktor, 1982; Stieger et al., 1983).

2.3. AA transport in cell lines of renal origin

Several cell lines derived from vertebrate kidney tissue have been used to study renal AA transport and its regulation. These cell lines may form recognisable polarised monolayers but also display differences in AA transport phenotype: for example, sodium-dependent taurine uptake into LLC-PK1 cells was higher from the apical than the basolateral surface whereas, in MDCK cells, taurine uptake from the basolateral was larger than uptake from the apical surface. Results from studies using NRK, LLC-PK1, MDCK, A6, HEK293, OK and NBL-1 cell lines are included in this review (e.g. Rabito and Karish, 1982, 1983; Jessen et al., 1994, 1996; Hensley and Mircheff, 1994; Hirsch et al., 1998).

3. Molecular basis of AA transport

We present below a brief summary of the major families of AA transporters, focusing particularly on transporters expressed in the kidney. Where appropriate, we include the human gene family numbers allocated by the HUGO Gene Nomenclature Committee on its database at http://www.gene.ucl.ac.uk/nomenclature/genefamily.shtml. All transporters considered here belong to the Solute Carrier (*SLC*) superfamily. Further details on molecular structures and transporter-related genetic disorders can be found on the OMIM (Online Mendelian Inheritance in Man) database at http://www.ncbi.nlm.nih.gov/Omim/.

3.1. Excitatory AA transporter family (SLC1)

3.1.1. Anionic (acidic) AA transporters (EAATs)

The glutamate transporter family includes at least five isoforms with properties of System X_{ag}-(Kanai et al., 1993; Palacin et al., 1998a). A glycoprotein purified from rat brain was identified as the glutamate/aspartate transporter EAAT1 (originally named GLAST or GluT-1). EAAT1 was reported to be of glial origin (Pines et al., 1992). EAAT1 (*SLC1A3*) is specific for glutamate and aspartate transport and shows strict dependence on Na^+ (Storck et al., 1992). Glutamate uptake is coupled to the co-transport of two Na^+ ions, and the counter-transport of one K^+ ion and one OH^- ion, resulting in an inward movement of one positive charge (Bouvier et al., 1992; Kanai et al., 1995). The transporter is present in brain and testis, whereas only a very small signal is detected in the kidney (Tanaka, 1993). Mouse glutamate transporter (mEAAT2; originally named GLT-1) from adult whole-brain total RNA shares a 93% identity with hEAAT2 (*SLC1A2*; Sutherland et al., 1995). Although functional properties are not changed by the alteration of N-termini and C-termini when expressed in oocytes, co-expression of two liver-type EAAT2 with different C-termini (mGLT-1A and mGLT-1B) has been found. These results suggest the tissue specific alternative splicing at 5′-ends of EAAT2 (GLT-1) messages and the association of spliced variants with different C-termini (Utsunomiya Tate et al., 1997).

A complementary DNA encoding an electrogenic Na^+- but not Cl^--dependent high-affinity glutamate transporter EAAT3 (originally termed EAAC1) was obtained from rabbit small intestine by expression in oocytes (Kanai and Hediger, 1992). They found EAAT3 transcripts in the kidney as well as the central nervous system, small intestine, liver and heart. The function and pharmacology of the expressed protein are characteristic of the high-affinity glutamate transporter already identified in neuronal tissues (Danbolt, 2001). Fairman et al. (1995) reported the primary structure and functional characterisation of the human high affinity glutamate transporter hEAAT3 (*SLC1A1)*. Transport is mediated by a Na^+- and K^+-coupled electrogenic mechanism. Northern analysis using the labelled hEAAT3 cDNA confirmed expression in the brain, heart, liver, kidney, and intestine (Kanai et al., 1994). Expression of EAAT1 as well as EAAT3 is not restricted to the brain (Arriza et al., 1994).

To investigate the specific role of EAAT3, Peghini et al. (1997) have generated two mouse lines lacking EAAT3 (EAAC1). These eaac-1$^{-/-}$-mice develop dicarboxylic aminoaciduria, highlighting the importance of EAAT3 to renal reabsorption of anionic AAs. *In situ* hybridisation allowed the cellular localisation of glutamate transporter mRNA in the rabbit kidney to be visualised, especially in the outer stripe of the outer medulla and in the S3 segment of proximal tubules (Meister and Jacobsen, 1994; Shayakul et al., 1997).

3.1.2. Zwitterionic AA transporters (ASCT1, 2; ATB^0)

The excitatory amino acid transporter (*SLC1*) gene family also includes the ASCT and ATB^o transporters. System ASC is characterised by a preference for small neutral AAs. Northern blot analyses revealed ubiquitous expression of ASCT1 (*SLC1A4*), consistent with the general metabolic role ascribed to System ASC (Arriza et al., 1993). ASCT1 is now known to function as an electroneutral exchanger, because the electrical currents initially reported to be associated with ASCT1-mediated transport result from activation of a thermodynamically uncoupled chloride conductance with permeation properties similar to those described for the glutamate transporter subfamily. Like glutamate transporters, ASCT1 activity requires extracellular Na^+. However, unlike glutamate transporters, which mediate net flux and complete a transport cycle by counter-transport of K^+, ASCT-1 mediates only homo- and heteroexchange of AAs and is insensitive to K^+ (Zerangue and Kavanaugh, 1996). A unique property of System ASC activity is an altered substrate selectivity such that at pH values below 7.4 anionic AAs function as inhibitors and substrates. Following transfection of HeLa cells with the ASCT1 cDNA, transport strongly favoured zwitterionic AAs when uptake was measured at a physiologic pH. However, lowering the pH to 5.5, significantly enhanced the interaction of the ASCT1 carrier with anionic AAs (Tamarappoo et al., 1996).

A cDNA was subsequently isolated from mouse testis encoding a Na^+-dependent transporter ASCT2 with sequence similarity to ASCT1. ASCT2 RNA was detected in lung, skeletal muscle, large intestine, kidney, and testis, but not in brain, liver, heart or small intestine (Endou et al., 1997; Kanai, 1997a, b). When expressed in oocytes, ASCT2 *(SLC1A5)* exhibited uptakes of neutral amino acids with high affinity. The substrate selectivity of ASCT2 was typical for System ASC, which prefers neutral AAs without bulky or branched side chains. Glutamate transport was enhanced by lowering extracellular pH, suggesting that glutamate was transported in protonated form. ASCT2 mediated transport is electroneutral and Bröer et al. (2000) showed that ASCT2 is strongly expressed in rat astroglia. In oocytes expressing rat ASCT2, these authors observed high-affinity uptake of glutamine that was Na^+-dependent, concentrative, and unaffected by membrane depolarisation. Addition of different AA to the extracellular solution resulted in a rapid release of glutamine from the ASCT2-expressing oocytes, indicative of a role for ASCT2 in cellular efflux of glutamine: glutamine release from astrocytes is an essential part of the glutamate-glutamine cycle in the brain (Bröer et al., 1999).

