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Amino acid transport in the renal proximal tubule

Review Article

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Summary. In the kidney the proximal tubule is responsible for the uptake of amino acids. This occurs via a variety of functionally and structurally different amino acid transporters located in the luminal and basolateral membrane. Some of these transporters show an ion-dependence (e.g. Na^+, Cl^- and K^+) or use an H^+-gradient to drive transport. Only a few amino acid transporters have been cloned or functionally characterized in detail so far and their structure is known, while little is known about a majority of amino acid transporters. Only few attempts have been untertaken looking at the regulation of amino acid transport. We summarized more recent information on amino acid transport in the renal proximal tubule emphasizing functional and regulatory aspects.

Keywords: Amino acids – Kidney – IHKE-1-cells – LLC-PK_1-cells – Regulation – DOG – PKC

Introduction

Vectorial transcellular transport of amino acids in the renal proximal tubule is mediated via functionally and structurally different amino acid transporters at both sides of the epithelial cells. Several kidney preparations, a variety of cultured renal epithelial cells and expression models have been used to study renal amino acid transport. Na^+-dependent and -independent transporters, Cl^--dependent and H^+-gradient driven transporters and amino acid exchangers are present in renal epithelial cells. Some of these transporters have been functionally analyzed in detail and the molecular structure is known, whereas structure and function of others still remain unclear. Regulation of renal amino acid transport has only recently been under investigation. This review summarizes more recent information on amino acid

transport in the renal proximal tubule emphasizing functional and regulatory aspects.

Methodological approaches

Earlier studies using mircoperfusion and microimpalement techniques demonstrated that renal reabsorption of neutral, basic, and acidic amino acids is located in the proximal tubule of the nephron (Ullrich, Greger, 1985; Young, Freeman, 1971; Barfuss and Schafer, 1979; Silbernagl, 1988; Schafer and Barfuss, 1980). Further examination of amino acid transport on the cellular level was mainly investigated using tracer flux measurements (Jessen et al., 1996; Jessen and Sheikh, 1991; Fass et al., 1997; States et al., 1987; Lynch and McGivan, 1987; Mora et al., 1996) and electrophysiological techniques such as microelectrode impalements and more recently, the patch clamp technique (Samarzija et al., 1982; Samarzija and Frömter, 1982a, 1982b, 1982c; Frömter, 1982; Hirsch et al., 1998). All these techniques have advantages and disadvantages. While electrophysiological techniques only allow the measurement of electrogenic transport systems, a quantitative analysis of all amino acid transport systems can be obtained from tracer flux studies. Nevertheless, the advantage of the electrophysiological approach over a biochemical method is the noninvasiveness of the technique that enables monitoring indirectly transport in a living cell.

Na^+-dependent amino acid transport systems

Neutral amino acids

Electrophysiological analysis showed that the transport of amino acids across the luminal membrane of proximal tubule cells induced a depolarization of this membrane, a reduction of the membrane resistance and a shift to a more lumen negative transepithelial voltage (Lang et al., 1986; Hoshi et al., 1976; Frömter, 1982). The driving force for the electrogenic amino acid transport is due to 1) a concentration gradient for the amino acid, 2) the membrane potential, and 3) the inwardly directed Na^+-gradient (Zelikovic and Chesney, 1989; Lang et al., 1986; Schafer and Barfuss, 1980; Silbernagl, 1988).

Na^+-dependent amino acid transport systems have been described in proximal tubule cell lines of pig (LLC-PK_1), opossum (OK), and human kidney (IHKE-1) as well as in isolated proximal tubules of the rat (Hirsch et al., 1998; Samarzija et al., 1982; Samarzija and Frömter, 1982a, 1982b, 1982c; Handler, 1986; Schwegler et al., 1989). On a molecular level several different major amino acid transport systems have been reported for the Na^+-dependent uptake of neutral amino acids (see also Fig. 1): the systems *A, ASC, B,* B^0, B^{0+} and N (Castagna et al., 1997). The ubiquitous systems *A* and *ASC* mediate Na^+-dependent transport of neutral amino acids via the basolateral membrane of epithelial cells leading to an intracellular accumulation of neutral amino acids. System *ASC* favors alanine, threonine,

