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MOLECULAR PHYSIOLOGY IN THE TRANSPORT SYSTEM

Proximal Tubular Transport of Organic Compounds

Polyspecific cation transporters in the proximal tubule

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The homeostasis of endogeneous organic cations, such as choline, monoamine neurotransmitters, nucleosides, cationic drugs and cationic xenobiotics, is controlled by polyspecific cation transporters in small intestine, liver, kidney, brain and heart [1–3]. In 1994, some of us cloned the polyspecific cation transporter rOCT1 from rat kidney, which proved to be the first member of a rapidly growing family of polyspecific transporters with 12 predicted α-helical transmembrane domains and a large extracellular loop between the first and second presumed transmembrane domain [4,5]. This family includes a subfamily of electrogenic cation transporters (OCT1, OCT2 and OCT3), a subfamily containing cation transporters and the sodium carnitine co-transporter (OCTN1 and OCTN2), a subfamily of anion transporters (OAT1, OAT2 and OAT3) and various integral membrane proteins with non-identified functions [5]. OCT2 and OCT3 have ∼70 and 50% amino acid identity to rOCT1, respectively; OCTN1/2 have ∼40% amino acid identity to rOCT1, whereas OAT1–3 have ∼30% amino acid identity to rOCT1. The functional properties of OCT1, OCT2 and OCT3 from rat and human have been studied in detail [4,6–9]. These transporters are sodium-independent facilitated diffusion systems which may operate in both directions [6,7]. Since cation uptake by these transporters can be driven by the membrane potential and/or by the chemical cation gradient, under normal metabolic conditions, the influx of cations into cells is mediated much more effectively than the efflux of cations. However, in depolarized cells, cation efflux may become important.

OCT1, OCT2 and OCT3 are polyspecific transporters which translocate a variety of organic cations with different structures and are inhibited by some hydrophobic cations which are not transported. Inhibitory cations are, for example, quinine, tetrabutylammonium and cyanine 863. However, *N*-methylquinine, which contains a constant positive charge at variance to quinine, and tributylmethylammonium are transported by rOCT1 (unpublished data). OCT1, OCT2 and OCT3 may also be inhibited by non-charged compounds such as corticosteroids and by anions such as probenecid or α-ketoglutarate (unpublished data). The substrate and inhibitor specificity of OCT1, OCT2 and OCT3 overlaps. For some cations, significant differences in affinity and maximal transport rates between OCT1, OCT2 and OCT3 within identical species have been observed. For example, after expression of rOCT1 and rOCT3 in *Xenopus laevis* oocytes, apparent K_m values of 95 μM (rOCT1) and 2.5 mM (rOCT3) were determined for the uptake of [^{14}C]tetraethylammonium (TEA) [4,9]. The IC_{50} values for the inhibition of [^{14}C]TEA uptake by procainamide were 29 μM and 0.42 mM for rOCT1 and rOCT2, respectively (unpublished data). In different species, the affinity of substrates and inhibitors for the same transporter subtype may differ significantly. For example, for the inhibition of [^{14}C]TEA uptake by mepiperphenidol, IC_{50} values of 0.83 mM (rOCT2) and 4.8 μM (hOCT2) were determined. Also, the IC_{50} values for corticosterone inhibition of [^{14}C]TEA uptake mediated by rOCT3 or hOCT3 were 4.9 and 0.12 μM, respectively [10,11]. The species differences in substrate affinity of the cation transporters highlights the limitations and risks of using animal models for studying the excretion and toxicity of cationic drugs during drug development. Another recent observation may be of great biomedical importance. We observed that rOCT1 stably expressed in human embryonic kidney (HEK) cells is up-regulated when protein kinase C is stimulated, and that this up-regulation is combined with an ∼10-fold increase in affinity of rOCT1 for TEA [12].