Kekuda et al. (1996) described hATB^o cDNA (also designated *SLC1A5* alongside ASCT2), which induces a Na^+-dependent AA transport with preference for zwitterionic AAs. In further experiments the authors cloned the sodium-dependent neutral AA transporter B^o (ATB^o) from rabbit jejunum and from human intestinal cells. Mammalian intestine and kidney proximal tubular cells express an ATB^o which is identical to the ATB^o expressed in Caco-2 cells (Kekuda et al., 1997; Avissar et al., 2001). ATB^o is a potential candidate for the defective protein in Hartnup disease (Kekuda et al., 1997; Milovanovic et al., 2000). The transport characteristics of ATB^o are broadly consistent with those described for the AA transporter System B^o (anionic, cationic and N-methylated AAs are excluded) and differ from those of classical ASCT2. Very recently Meredith et al. (2002) have identified a distinct ATB^o clone (named NBL-1 B^o) from the bovine renal cell line NBL-1, which has an identical functional phenotype to the original description of System B^o: that is, substrate AA transport is inhibited by phenylalanine (which itself is a marginal substrate) but does not interact with anionic AAs. It appears that a small family of ASCT2/B^o transporter isoforms with subtle differences in transport properties may exist. NBL-1 B^o activity is found at the basolateral membrane of distal tubule-like NBL-1 cells (Doyle and McGivan, 1992).

3.2. Cationic AA transporter (SLC7 and SLC3/SLC7 heteromer) family

The diverse *SLC7* family includes the CAT and gpaAT transporter groups belonging to a phylogenetic cluster within the AA/polyamine/choline (APC) superfamily of transporters (Verrey et al., 1999).

3.2.1. CAT transporters

The founder members of the SLC7 gene family, CAT1, CAT2, CAT3 and CAT4 (*SLC7A1, SLC7A2, SLC7A3, SLC7A4* respectively) encode proteins mediating System y^+ for the cationic AAs arginine, lysine, and ornithine. The murine CAT1 transporter is the receptor of the murine leukaemia virus (Kim et al., 1991; Wang et al., 1991). At least two subtypes of the CAT-2 System are known which differ in their affinities for cationic AAs and exhibit differential tissue expression (Closs et al., 1993, 1997; Van Winkle et al., 1995; MacLeod, 1996). mCAT-2B is satured at one-fifth the arginine concentration and has a lower apparent V_{max} than mCAT-2A. The identification of mCAT-2B in murine macrophages showed that the AA sequence is the nearly same as mCAT-2A, the low affinity transporter expressed in hepatocytes. These transporters apparently result from differential splicing of transcripts from a single gene. The transport properties are otherwise similar to mCAT-1 and coincide with the y^+-phenotype (Closs et al., 1993). Ito and Groudine (1997) isolated a novel member (CAT3) of the CAT family. In oocytes, CAT3 cRNA exhibited a saturable, sodium ion-independent transport activity with high affinity for arginine and lysine. Results are consistent with the idea that CAT3, along with CAT1 and CAT2, constitutes the transport activity originally assigned to System y^+. Like CAT2, but unlike CAT1, the expression of CAT3 is regulated in a highly tissue-specific manner: when various adult tissues were examined, significant levels of CAT3 transcript were detectable only in brain. These results indicated a role of rat CAT3 in the System y^+ transporter activity in the nervous tissue (Hosokawa et al., 1997, MacLeod, 1996).

Both CAT1 and CAT2 are expressed in the kidney. Within the rat kidney, CAT-2 mRNA was more abundant in the medulla than in the cortex. Because CAT mediates the uptake of the NO precursor arginine, this transporter may have a role in the regulation of NO production in kidney cells: it is conceivable that the cells exhibiting CAT-2 mRNA staining also express NO synthase (Burger-Kentischer et al., 1998). The functional importance of CAT1-mediated transport was highlighted by the production of knockout mice, which were severely anaemic and runted and died within 12 h of birth (Perkins et al., 1997); renal function in these mice was not studied. CAT3 compensated for the loss of functional CAT1 in embryonic fibroblasts derived from these CAT1 knockout mice. Northern blot analysis documented that CAT2 mRNA increased 2-fold, whereas CAT3 mRNA levels increased 11-fold in $Cat1^{-/-}$ relative to $Cat1^{+/+}$ fibroblasts. The low affinity CAT2A mRNA was not detectably expressed in these cells (Nicholson et al., 1998).

3.2.2. Glycoprotein-associated AA transporter complexes (SLC7/SLC3 family member heteromers)

The glycoprotein-associated AA transporters (gpaATs) are a group of AA permeases within the *SLC7* family which form functional transporters as disulfide-linked heteromeric complexes with one or other of the two glycoproteins (BAT and 4F2hc/CD98) constituting the *SLC3* family. These *SLC3* proteins form a "heavy chain" linked to one of several discrete and functionally-distinct gpaAT "light chains" to produce key AA transport activities including Systems L, y^+L (both involving 4F2hc; *SLC3A2*) and $b^{o,+}$ (involving BAT; *SLC3A1*). The *SLC3* gene family proteins have one or, in the case of BAT, up to four transmembane domains whereas the gpaAT proteins are typical 12-transmembrane domain transporters. The *SLC3* glycoproteins function largely to chaperone the catalytic *SLC7* gpaAT subunits to the plasma membrane. These catalytic subunits can be transported to the cell surface only in the presence of 4F2hc or BAT. The experimental evidence supporting these conclusions is reviewed by Palacin et al. (1998a, b), Mastroberardino et al. (1998), and Verrey et al. (2000). Expression of the membrane proteins of the BAT/4F2hc family in *Xenopus* oocytes alone results in the induction of AA transport activity, presumably because they interact with gpaAT light-chain subunits endogenous to oocytes (Ahmed et al., 1995, 1997; Bröer et al., 1998; Peter et al., 1999).

3.2.2.1. BAT-gpaAT heteromers. The protein product of the BAT gene (*SLC3A1:* also known as NAA-Tr, rBAT, D2, NBAT) is expressed specifically in microvillous domains of kidney and intestine and induces a

high-affinity transport system for cystine, basic AAs, and some neutral AAs when expressed in *Xenopus* oocytes (Kanai et al., 1992; Wells and Hediger, 1992; Bertran et al., 1992; Mosckovitz et al., 1993, 1994; Nirenberg et al., 1995). This transport activity resembles the functionally defined System $b^{o,+}$ and accounts for the acronym rBAT, meaning "related to $b^{o,+}$ AA transporter" (Palacin et al., 2000). In the kidney, BAT is associated with a second (approximately 45 kDa) protein as a heterodimer proposed to be the minimal functional unit of the renal cystine transporter (Deora et al., 1998). This second protein was recently identified as the $b^{0,+}$AT permease (*SLC7A9*) of the gpaAT family (Pfeiffer et al., 1999b). System $b^{o,+}$-like is an obligatory AA exchanger with a 1 : 1 stoichiometry for exchange of dibasic with neutral AAs. This transport activity generates voltage-dependent outward currents with neutral AA uptake but inward currents with cationic AA uptake (Coady et al., 1994; Busch et al., 1994; Ahmed et al., 1995). Sodium is not necessary for the accumulation of substrates via System $b^{o,+}$. *In vivo* the BAT- $b^{0,+}$AT transporter complex is believed to be involved in re-uptake of AAs into the bloodstream across the apical brush border membrane of polarized renal and intestinal epithelial cells (Tate et al., 1992), utilising a tertiary active transport mechanism (Verrey et al., 2000). The specific location of BAT in the microvilli of epithelial cells from S3 segment of the proximal tubule coincides with the site of high-affinity resorption of cystine in the kidney. This was consistent with the involvement of BAT in a $b^{o,+}$-like high-affinity resorption system for cystine in the proximal straight tubule (Furriols et al., 1993).