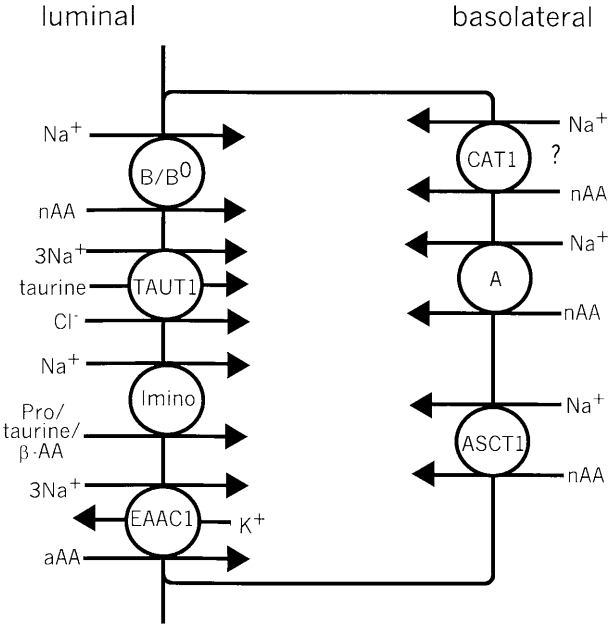

Fig. 1. Model of a proximal tubule cell showing the Na^+-dependent amino acid transport systems. *nAA* neutral amino acids, *aAA* acidic amino acids, *β-AA* β-amino acids, *Pro* proline, *?* unclear membrane localization

serine and cysteine as substrate, but it can also transport homologs which contain an additional carbon atom and even anionic amino acids at low pH (Christensen, 1984). The main difference between system *A* and *ASC* is the pH-insensitivity of the latter one. Furthermore, system *ASC* does not recognize N-methylamino-α-isobutyric acid (MeAIB) as a substrate (Castagna et al., 1997). These systems were predominantly studied in erythrocytes and hepatocytes where amino acid uptake is required for metabolic reasons (Stevens et al., 1984; Tate et al., 1996). It has also been suggested that systems *A* and *ASC* are responsible for the Na^+-dependent amino acid transport in MDCK cells derived from canine kidney (Boerner, Saier, 1982) while in LLC-PK_1 cells of porcine this transport is almost exclusively mediated by system *ASC* (Sepúlveda, Pearson, 1982). Two ASC-type transport proteins have been cloned (ASCT1, ASCT2) and they show a widespread tissue distribution with the transport characteristics mentioned above (Zerangue and Kavanaugh, 1996).

The Na^+-dependent systems *B, B^0*, and *B^{0+}* are responsible for the absorption of aliphatic, branched-chain and aromatic amino acids and are located in intestinal and renal epithelial brush border membranes. System *B^{0+}* also transports dibasic amino acids. Their specificity for neutral amino acids is broader than that of systems *A* and *ASC* (Stevens et al., 1984; Doyle and McGivan, 1992; Lynch, McGivan, 1987; Maenz and Patience, 1992). Data obtained from human embryonic (IHKE-1) and adult (AHKE) proximal tubule cells as well as data from rat proximal tubule correspond well to the

system *B* and B^0 making it most likely the system in the proximal tubule responsible for the main entry of neutral amino acids (Hirsch et al., 1998; Samarzija and Frömter, 1982a; Jessen et al., 1996). The system B^0 transporter was cloned from rabbit jejunum (riATB^0) and from the human intestinal cell line Caco-2 (ATB^0). When expressed in a human kidney cell line or in human HeLa cells a Na^+-dependent transport of neutral, but not of acidic, basic or N-methylated amino acids was seen (Kekuda et al., 1997).

Besides the systems mentioned above there is at least one more important system that transports neutral amino acids across renal brush border membranes. The *imino* system is supposed to be a specific transporter for taurine, β-amino acids and proline in the luminal membrane of proximal tubule cells (Bertran et al., 1994; Uhl and Johnson, 1994). These findings were supported by tracer flux studies in IHKE-1 and AHKE cells (Jessen et al., 1994, 1996; Jessen, 1994) as well as in luminal membrane vesicle preparations from the proximal tubule of rabbit and rat (Fass et al., 1977; Jessen and Sheikh, 1991; Zelikovic and Chesney, 1989; Chesney et al., 1999). This system accounts for 60% of the Na^+-dependent proline uptake in renal brush-border membranes (Castagna et al., 1997). Our recent data from IHKE-1 cells also demonstrate that such a system exists in these cells since AIB strongly depolarized membrane voltage when being applied to the apical surface of the cells (Hirsch et al., 1998). The system *A* also has the capability of transporting AIB but is located in the basolateral membrane. Since in our study IHKE-1 cells were grown on glass cover slips and therefore, the accessibility to the basolateral membrane was restricted, we could exclude system *A*.