To understand the physiological function of the organic cation transporters in the kidney, their nephron distribution and membrane localization must be known. Throughout the last 20 years, functional measurements have been performed with microperfused proximal tubules and isolated plasma membrane vesicles, trying to distinguish and characterize the different polyspecific cation transport systems [1,2,5]. In Figure 1, transport systems which have been character-

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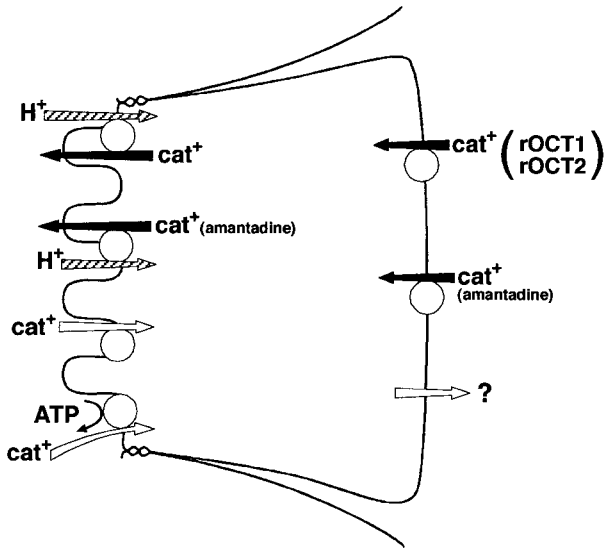

Fig. 1. Functionally distinguished and identified transport systems in the proximal tubule. In rat, rOCT1 is expressed in the S1 and S2 segments, whereas rOCT2 is expressed in the S2 and S3 segments.

ized in the proximal tubule are summarized. In the basolateral membrane, two potential-dependent cation uptake systems have been distinguished which mediate the first step in cation secretion [5,13]. The cation efflux from the tubular epithelial cells at the luminal membrane is mediated by two proton cation antiporters with different cation specificities that operate in an electroneutral fashion. The reabsorption of cations from the tubular lumen is mediated by a polyspecific potential-dependent cation uptake system which has some preference for choline. In addition, an ATP-dependent cation transporter may be engaged [5]. The transport system(s) which are responsible for cation efflux at the basolateral cell side have not been characterized.

By northern blotting, it has been demonstrated that OCT1, OCT2, OCT3, OCTN1 and OCTN2 are transcribed in kidney [5]; however, the nephron and membrane localization has only recently been determined for rOCT1 and rOCT2 (unpublished data). We detected large amounts of rOCT1 and rOCT2 mRNA in S1, S2 and S3 segments of proximal tubules by *in situ* hybridization. By polymerase chain reactions with dissected nephron segments, small amounts of rOCT1 mRNA and rOCT2 mRNA were also found in glomeruli, and a small amount of rOCT2 mRNA was detected in the distal convoluted tubule. Employing specific antibodies, rOCT1 protein was found only in the S1 and S2 segments of proximal tubules, whereas rOCT2 protein was observed in S2 and S3 segments. The data indicate post-transcriptional regulation of OCT1 and OCT2, as has been described for rOCT1 in the liver [14]. By immunohistochemistry, both OCT1 and OCT2 were localized to the basolateral membrane of the proximal tubules. This indicates that these transporters are engaged in electrogenic cation uptake over the basolateral membrane. The functional characterized bicarbonate-sensitive amantadine transporter which does not interact with TEA [13] has not been identified. Also the previously characterized proton cation antiporters in the brush border membrane which mediate cation efflux into the tubular lumen [5] are unknown. The same is true for the potential-dependent cation transporter in the brush border membrane which has been described mainly as a choline transporter [5]. In addition, the transport systems which are responsible for the basolateral release of organic cations into the interstitium are unknown (see Figure 1). It still remains a challenging task to identify the different organic cation transporters in the proximal tubule and other segments of the nephron, and to determine the functional significance of these transporters for the reabsorption and secretion of different organic cations. In addition, the regulation of the cation transporters must be investigated in detail. Furthermore mutations in these transporters have to be elucidated which influence the excretion, reabsorption and toxicity of cationic drugs.

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