3.2.2.2. 4F2hc-gpaAT heteromers. 4F2hc (*SLC3A2*: the heavy chain of the human 4F2 cell surface antigen; also known as CD98) is an almost ubiquitous transmembrane glycoprotein in mammalian cells. The 4F2hc protein has 30% sequence identity with BAT (Coady et al., 1996). System L is one of the major AA-transport systems in almost all mammalian cells. The L-type AA permease LAT1 (*SLC7A5* – also known as AAT-L-lc, E16, IU12) has been identified as being a disulfide-linked "light chain" of the ubiquitously expressed 4F2hc (Kanai et al., 1998; Pfeiffer et al., 1998, 1999a). The functional holotransporter mediates Na^+-independent exchange of large neutral AAs (and AA-related drugs such as L-DOPA) corresponding to transport System L (Kanai et al., 1998; Kageyama et al., 2000). Thyroid hormones are also substrates of System L (Ritchie et al., 1999). Northern blot analysis for LAT1 mRNA showed high concentrations in brain, spleen, and placenta, but in low amounts in testis and colon; it is not expressed in liver and kidney, but in some tumour cell lines. LAT2 (*SLC7A8*) shows AA sequence similarity to LAT-1 and also interacts with 4F2hc to induce System L-like AA transport activity (Segawa et al., 1999; Pineda et al., 1999; Rajan et al., 2000a). The AA transport activity induced by the co-expression of 4F2hc and LAT-2 was sodium-independent but differed from that expressed by LAT1 by showing broad specificity for small and large zwitterionic AAs. LAT2 is expressed primarily in the small intestine and kidney. The high expression of LAT-2 mRNA in epithelial cells of proximal tubules, the basolateral location of 4F2hc in these cells, and the AA transport activity of LAT-2 suggest that this transporter contributes to the renal reabsorption of neutral AAs in the proximal tubule cells. Neither LAT1 or LAT2 interact with rBAT, the second member of the *SLC3* family. The System L transporters extend the specificity range of vectorial AA transport when located in the same membrane as carriers that unidirectionally transport one of the exchanged substrates (Meier et al., 2002). For example, SAT transporter activity is believed to drive accumulation of AA substrates into cells, enabling uptake in parallel of indispensable amino acids such as leucine or phenylalanine, by hetero-exchange through System L (so-called "tertiary active transport"). As a footnote, we point out that, although System T for aromatic amino acids is superficially similar to System L at a functional level, the first cloned System T transporter (TAT1) has surprisingly turned out to be a member of the monocarboxylate (*SL16)* transporter family. TAT1 (*SL16A10*) is expressed strongly in certain epithelial tissues (intestine, placenta) but is not detected in the kidney (Kim et al., 2001).

Torrents et al. (1998) identified a human y^+L AA transporter-1 (y^+LAT-1; *SLC7A7*) that induces System y^+L transport activity (Deves et al., 1992) when co-expressed with 4F2hc in *Xenopus* oocytes. The heteromer forms an exchanger which mediates the Na^+-independent efflux of cationic AAs and the Na^+-dependent uptake of large neutral AAs, as is typical of System y^+L-mediated transport; i.e. obligatory AA exchange that mediate efflux of dibasic AAs and, in the presence of sodium, influx of neutral AAs (Pfeiffer et al., 1999a). Human y^+LAT-1 forms a 135-kDa, disulfide bond-dependent heterodimer with 4F2hc and

is expressed in kidney ≫ leukocytes ≫ lung > placenta = spleen > small intestine. The 4F2hc (and presumably also y^+LAT1) protein is localized at the basolateral membrane of transporting epithelia, indicating that the y^+LAT1-4F2hc heterodimer is the basolateral System y^+L AA exchanger involved in transepithelial reabsorption of cationic AAs. A second System y^+L isoform y^+LAT-2 (*SLC7A6*) (Torrents et al., 1998; Boyd et al., 2000) is not expressed in kidney.

Rajan et al. (1999, 2000b) provide evidence that the $b^{0,+}$ AA transporter ($b^{0,+}$AT) can interact with 4F2hc as well as with BAT to constitute a functionally competent $b^{0,+}$-like AA transport system. Even though both the 4F2hc x $b^{0,+}$ complex and the rBAT x $b^{0,+}$ complex exhibit substrate specificity that is characteristic of System $b^{0,+}$, these two complexes differ significantly in substrate affinity. The ability to interact with 4F2hc and BAT is demonstrable with mouse as well as with human $b^{0,+}$AT. This may be of functional significance because the regional distribution pattern of mRNA in the kidney is identical for $b^{0,+}$ and 4F2hc whereas the pattern of rBAT mRNA expression is different, although there are regions in the kidney where $b^{0,+}$AT mRNA expression overlaps with BAT mRNA expression as well as with 4F2hc mRNA expression.

Two other gpaAT transporters have been reported which require 4F2hc for functional expression at the cell surface. Asc-1 (*SLC7A10*) exhibits the functional properties of System asc which transports small neutral AAs Na^+-independently (Nakauchi et al., 2000). Asc-1 mRNA was detected in the brain, lung, small intestine, and placenta. Sato et al. (1999) have isolated cDNA (xCT) encoding the cystine/glutamate transport System x^-_c from mouse. Co-expression of human xCT (*SLC7A11*) with 4F2hc in HeLa cells leads to the induction of cystine and glutamate uptake with characteristics similar to that of x^-_c (Bridges et al., 2001). Transport of cystine via System x^-_c may be a regulatory factor for the intracellular glutathione level, and its transport activity is induced in response to oxygen by enhancing the expression of both xCT and 4F2hc mRNAs (Sato et al., 2000).

3.3. Neurotransmitter transporter family (SLC6)

3.3.1. Taurine and β-AA transporter

The neurotransmitter transporter family (*SLC6*) includes transporters for transmitters including norepinephrine, serotonin, GABA and glycine (Amara and Kuhar, 1993). The taurine and β-alanine transporter TAUT (*SLC6A6*) is a member of this same gene family (Yamauchi et al., 1997; Benyajati and Johnson, 1991; Burnham et al., 1996; Guastella et al., 1990). TAUT cDNA was cloned from mouse and rat brain (Smith et al., 1992) and mouse retina. The deduced protein sequence of the mouse retinal taurine transporter revealed sequence identity to the canine kidney, rat brain, mouse brain, and human placental taurine transporters. Within the kidney, taurine transporter mRNA was localized to the S3 segment of the proximal tubule, to the loop of Henle, and to the glomerular epithelial cell layer. TAUT mRNA induced Na^+- and Cl^--dependent taurine transport activity in oocytes. Two Na^+ and one Cl^- are required to transport one taurine molecule via the taurine transporter. The transport is pH-dependent and taurine uptake was inhibited by β-alanine and GABA.

3.3.2. GABA transporters

GAT2 is the only member of the high-affinity GABA transporters expressed significantly in peripheral tissues in addition to the CNS (Liu et al., 1993). However, in the MDCK renal cell line two GABA transporter isoforms (GAT-2 and GAT-3; *SLC6A13* and *SLC6A11* respectively) are localized to the basolateral and apical surfaces respectively. A sequence of 22 AAs at the C-terminus of GAT-2 is required for the transporter's basolateral distribution and is capable of directing GAT-3 to the basolateral surface when appended to the C terminus of this normally apical polypeptide (Rasola et al., 1995; Clark, 1997; Kwon, 1996).