A system β has also been described as a specific transport system for Na^+-dependent uptake of β-amino acids and taurine which seems to play a role as an osmolyte transporter in the kidney (Castagna et al., 1997; Garcia-Perez and Burg, 1991). Isoforms of the taurine transporter (TAUT) have been cloned from various tissues and species including dog kidney (Uchida et al., 1992) and LLC-PK_1 cells of the pig (Han et al., 1998).

The GLY-like system which has been demonstrated to be important in brain, liver, and erythrocytes (Kilberg et al., 1980) might also be located in IHKE-1 cells derived from the human proximal tubule since glycine depolarized membrane voltages of these cells (Hirsch et al., 1998). Finally, a system known as *N* which is mainly expressed in muscle and liver transports glutamine, asparagine, and histidine. It plays a key role in glutamate metabolism (Kilberg et al., 1980).

Acidic amino acids

The system responsible for the uptake of glutamate and aspartate is known as system X^-_{AG}. A cloned high affinity transporter resemblembing this system in the kidney, intestine, and brain was named EAAC1 (Kanai and Hediger, 1992). Immunolocalization of EAAC1 revealed that this transporter is predominantly expressed in the S2 and S3 segment of the proximal tubule and weaker in the S1 segment, descending thin limb, medullary thick ascending

limb and distal convoluted tubule (Shayakul et al., 1997). Na^+-dependent transport of acidic amino acids was also found in the apical membrane of LLC-PK_1 cells (Rabito and Karish, 1982). From electrophysiological and tracer uptake experiments in OK cells it was reported that the transport of glutamate and aspartate was Na^+-dependent, but electroneutral (Schwegler et al., 1989; Malström et al., 1987). In apical membrane vesicles of these cells it was demonstrated that glutamate uptake depended on an outwardly directed K^+-gradient. Therefore, a stoichiometry of 2 Na^+ + 1 glutamate against 1 K^+ was proposed. Electroneutral, but Na^+- and K^+-dependent uptake of glutamate was already suggested in tracer flux studies with rabbit renal brush border membrane vesicles (Schneider et al., 1980; Schneider and Sacktor, 1980). In these studies the existence of a high and low affinity glutamate transport system was claimed. Contrary to these findings transport of glutamate and aspartate evoked membrane depolarizations in proximal tubule cells of rat kidney (Samarzija and Frömter, 1982c) and small, but significant depolarizations of membrane voltages in IHKE-1 cells (Hirsch et al., 1998). To explain the glutamate induced depolarization of the membrane there has to be a cotransport of at least 3 Na^+ with one glutamate against 1 K^+.

Simultaneous luminal and peritubular perfusion of proximal tubule from rat kidney demonstrated that peritubular transport of acidic amino acids is similar to the luminal transport mechanism thus, leading to high intracellular accumulation of these amino acids (Samarzija and Frömter, 1982c). The physiological meaning might be that glutamate transport in kidney cells represses cellular glutaminase activity and hence, regulates glutaminase utilization (Carter and Welbourne, 1997).

Basic amino acids

In contrast to systems *B* and B^0 amino acid transport mediated by system B^{0+} is Na^+-dependent and optimal transport activity appears to require Cl^- (Devés and Boyd, 1998). System B^{0+} accepts neutral and dibasic amino acids as substrates. Evidence for system B^{0+} mediated transport was found in the apical membrane of MDCK cells which showed Na^+-dependent interaction with arginine, alanine and leucine with comparable affinities (Boerner et al., 1986). Similarly, in rat renal brush border membrane vesicles Na^+-dependent transport of lysine was found to be mediated by the same transport system as arginine, cystine and phenylalanine (Stieger et al., 1983). Depolarization of cell membranes induced by Na^+-dependent transport of basic amino acids altered when Na^+ was replaced in the experimental solutions in electrophysiological experiments using rat proximal tubules as well as IHKE-1 cells (Samarzija and Frömter, 1982b; Hirsch et al., 1998). In IHKE-1 cells evidence for a Na^+-dependent and a Na^+-independent transport system for basic amino acids was found. The Na^+-independent system is known as system b^{0+} (rBAT) and will be introduced below.

Na^+-independent amino acid transport systems

Several Na^+-independent transport systems of amino acids have been characterized so far. Among them is the b^{0+} system which exchanges neutral amino acids against dibasic and vice versa and is highly expressed in kidney and intestine (Mora et al., 1996; Kakuda and MacLeod, 1994; Mircheff et al., 1982). The related cloned proteins are known as rBAT, NBAT, and D2 (Castagna et al., 1997; Kakuda and MacLeod, 1994). Closely related to the b^{0+} system is the y^+L system which is known to transport dibasic amino acids in the absence of Na^+ and neutral amino acids in the presence of Na^+. The related cloned protein is 4F2hc (Kakuda and MacLeod, 1994). A rather similar transport pattern shows the y^+ system which is responsible for the Na^+-independent uptake of cationic amino acids but in the presence of Na^+ some neutral amino acids are also transported. The cloned proteins related to that system are part of the CAT-family (Castagna et al., 1997). Both systems, y^+L and y^+, show a widespread tissue distribution (Castagna et al., 1997; Kakuda and MacLeod, 1994). Another Na^+-independent system with a widespread tissue distribution is the system L which transports aromatic and branch-chained amino acids (Castagna et al., 1997). In the blood-brain barrier and in glial cells system L is the major amino acid transport system (Pardridge and Oldendorf, 1977).

In IHKE-1 cells two different Na^+-independent amino acid transport systems could be described by us, a Cl^--dependent and a Cl^--independent system. The latter one showed transport characteristics of rBAT and the

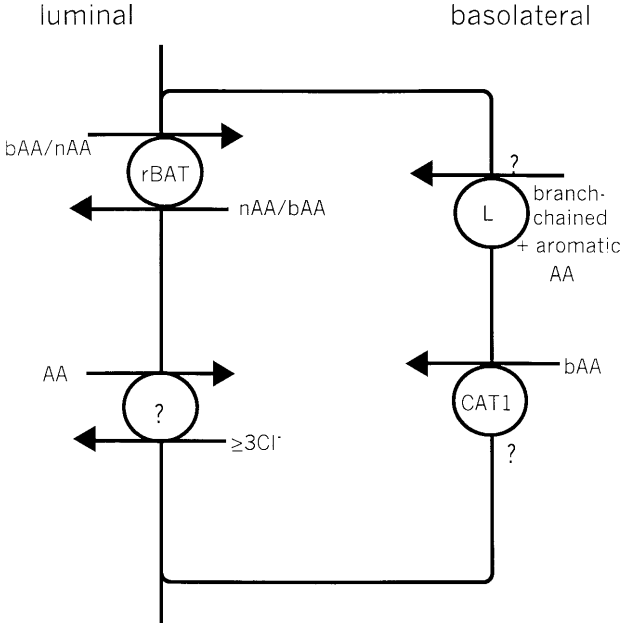

Fig. 2. Model of a proximal tubule cell showing the Na^+-independent amino acid transport systems. *nAA* neutral amino acids, *bAA* basic amino acids, *?* unclear membrane localization

existence of its mRNA was demonstrated by northern blotting (Hirsch et al., 1998). The second, Cl^--dependent system will be discussed below.

Cl^--dependence of amino acid transport

For a few Na^+-dependent amino acid transporters a participation of Cl^- was demonstrated. Members of the neurotransmitter transporter family which includes the proline transporter PROT are Cl^- and Na^+-dependent and display a stoichiometry of 2 Na^+ and 1 Cl^- of 1 GABA. Homologs of these transporters have been detected in mouse kidney and MDCK cells (Kilberg et al., 1997; Malandro and Kilberg, 1996). Studies on luminal membrane vesicles of rabbit proximal tubules revealed a Cl^--dependence of the Na^+-dependent β-amino acid specific transport system (Jessen and Sheikh, 1991; Wolff and Kinne, 1988). The Cl^--dependence of Na^+-dependent taurine transport and β-amino acids was also confirmed in experiments with rat renal brush border membrane vesicles (Chesney et al., 1999; Zelikovic et al., 1989). Uptake of taurine in IHKE-1 cells showed a specific requirement for Cl^- especially at low amino acid concentrations (Jessen, 1994). A coupling ratio of 3 Na^+/1Cl^-/1 taurine was proposed in all three species. In brush border membrane vesicles of pig kidney cortex two L-glycine transport systems were found where the high-affinity transport system depended on an inwardly directed Cl^--gradient and showed saturation at increasing Cl^--concentrations (Scalera et al., 1987).