3.3.3. System $B^{0,+}$ transporter

The first cloned B^{0+} AA transporter was $hATB^{0+}$ (Sloan and Mager, 1999). $hATB^{0,+}$ (*SLC6A14*) was found to transport both neutral and cationic AAs by a Na^+ and Cl^--dependent mechanism. Ugawa et al. (2001) have shown that a System B^{0+} transporter in cultured colonic epithelial cells mediates amino acid absorption. *In vivo* glycine uptake assay demonstrated that a System B^{0+}-like transporter protein was expressed on the apical surface of the colonic absorptive cells. The authors cloned a mouse colonic amino acid transporter, designated $mCATB^{0+}$, which exhibited a Na^+-dependent stereoselective mechanism with broad

specificity for neutral and cationic AAs when expressed in oocytes. Hatanaka et al. (2001b) cloned the Na^+ and Cl^- coupled AA transport System $B^{0,+}$ from the mouse colon and investigated its ability to transport NOS inhibitors. When expressed in mammalian cells, it can transport a variety of zwitterionic and cationic AA. Each of the NOS inhibitors tested compete with glycine for uptake through this transport system. These data represent the first identification of an ion gradient-driven transport system for NOS inhibitors in the intestinal tract.

3.3.4. Glycine (GLYT) transporters

Glycine transporters exhibit their greatest expression in the central nervous system. The open reading frame of the high affinity glycine transporter GLYT1 cDNA predicts a protein containing 633 AAs with a molecular mass of approximately 70 kDa (Guastella et al., 1992). In common with other Na^+/Cl^--dependent transporters, GLYT1 (*SLC6A9*) possesses 12 putative transmembrane domains, according to its hydropathicity profile. This protein is the human homologue of a glycine transporter previously isolated from rat (GlyT-1b). In addition to GlyT-1b, a further isoform could be characterized by alternative splicing. This isoform, GlyT-1c, which is distinct from GLYT-2 described in rat, contains an additional exon encoding 54 AAs in the amino-terminal part of GlyT-1b and is mainly expressed in brain (Borowsky et al., 1993). The high affinity, Na^+/Cl^--dependent glycine transporter GLYT-2 (*SLC6A5*) is about 50% homologous to GLYT1 and the rat proline transporter (PROT). GLYT-2 is specific to the CNS, whereas GLYT1 is found in the CNS as well as in peripheral tissues.

3.3.5. Proline transporter

The Na^+/Cl^--dependent, high-affinity proline transporter (PROT; *SLC6A7*) is expressed in regions of the brain that contain glutaminergic neurons (Malandro and Kilberg, 1996). The cloned transporter cDNA predicts a 637 AAs containing protein with 12 putative transmembrane domains which exhibits about 45% AA sequence identity with certain other members of the family of neurotransmitter transporters (Fremeau et al., 1992). The human brain proline transporter (hPROT) was also detected on immunoblots of membranes from human liver, kidney and heart (Shafqat et al., 1995).

3.4. Neutral AA transporter family (SLC38)

Three System A transporter isoforms (SAT1-3, also known as ATA1-3 and SA1-3) and two System N transporter isoforms (SN1, SN2) have been identified to date (see Table 1). SAT and SN transporters are structurally related proteins with Na^+-dependent mechanisms. They are members of a larger transporter gene family including the vesicular GABA/Gly transporter VGAT (Reimer et al., 2000). There is at least 45% amino acid sequence identity throughout SAT and SN isoforms and the transporter proteins are modelled typically as 11 TMD proteins.

The SN1 transporter was cloned by Chaudhry et al. in 1999. Functional analysis shows that SN1 (*SLC38A3*) involves H^+ exchange as well as Na^+ co-transport and may mediate either glutamine uptake or release under appropriate conditions (Chaudhry et al., 1999). Fei et al. (2000) subsequently cloned the human Na^+ and H^+-coupled AA transport System N (hSN1) from HepG2 liver cells and a closely-related SN2 isoform (*SLC38A5)* was identified (Nakanishi et al., 2001). The Na^+ -glutamine coupling stoichiometry of SN1 is controversial and has alternatively been estimated as $(2Na^+ + \text{amino acid})_{in}/H^+_{out}$) (Fei et al., 2000) or $(1Na^+ + \text{amino acid})_{in}/H^+_{out}$) (Chaudry et al., 1999; Reimer et al., 2000). Very recent reports indicate that an electroneutral $(1Na^+ + \text{amino acid})_{in}/H^+_{out}$) transport mechanism is associated with an uncoupled H^+ conductance (Chaudry et al., 2001). Both SN1 and SN2 are expressed at mRNA level in the kidney.

Varoqui et al. (2000) isolated a complementary DNA clone SAT1/ATA1 (*SLC38A1*; originally designated GlnT) encoding a plasma membrane glutamine transporter in neural tissue. This transporter displayed the functional characteristics of System A, that is AA transport is Na^+-dependent and inhibited by MeAIB. Sugawara et al. (2000a) and Hatanaka et al. (2000) subsequently reported the identification of SAT2/ATA2 (*SLC38A2*), a transporter responsible for the System A activity expressed in most human tissues. SAT2/ATA2 bears significant homology to GlnT/ATA1 and System N (SN1) and is almost ubiquitously expressed in rat and human tissues (Reimer et al., 2000). Sugawara et al. (2000b) and Hatanaka et al. (2001a) reported on the cloning and functional characterization of a third subtype of amino acid transport System A, designated ATA3 (*SLC38A4*). SAT3 is extremely unusual in that it also transports cationic amino acids in a Na^+ independent manner, in fact the

affinity of hATA3 for cationic amino acids is higher than for neutral amino acids. hATA1 and hATA2, the other two known members of the System A subfamily, show little affinity toward cationic amino acids. hATA3 also differs from hATA1 and hATA2 in exhibiting low affinity for MeAIB. The transport characteristics of ATA3 are thus at least superficially similar to those classified as System y^+L (although the actual mechanism may differ, because y^+LAT transporters operate as obligatory AA exchangers; see section 3.4). Since the liver does not express any of the previously known high-affinity cationic amino acid transporters, ATA3 may provide the major route for the uptake of arginine in this tissue.

SAT and SN transporters appear capable of mediating net amino acid transfer across cell membranes and their mechanisms are not limited by a requirement for obligatory exchange with other amino acids. This affords them utility in accumulating cellular amino acids involved in protein metabolism as well as possible roles in cell volume regulation. In addition, SAT and SN transporters are sensitive to changes in cell membrane potential, ion gradients and local pH, rendering them particularly sensitive to changes in metabolic status of tissues. Furthermore both SN1 and SN2 (although not SAT2) appear capable of mediating bidirectional amino acid transport, although the mechanism involved here is not yet clear. These unusual functional properties may enable SN transporters to release rather than accumulate amino acid substrates under certain specific conditions (e.g. plasma acidosis).

4. Disorders of renal AA transport

Cystinuria and lysinuric protein intolerance are two genetic disorders related to mutations in epithelial AA transporters. Cystinuria results from a failure in renal reabsorption of cystine which, due to the low solubility of cystine, leads to development of cystine stones within the renal tubules and concomitant impairment of renal function. Three cystinuria types can be distinguished by the mode of inheritance and by differences in absorption/clearance of an oral cystine load, although phenotype and genotype analyses provide evidence for only two types of cystinuria related to genetic defects in the two subunits of the System $b^{0,+}$ transporter complex effecting high-affinity cystine reabsorption in the kidney (Langen et al., 2000; Leclerc et al., 2001). Mutations in the human BAT gene *SLC3A1* result in Type 1 cystinuria (Zhang et al., 1994; Calonge et al., 1995; Saadi et al., 1998). In contrast, mutations of the human $b^{0,+}$AT gene *SLC7A9* are responsible for non-type 1 cystinuria (Feliubadalo et al., 1999; Pfeiffer et al., 1999b). Lysinuric protein intolerance (LPI), a rare autosomal recessive defect of cationic AA transport found mainly in Finland and Italy, results from the absence of the recently described y^+LAT-1 isoform (*SLC7A7*) of the y^+L AA transporter (Kamada et al., 2001; Dall'Asta et al., 2000; Font et al., 2001). The transport defect leads to brain dysfunction caused by hyperammonemia resulting from impaired absorption of AA substrates of the urea cycle. Mutations in the human cDNA coding for y^+LAT-1 were reported to be responsible for LPI; for example an identified missense mutation abolishes y^+LAT-1 amino-acid transport activity when co-expressed with the heavy chain of 4F2hc in *Xenopus laevis* oocytes (Mykkanen et al., 2000; Sperandeo et al., 1999, 2000; Borsani et al., 1999; Torrents et al., 1999).

Several other renal diseases are associated with aminoaciduria (Palacin et al., 2000), which could threaten the patient's survival; e.g. Hartnup disorder is an human hyperaminoaciduria of unknown aetiology, although it has been suggested that the AA transporter B^o is the most likely candidate for the defect associated with this disease (Mailliard et al., 1995). Hartnup disorder affects the transport of a large number of neutral AAs (Scriver et al., 1987). Vitamin D deficiency results in a generalized aminoaciduria for a variety of AAs from several classes. The molecular basis of this effect remains unclear, but the aminoaciduria is independent of PTH or cAMP generation and depends on plasma phosphate concentration (Dabbagh et al., 1989).

5. Regulation of AA transport in the kidney

The observation that aminoaciduria is associated with several renal diseases makes it of clinical relevance to elucidate mechanisms for regulation of AA transport in the kidney, in order to try to prevent or treat these diseases. For example, the stimulation of renal AA reabsoprtion would be beneficial in the treatment of both cystinuria and LPI. Known and putative mechanisms which may contribute to regulation of renal AA transport activity are summarised below.

5.1. Growth factors, peptide hormones and protein phosphorylation

Insulin and glucagon stimulate transport of AAs in a variety of cell systems by mechanisms which include changes in protein synthesis and (at least for System A) recruitment of transporters from intracellular pools (Su et al., 1998a, b; Hyde et al., 2002), although their direct effects on AA transport in epithelial tissues are not well-studied. Only little information exists in the literature describing hormonal control of renal AA handling. However the insulin-like growth factor system is intimately involved in renal development, growth, function and the pathophysiology of several disease states and growth factors such as IGF-1 and EGF are reported to have stimulatory effects on AA transport. For example, the intestine is an important target organ for insulin-like growth factor-I (IGF-I). Levels of IGF-I are reduced in cirrhosis and transport of AAs in intestinal brush-border membrane vesicles is impaired in cirrhotic rats: low doses of IGF-I can correct this defect (Pascual et al., 2000). The investigation of the influence of epidermal growth factor (EGF) on renal AA handling in glutamine, arginine, or alanine loaded rats showed that EGF pre-treatment was followed by a stimulation of renal AA reabsorption. EGF also decreased GFR and increased sodium excretion. Interestingly, simultaneous administration of PAH and AAs reduces AA reabsorption capacity. Probably this is an effect at the level of energy supply – overload of PAH is followed by increased PAH secretion, an energy consuming process which exhausts the energy resources of the renal cells resulting in a reduced AA reabsorption capacity. This phenomenon can be normalised after pre-treatment with EGF as a signal to stimulate AA transport (Fleck and Pertsch, 1998). A stimulation of MeAIB and arginine transport in NRK cells by TGF β and EGF could be shown (Boerner and Saier, 1985). Treatment of intestinal epithelial (CaCo) cells with EGF, TGFα and PKC activator phorbol ester stimulated System y^+-mediated arginine transport activity, this upregulation of transport being blocked by cycloheximide or by the PKC inhibitor chelerythrine (Pan and Stevens, 1995b). PKC activity may therefore be involved in EGF modulation of AA transport. Within the kidney, specific, high-affinity EGF receptors have been demonstrated in mesangial cells, proximal tubule, and cortical and inner medullary collecting duct, as well as in medullary interstitial cells. In the proximal tubule, EGF binding and EGF receptor-associated tyrosine kinase activity are localized at the basolateral membrane. Addition of EGF to cultured kidney cells may stimulate a number of intracellular responses, including increased Na^+/H^+ antiport activity, activation of glycolysis, increased DNA, RNA, protein and hyaluronic acid synthesis (Harris, 1991). Growth hormone (rhGH) decreases net renal uptake of 2 mM glutamine by 84% with basolateral uptake reversing to release. In response to rhGH in the kidneys the glutamine uptake is diminished and both glutamine and alanine release are enhanced (Welbourne et al., 1992) – glutamine formation increased as ammonium production decreased (Welbourne and Fuseler, 1993). A macrophage factor, interleukin 1 (IL 1), modulates proximal tubular cell sodium-dependent glucose and AA transport, but this effect is not associated with detectable effects on cell growth or differentiation. IL 1 led to 2-fold increased uptake of glucose and aspartate, but was without influence on the uptake of leucine and arginine. This effect was prevented by cycloheximide and actinomycin D (Kohan and Schreiner, 1988). Parathyroid hormone also increases AA excretion by inhibition of AA reabsorption (Scriver and Bergeron, 1974).

In adults with oxidative phosphorylation diseases (ATP production deficiency) the fractional excretions of neutral AAs and other solutes reabsorbed by sodium-coupled transport processes are enhanced (Shoffner et al., 1995). This presumably reflects a reduced energy supply for active solute reabsorption but may also result at least partly from impaired protein phosphorylation (Zelikovic and Przekwas, 1993). There are many instances showing that AA transport may be modulated by activity of protein kinases, frequently these studies employ phorbol esters such as 12-myristate-13-acetate (phorbol ester), a potent activator of protein kinase C (PKC) (Klip et al., 1986; Saier et al., 1988; Chesney et al., 1993; Shotwell et al., 1983). The phorbol ester produces dose- and time-dependent decreases of glutamate transport in human embryonic kidney cells expressing the cloned EAAT-1 cDNA. The AA sequence of rat EAAT-1 reveals three consensus sequences of putative phosphorylation sites for PKC. However, removal of all putative PKC sites of wild-type EAAT-1 by site-directed mutagenesis did not abolish inhibition of glutamate transport by phorbol ester. Immunoprecipitation of ^{35}S-methionine-labeled transporter molecules indicates a similar stability of phosphorylated and

nonphosphorylated EAAT-1 protein. These data suggest that the transport activity of EAAT-1 is inhibited by phosphorylation at a non-PKC consensus site (Conradt and Stoffel, 1997). In contrast, purified glutamate transporter from pig brain is phosphorylated by PKC in a direct way, predominantly at serine residues. Stimulation of PKC by phorbol ester stimulates glutamate transport within 30 minutes (Casado et al., 1993). Mutation of Ser113 to Asn113 in the EAAT2 sequence abolished the stimulation of transport activity by the phorbol ester 12-O-tetradecancoylphorbol-13-acetate, suggesting a possible role of PKC phosphorylation in the regulation of EAAT activity (Casado et al., 1993). Pre-incubation with phorbol ester of C6 glioma cells increased transport activity of Na^+-dependent EAAT3-mediated glutamate transport. Cycloheximide had no effect on the increase. The increase in transport activity was due to an increase in V_{max} with no change of K_m. The inactive phorbol ester 4α-phorbol 12,13-didecanoate did not stimulate glutamate transport activity, and the PKC inhibitor chelerythrine blocked the stimulation caused by phorbol ester. These studies suggest that activation of protein kinase C causes a rapid increase in EAAT3-mediated transport activity. Phorbol ester may increase the turnover number of pre-existing transporters or phorbol ester may increase the number of functional transporters on the plasma membrane (Dowd and Robinson, 1996). Phorbol ester activated PKC also phosphorylates a subunit of Na^+-K^+-ATPase and increased its activity (Pedemonte et al., 1997); this may in turn influence ion-couple AA transport mechanisms.