As already mentioned above in IHKE-1 cells a second Na^+-independent but Cl^--dependent amino acid transport system was found (Hirsch et al., 1998). Depolarizations of membrane voltage induced by this Cl^--dependent system could be reduced by the Cl^--channel and Cl^--exchanger blockers NPPB (5-nitro-2(3-phenylpropylamin)benzoat) and DIDS (4,4′-di-isothiocyanato stilben-2-2′-disulfonic acid). This amino acid transport system doesn't fit to any described system so far. It transports neutral, acidic and basic amino acids and always induces a depolarization of membrane voltage. Reduction in extracellular Cl^- from 145 to 5 mM abolished the depolarization of membrane voltage due to neutral and acidic amino acid transport. Basic amino acids showed a significant weaker depolarization under these experimental conditions (Hirsch et al., 1998), suggesting that this system exchanges amino acids against Cl^-. It could be compared to the Cl^-/formate or the Cl^-/HCO_3^--transporter described for brush border membrane vesicles for the proximal tubule of rabbit. Both systems can also be blocked by DIDS (Soleimani and Bizal, 1996).

The only described Na^+-independent amino acid transport system for which a Cl^--dependence is discussed is system x^-_c in fibroblasts, hepatocytes, endothelial cells, glial cells and macrophages which specifically transports L-cystine in exchange with glutamate and serves as a protector for oxidative stress (Bertran et al., 1994; McGivan, Pastor-Anglada, 1994; Kakuda, MacLeod, 1994; Bannai, 1984) and thus, is different from the novel system described in electrophysiological studies in IHKE-1 cells (Hirsch et al., 1998).

Regulation of renal amino acid transport

Osmotic influences, cellular stress, and adaptive regulation

In rat tubule cells the transport of phenylalanine was inhibited by hyperosmolar solutions (Samarzija et al., 1982) supposedly via increased synthesis of specific regulatory proteins (McGivan and Pastor-Anglada, 1994). While hyperosmotic solutions had no effect on the glutamate transport in IHKE-1 cells, reduced transport activity for glutamate was seen when a hypotonic surrounding was generated (Hirsch et al., 1998). This reduction is probably not based on a specific regulatory mechanism but rather on a general inhibition of substrate uptake for cell protection.

Deprivation of amino acids stimulates protein synthesis of system *A* and system X^-_{AG} in NBL1 cells from bovine kidney and together with system X^-_{AG} a novel glycoprotein is upregulated due to the triggering of amino acid transport and stress proteins (McGivan et al., 1996; Burston and McGivan, 1997). Positive and negative feedback regulation was demonstrated for taurine transport in LLC-PK_1 and MDCK cells where taurine depreviation led to an increase in taurine transport and uptake capacity of taurine was decreased when the cells were supplied with excessive L-taurine (Jones et al., 1990). Adaptive regulation was also found for systems *GLY*, *N* and x^-_c (Christensen, 1984).

Hormones

Hormonal regulation has been shown for system *A*, where glucagon and insulin induce protein synthesis in hepatocytes and other cells (Kilberg et al., 1997; Castagna et al., 1997). It was concluded that cAMP and glucagon exert their stimulative effect by activating the Na^+/H^+-exchanger, causing an increased cellular accumulation of Na^+ and activation of the Na^+/K^+-ATPase which then leads to a hyperpolarization of the cell and stimulation of Na^+-dependent alanine transport (McGivan and Pastor-Anglada, 1994). This regulation serves as an adaptive mechanism of amino acid uptake in order to adjust the cell to the actual offer of amino acids. *In vivo* application of parathyroid hormone led to an increase in alanine uptake in basolateral membrane vesicles prepared from rabbit kidney tubules, but had no effect on alanine uptake in the luminal membrane vesicles (Bidot-Lopez et al., 1982). Thus, it can be assumed that alanine uptake in rabbit kidney tubule cells is mediated by system *A* as the major regulated Na^+-dependent amino acid transport system.