Members of the neurotransmitter transporter family are also regulated through PKC. The taurine transporter TAUT has five putative protein kinase A and six putative protein kinase C recognition sites intracellularly. Tyrosine residues are essential for optimal transport function of the human placental taurine transporter and these critical tyrosine residues are located at or near the Na^+-binding site of the transporter (Kulanthaivel et al., 1989). Treatment of kidney cells with a diacylglycerol kinase inhibitor, which results in increased intracellular diacylglycerol, a natural stimulant of PKC, inhibited taurine uptake, providing evidence for a specific effect of PKC activation (Jessen and Jacobsen, 1997). It is possible that the reduction in taurine uptake is a result of enhanced efflux in response to PKC activation, because exposure of LLC-PK1 cells to active phorbol esters increased taurine efflux in cells loaded with taurine. PKC might exert its action on taurine transport by phosphorylation of a membrane-bound protein or a regulatory protein involved in the control of β-AA transport (Jones et al., 1991; Zelikovic and Przekwas, 1993). Investigation of Ca^{2+}- and phospholipid-dependent protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) on Na^+- and Cl^--dependent proline transport suggest that Ca^{2+}-dependent protein kinase-mediated phosphorylation inhibits NaCl-dependent proline transport (Zelikovic and Przekwas, 1995). Treatment of human embryonic kidney cells (HEK 293 cells) expressing the mouse glycine transporter 1 (GLYT1b) with PKC activator phorbol ester decreased specific glycine uptake. This down-regulation resulted from a reduction of the maximal transport rate and was blocked by the PKC inhibitor staurosporine. The inhibitory effect of phorbol ester treatment was also observed after removing all five predicted phosphorylation sites for PKC in GLYT1b by site-directed mutagenesis. These data indicate that glycine transport by GLYT1b is modulated by PKC activation (Gomeza et al., 1995).

In LLC-PK1 cells, phorbol ester stimulates System A transport, an effect that was blocked by cycloheximide (Dawson and Cook, 1987). LLC-PK1 have a low rate of Na^+-dependent System A transport capacity. Cells respond to phorbol ester with brief enhancement of A-System activity that returns to control levels within 10–20 min. The response is followed some 30 min later by a large and prolonged elevation of transport activity. The initial transport response appears to be a consequence of a protein kinase-C-dependent phosphorylation event, while the delayed response is dependent on the synthesis of new protein (Dawson and Cook, 1988).

Alanine uptake via transport System B in intestinal cell line Caco-2 was stimulated by phorbol ester after at least 6 h of continuous exposure, and was blocked by the protein kinase inhibitors chelerythrine or photoactivated calphostin C. The protein synthesis inhibitor cycloheximide blocked the phorbol ester up-regulation of System B activity, suggesting that de novo synthesis was involved in up-regulating System B activity. Actinomycin D also blocked the phorbol ester stimulation of System B, suggesting possible transcription involvement. It is concluded that Caco-2 cells regulate carrier-mediated sodium-dependent transport of alanine by changing the membrane capacity to transport alanine via System B and that this regulation involves de novo protein

synthesis under the control of protein kinase C (Pan and Stevens, 1995a).

The activity of System L in human placental choriocarcinoma cells is stimulated by activation of PKC, unaffected by agents which elevate intracellular levels of cAMP, and stimulated by agents which interfere with calmodulin-dependent processes. Ramamoorthy et al. (1992) investigated the regulation of the activity of System L in this epithelial cell line by agents which are known to modulate the activities of three different classes of protein kinases: A-, C-, CaM-kinase. Calmodulin antagonists like calmidazolium stimulated System L activity markedly. This stimulatory effect was specific for System L because Systems A and ASC were not stimulated by these agents. The stimulation was primarily due to an increase in the V_{max}, the apparent K_m being only minimally affected. It was concluded that the activity of System L is stimulated by PKC, inhibited by CaM-kinase and unaffected by A-kinase.

5.2. *Osmotic shock*

Renal epithelial cells may be subject to large, rapid variations in external osmolarity depending on the osmoregulatory demands placed on the kidney. They therefore have effective volume regulatory mechanisms, which include the ability to gain or lose osmotically active molecules such as AAs under appropriate circumstances. Accumulation of osmolytes by renal cells is due to increased uptake via specific transporters and certain AA transporters play a prominent part in this process. For example, the basolateral surface of MDCK cell monolayers responded to hyperosmolality with increased taurine accumulation (Jones et al., 1992, 1994). Taurine accumulation by the MDCK cell appears to be a mechanism for adaptation to osmotic stress (Jones et al., 1993). The response of MDCK cells to medium osmolality requires both protein synthesis and RNA transcription and is expressed in the presence of microtubular toxins. There are at least two distinct mechanisms involved in the regulation of taurine transport: external taurine concentration and external osmolality, with taurine concentration seeming to be the predominant stimulus (Jones et al., 1995). Cellular taurine uptake systems are very different from swelling activated taurine release systems. The uptake systems have a low tolerance for changes in the chemical structure of the transported substrate, i.e. a high degree of transport specificity, whereas the release systems apparently tolerate such diverse molecules as chloride, taurine, and myo-inisitol and can be considered as a channel-like pathway or pore (Haynes and Goldstein, 1993; Kinne et al., 1998). Regulatory volume decrease in MDCK cells was related to a loss of K^+, Cl^-, and AAs. The fluxes of glutamate, taurine and glycine during regulatory volume decrease depended linearly on their external concentrations, implying that the transport process was diffusional (Roy and Banderali, 1994).

Besides taurine transport, System A activity in the hypertonic MDCK cells (measured as sodium-dependent MeAIB uptake) increased about 60-fold relative to the uptake in isotonic cells. This indicate that AAs, especially neutral AAs, and not only taurine, can function as volume-regulating osmolytes and System A appears to contribute to the accumulation of neutral AAs in hypertonic MDCK cells. System A activity for neutral AA transport is increased after hypertonic shock by a mechanism which is consistent with the synthesis of a regulatory protein that activates pre-existing System A carrier proteins (Felipe et al., 1992). Ruiz-Montasell et al. (1994) showed that the increase in System A activity after hypertonic treatment requires the integrity of cytoskeletal structures. System A transport decreases when cells return to isotonic conditions by a mechanism that is insensitive to inhibitors of protein synthesis. The increase in System A transport activity is also followed by the accumulation of neutral AAs, which is totally blocked by cycloheximide and actinomycin. The content of acidic AAs also increased rapidly in hypertonic cells. This suggested that AA transporters other than System A might also be induced in hypertonic conditions or that hypertonicity might modulate AA metabolism (Horio et al., 1997). Indeed the high-affinity Na^+-dependent glutamate transport System Xag- is induced (increase in V_{max} with no change in K_m) by hypertonicity in the renal epithelial cell line NBL-1. This effect is dependent on protein synthesis and glycosylation and is combined with an increase in EAAT3 mRNA levels. This was the first evidence that hypertonicity may increase the expression of a gene for an AA transport protein itself (Ferrer-Martinez et al., 1995). Burston and McGivan (1997) identified a protein of 110 kDa in the renal epithelial cells which is induced by incubation in an amino-acid-free medium. The protein did not correspond to any protein in the databases and was found also in rat liver plasma membranes. Kempson (1998) found that differential

upregulation of System A and the betaine/GABA transporter BGT1 by hypertonic stress is due to intrinsic changes in these transporters at the membrane level. In contrast, the downregulation of System A in intact cells is likely due to the action of an intracellular repressor that is not present in the isolated membranes.

5.3. Dietary and adaptive regulation

The AA reabsorptive mechanisms of the kidney are responsive to changes in renal AA load (e.g. when dietary AA intake is altered). The nephron can respond rapidly to changes in the intake of AAs, for example conserving taurine in periods of nutrient lack and excreting excess taurine within 4 h in periods of load. When dietary intake of taurine is reduced, urinary taurine levels decline due to enhanced renal tubular reabsorption of taurine (Chesney et al., 1986; Rozen and Scriver, 1982). Adaptive responses to maintain whole-body taurine homeostasis occur predominantly via changes in the activity of the high-affinity taurine transport. At least two types of adaptive response to taurine diet are evident: a rapid response, best examined in MDCK cells (Jones et al., 1992) and a prolonged response, best observed *in vivo* and in slices, isolated tubules, or BBMV (Zelikovic and Chesney, 1989b). In MDCK or LLC-PK1 cells, the short-term adaptive response is unaffected by inhibition of protein and nucleic acid synthesis by cycloheximide and actinomycin D respectively. The inositol phospholipid pathway involving diacetylglycerol and IP3 also does not appear to be linked directly to this adaptive regulation (Jessen and Jacobsen, 1997). Only colchicine, which impairs the movement of proteins along the microtrabecular network (Andreu and Timasheff, 1986), was capable of blocking the response: the effect of colchicine indicates that the increase or reduction in Na^+-taurine symport activity is due to incorporation of transporter into the plasma membrane rather than to de novo synthesis (Chesney et al., 1989). In the longer term, taurine transport activity in LLC-PK1 cells is evidently regulated by medium taurine concentration and occurs at the level of mRNA transcription. In rats the long-term adaptive response also occurs at the level of mRNA, and is blocked by actinomycin D. With taurine restriction, taurine transporter mRNA expression was upregulated, but this regulation is limited to the S3 segment of the proximal tubule (Matsell et al., 1997). The nature of the intracellular stimulus for increased transcription is depending on a subcellular taurine pool, which regulates gene transcription or modifies a regulatory protein (Han et al., 1996, 1997).

AA starvation enhances System A activity in MDCK cells as a sign of adaptive regulation. Insulin was found to be an absolute requirement for this response and PGE_1 was a negative controlling factor for the transport system (Boerner and Saier, 1885). An increase in the expression of the SAT2/ATA2 System A transporter gene underlies adaptive upregulation of System A upon amino acid starvation in muscle cells (Hyde et al., 2001), fibroblasts (Gazzola et al., 2001) and liver (Varoqui and Erickson, 2002). SAT2 is expressed in kidney and might also be predicted to be the regulated isoform in this tissue. An investigation of AA transport by System A in the MDCK kidney cell line showed that azacytidine (an inhibitor of DNA methylation) and butyrate (an enhancer of histone acetylation) inhibited expression of System A. Phorbol ester stimulated System A activity in MDCK cells under normal growth conditions but did not stimulate its activity in AA-starved cells: the stimulation by phorbol ester was prevented by prior exposure to butyrate but not to azacytidine. The azacytidine sensitivity of System A suggests that one mechanism of control involves DNA methylation of either the gene encoding the transporter or of accessory genes. The data suggest that at least one of the genes involved in System A expression is phorbol ester inducible, is activated by AA starvation, and is butyrate sensitive (Boerner and Saier, 1985, 1988).

In a renal bovine epithelial cell line derived from distal tubule, AA deprivation induces an increase in aspartate transport with a doubling of V_{max} and no change in K_m. However, there is no increase in EAAT3 mRNA during induction of transport activity by AA deprivation (Ferrer-Martinez et al., 1995) nor is there any change in the total amount of transporter protein (Nicholson and McGivan, 1996). These results suggest that transporter activity is regulated at the post-transcriptional level and it is proposed that low intracellular glutamate levels lead to the induction of a putative protein that activates the EAAT3 transporter.

In NRK kidney cells, CAT-1 mRNA levels increased following AA starvation. The transcription rate of the CAT-1 gene remained unchanged during AA starvation, also suggesting a post-transcriptional mechanism of regulation (Aulak et al., 1996, 1999).

Treatment of rats with cycloheximide (which inhibits protein synthesis) superinduces the level of the 7.9-kb CAT-1 mRNA in the kidney, spleen, and brain, but not in the liver, suggesting that cell type-specific labile factors are involved in its regulation (Aulak et al., 1996).

The amount of BAT mRNA in mouse kidney increases during postnatal development, consistent with an increase in renal cystine and dibasic transport activity. Dietary aspartate induced a marked increase in cystine transport via the $b^{0,+}$-System in mouse ileum, in conjunction with an increased expression of BAT mRNA (Segawa et al., 1997).

5.4. Steroid, thyroid and other hormones

Steroid hormones have been noted to enhance or diminish transport of AAs depending on the nature of the hormone. These agents may act at the transcriptional or post-transcriptional levels. While stimulation may involve either increased carrier synthesis or rapid non-genomic effects at the cell membrane, inhibition may involve synthesis of a labile protein which either decreases the rate of synthesis or increases the rate of degradation of a component of the transport system (Lerner, 1985). Testosterone induces rapid stimulation of AA transport in mouse kidney. The hormonal increment in uptake persisted for at least 60–120 min, showed time-, energy-, and Na^+-dependence, and varied with substrate and testosterone concentration. In contrast, dexamethasone had little effect under the same conditions (Koenig et al., 1982), although dexamethasone does stimulate taurine uptake by flounder renal tubules: this taurine transport was not affected by second messengers like cAMP, cGMP, adenosine, or the Ca^{2+}-ionophore A12384 (King et al., 1982). Glucocorticoid hormones (which are attendant with metabolic acidosis) also enhance renal glutamine extraction coupled to ammoniagenesis (Waybill et al., 1994; Welbourne and Dass, 1988). Glucocorticoids and insulin induce expression of the CAT-1 gene in liver cells through induction of transcription and stabilization of the mRNA; it is possible that a similar mechanism operates in kidney. In our own experiments (Fleck, 1992a, b), repeated administration of dexamethasone or triiodothyronine (T_3) caused significant changes of AA plasma concentrations in young (10 days) and adult (2 months) rats. In young animals the fractional excretion of endogenous AAs was reduced in 15 of 22 AAs after dexamethasone and in 12 of 23 AAs after T_3 treatment, indicating stimulatory effects of both hormones on tubular AA carrier systems in immature animals. In adult rats the stimulatory effects of hormone treatment could not be found under physiological conditions. However, after administration of an AA mixture containing various AAs in high doses to overload the renal AA reabsorption capacity, the reabsorbed amounts of AAs were higher in rats treated with dexamethasone, but not after treatment with T_3 (Fleck and Nußbaum, 1996). On the other hand, in both immature and adult rats it is possible to stimulate the renal excretion of p-aminohippurate after treatment with the two hormones (Bräunlich et al., 1986). The effects of T_3 treatment on renal AA reabsorption were subsequently investigated after loading the animals with high amounts of AAs, under which conditions it was possible to prove stimulating hormone effects, because AA reabsorption is employed to capacity. In these studies, leucine and glutamine were chosen as bolus injections to overload AA reabsorption carriers. Bolus injections of both AAs were followed by temporary increase in fractional excretion of the administered AAs as well as endogenous AAs which were not administered. The influence of T_3 or dexamethasone on renal AA handling was investigated in leucine or glutamine loaded animals. Under these load conditions, dexamethasone treatment was followed by a stimulation of renal AA reabsorption: the increase in fractional AA excretion after AA load was significantly lower than in untreated rats. The effect of T_3 was different in leucine and glutamine loaded animals: after leucine bolus injection a comparable stimulatory effect as shown for dexamethasone could be demonstrated, but after glutamine administration the stimulatory action of T_3 was masked. T_3 even increases fractional AA excretion in glutamine loaded rats, indicative of enhanced "housekeeping" in the renal tubular cells. These results indicate different effects of both hormones on the renal handling of AAs (Fleck et al., 1997; Schwertfeger et al., 2000). The Na^+-dependent uptake of proline was not affected by the thyroid status (Sacktor and Kinsella, 1985). Thyroid hormone also had no effect on Na^+-independent transport of arginine in renal proximal tubular brush border membrane and BAT mRNA levels were unaffected (Sorribas et al., 1995).