Protein kinases

Most of the investigations of regulation of amino acid transporters were done with cloned proteins expressed in *Xenopus laevis* oocytes or human epithelial kidney cells (HEK293), e.g. the taurine transporter TAUT1, the GABA transporter GAT1, and also the glycine transporter GLYT1b (Corey et al.,

1994; Loo et al., 1996; Sato et al., 1995). For the TAUT1 transporter it was shown that the transporter is not directly regulated via PKC and PKA, but the insertion of the protein into the plasma membrane was mediated by protein kinases (Loo et al., 1996) as it had been shown before for the Na^+/glucose cotransporter (Hirsch et al., 1996). A similar regulatory pattern can be seen for the GAT1 and the GLYT1b transporter. GAT1 was shown to be stimulated by PKC while GLYT1b was inhibited even after removel of all putative phosphorylation sites for PKC on both transport proteins (Corey et al., 1994; Sato et al., 1995). The Na^+/PO_4^{2-} cotransporter (Na/Pi-2) from the proximal tubule showed an identical regulatory pattern as GLYT1b (Hayes et al., 1995).

Table 1. Stimulation of amino acid transport in IHKE-1 cells

A. 5′ Preincubation of agonist

Substrate	Agonist	ΔV_m (mV)	n
Glycine (0.1 mM)	–	11 ± 1	5
Glycine (0.1 mM)	ANP (10 nM)	12 ± 1	5
Glycine (1 mM)	–	34 ± 3	7
Glycine (1 mM)	ANP (10 nM)	33 ± 3	7
Glycine (0.1 mM)	–	11 ± 1	6
Glycine (0.1 mM)	DOG (1 μM)	11 ± 1	6
Glycine (1 mM)	–	26 ± 3	15
Glycine (1 mM)	DOG (1 μM)	27 ± 3	15
Glycine (1 mM)	–	22 ± 3	13
Glycine (1 mM)	Forskolin (1 μM)	23 ± 4	13

B. 24 h Preincubation of agonist

Substrate	Agonist	ΔV_m (mV)	n
Glycine (0.1 mM)	–	7 ± 1	20
Glycine (0.1 mM)	ANP (10 nM)	8 ± 1	24
Glycine (1 mM)	–	20 ± 4	20
Glycine (1 mM)	ANP (10 nM)	26 ± 4	24
Glycine (0.1 mM)	–	6 ± 1	10
Glycine (0.1 mM)	DOG (1 μM)	8 ± 1	17
Glycine (1 mM)	–	19 ± 5	10
Glycine (1 mM)	DOG (1 μM)	21 ± 3	16

C. Direct influence of agonists in the presence and absence of extracellular Na^+

Substrate	$[Na^+]_o$ (mM)	Agonist	ΔV_m (mV)	n
Leucin (1 mM)	145	–	17 ± 2	5
Leucin (1 mM)	145	DOG (1 μM)	15 ± 2	5
Leucin (1 mM)	0	–	10 ± 2	6
Leucin (1 mM)	0	DOG (1 μM)	4 ± 2*	5

Vm membrane voltage; mean ± SEM, *$p < 0.05$.

In IHKE-1 cells neither forskolin nor dioctanoylglycerol (DOG) had an effect on the transport of glycine in the presence of Na^+. In the current clamp mode amino acid induced potential changes were measured electrophysiologically in IHKE-1 cells (Table 1). Preincubation of DOG (1 μM) and forskolin (1 μM) for 5 minutes did not show an alteration of the potential response induced by the transport of 0.1 mM or 1 mM glycine compared to the paired controls. Since it was shown that ANP inhibits Na^+-coupled transport processes in the proximal tubule of the kidney (Hammond et al., 1985) and furthermore, the existence of natriuretic peptide receptors which led to an increase in intracellular cGMP were demonstrated in IHKE-1 cells (Hirsch et al., 1998), it was supposed that ANP might also have an influence on Na^+-dependent amino acid transport in these cells. Preincubation experiments (5 min) with ANP (10 nM) had no effect on the Na^+-dependent glycine transport in IHKE-1 cells. 24 h incubation of IHKE-1 cells with ANP or DOG did also not influence Na^+-dependent glycine transport in IHKE-1 cells. The addition of 1 μM DOG did not alter the transport of 1 mM leucine in IHKE-1 cells in the presence of Na^+ whereas DOG did inhibit the transport of 1 mM leucine in these cells in the absence of Na^+. Conclusively, the Na^+-dependent transport of neutral amino acids was not influenced by stimulation of PKA, PKC or PKG in IHKE-1 cells (summarized in Table 1), but PKC may play a role in the regulation of Na^+-independent transport of amino acids in proximal tubule cells of the kidney.

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