A pre-treatment with T_3 or dexamethasone also stimulated the renal AA transport capacity of partly (5/6) nephrectomized rats after AA load (Fleck et al., 1999). Furthermore, under load conditions dexameth-

asone or T_3 treatment was followed by a stimulation of renal AA reabsorption in bile duct-ligated rats (Fleck and Engelbert, 1998). In a separate study, the influence of T_3 or dexamethasone on the renal handling of taurine was investigated in taurine loaded animals. T_3 treatment was followed by a slight stimulation of the renal taurine reabsorption, whereas dexamethasone was without significant effect (Fleck and Langner, 1998). Schwertfeger et al. (2002) have investigated the transport mechanisms involved in the stimulation of renal tubular reabsorption of AAs by glucocorticoids *in vivo* through the examination of activity and expression of specific transport Systems L and y^+L for leucine in membrane preparations of rat kidneys. Rats were treated with dexamethasone and brush-border and basolateral membranes were isolated. Functional analysis of leucine uptake revealed induction of a System y^+L transport component in the basolateral membrane in the dexamethasone-treated group. A minor System L transport component was unaffected by glucocorticoids. Expression of both subunits of the System y^+L transporter was increased in dexamethasone-treated rat kidneys: Western blot analysis showed a significant increase of 4F2hc protein abundance in the basolateral membrane and PCR revealed a 4-times induced expression of y^+LAT1 mRNA. Results indicate that System y^+L in rat kidney is regulated by glucocorticoids. The enhancement of both 4F2 heavy chain and y^+LAT1 light chain is necessary for induction of this transport system in the kidney.

Removing the adrenal glands impairs the elimination rate of glutamine from the blood by the kidney. Administering glucocorticoids to adrenalectomized rats reverses a small net release of glutamine to uptake in the kidney. Renal basolateral transport of glutamine is closely coupled to its oxidative metabolism. Supplementing these adrenalectomized animals with triamcinolone restored glutamine uptake into the cells (Welbourne, 1988, 1989).

5.5. Metabolic acidosis

Several key AA transporters expressed in the kidney (e.g. SAT and SN isoforms) are extensively regulated by changes in extracellular pH within the physiological range. There is also evidence that EAAT and SN transporters include movement of H^+ ions as part of the transport cycle, making their activity dependent on the magnitude and direction of any H^+ electrochemical gradient across the cell membrane. These properties may contribute to regulation of renal function during metabolic acidosis, which constitutes an important element of the physiological response to this metabolic insult. Renal glutamine utilization increases markedly during chronic metabolic acidosis, producing ammonia for H^+ excretion (as NH^+_4) in the urine as part of the compensatory process (Welbourne, 1988; Souba, 1993). The combined decrease in plasma pH and glutamine concentration during chronic metabolic acidosis also helps to redirect this amino acid from the intestine (which exhibits load- and pH-dependent glutamine uptake) to the kidneys (Welbourne, 1988). Relatively few AAs are metabolised in the kidney, notably glutamine deamidation can occur in the proximal tubule. Luminal ammoniagenesis from glutamine hydrolysis accounts to 5–10% of the normal renal ammonium excretion in the rat. The ratio between deamidation and reabsorption of glutamine in the early proximal tubule is 80 : 1 and in the late proximal tubule 10 : 1 (Silbernagl, 1986). An antiluminal glutamine uptake contributes to at least half of the overall glutamine consumption. Basolateral γ-glutamyl transferase (GGT) appears to be important for generating extracellular glutamate for renal uptake; approximately 40% of the glutamine extracted from plasma by the chronically acidotic rat kidney may enter renal cells as glutamate following extracellular glutamine hydrolysis by GGT at the basolateral membrane (Welbourne and Dass, 1988). Glutamine transported into kidney cells (including filtered glutamine) is coupled to activated glutaminase, yielding ammonia for tubular NH_4^+ excretion and bicarbonate, which is released into the plasma. A functional asymmetry (antiluminal vs. luminal) in glutamate transport may play an important role in modulating renal glutamine metabolism (Carter and Welbourne, 1998). The reabsorption of glutamine is neither influenced by 2-oxo-glutarate or by mercaptopicolinic acid nor by acute respiratory acidosis. Acidosis and inhibition of cellular gluconeogenesis does not influence resorption kinetics of glutamine (Silbernagl, 1980). This example with glutamine serves to highlight that when interferences with renal AA transport are investigated, it is important that AA metabolism in the kidney are considered.

5.6. Drugs, xenobiotics and varia

Cannon et al. (2001) tested the hypothesis that amino acid transport systems are involved in absorptive

transport of dicysteinylmercury. The data indicate that multiple amino acid transporters are involved in the luminal uptake of dicysteinylmercury, including the Na^+-dependent low affinity L-cystine-, B^0-, and ASC Systems and the Na^+-independent L-System. Pb^{2+} and Hg^{2+} inhibit rBAT-induced AA transport in a non-competitive, allosteric fashion and blockade of rBAT-induced AA transport may be involved in the aminoaciduria following mercury or lead intoxication (Waldegger et al., 1995). The effects of thallium, sodium dichromate and cis-platinum (CP) on renal amino acid excretion have been investigated in 10- and 55-day-old anaesthetised rats (Fleck and Appenroth, 1996; Fleck et al., 2001). In principle the renal fractional excretion of amino acids was distinctly higher in immature rats as a sign of lower amino acid reabsorption capacity. However, both chromate and CP significantly decreased amino acid reabsorption capacity as a sign of nephrotoxicity, most pronounced in adult rats after CP. Extrinsic denervation down-regulates carrier-mediated transport of AAs in the intestine: decreased in vitro glutamine transport was mediated in part by a decrease in number rather than affinity of sodium-dependent transporters (Foley et al., 1998). A similar phenomenon may occur in other epithelia such as the kidney.

Examining all data available in this field, the potential importance of the regulation of AA transport to renal function in health and disease is clear. Nevertheless, more and more parts of a large puzzle need to be collected for the full understanding of AA transport and its regulation by hormones, growth factors, drugs, xenobiotics, endogenous substrates like AA themselves and by the nervous system in the kidney and other tissues. Research in these areas is now proceeding apace. For example, we know from a colleague, quoted in the references, that a "state of the art" review article accepted by the editorial board of a "high-impact" journal was, at least in part, old-fashioned by the time it was published half a year later. In the meantime, other colleagues from a different lab wrote the next part of this story. And now we are attempting to continue with the same procedure.

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