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In vivo two-photon fluorescence microscopy opens a new area for investigation of the excretion of cationic drugs in the kidney

H Koepsell[1]

Polyspecific transporters mediate excretion and reabsorption of organic cations in kidney. With *in vivo* two-photon fluorescence microscopy, excretion and reabsorption of a fluorescent cation in rat renal proximal tubules was resolved. In combination with specific inhibitors, the contribution of individual cation transporters can be determined.

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The kidney plays an important role in excretion of organic cations. Organic cations include endogenous compounds such as choline, monoamine neurotransmitters, acetylcholine, cationic xenobiotics, and a variety of cationic drugs. Renal excretion of cationic drugs is of major clinical importance, because it determines the half-lives of many drugs in the body. Cationic drugs taken up with the food are absorbed in the small intestine. For very hydrophobic compounds, absorption may be passive; however, in absorption of more hydrophilic compounds, polyspecific cation transporters are involved. Very hydrophobic drugs are preferentially taken up by hepatocytes, where they may be metabolized and excreted into the bile. Less hydrophobic drugs often pass the liver and are excreted in the kidney. In the kidney, several excretory routes are used. Drugs that are not bound to large proteins may be filtered in the glomeruli. They may be reabsorbed in the nephron. Drugs that escape filtration reach the peritubular capillaries and may be secreted into the nephron.

[1]*Institut für Anatomie und Zellbiologie, Julius-Maximilians-Universität, Würzburg, Germany*
Correspondence: *H Koepsell, Institut für Anatomie und Zellbiologie der Universität Würzburg, Koellikerstrasse 6, 97070 Würzburg, Germany.*
E-mail: Hermann@Koepsell.de

Cloning and characterization of polyspecific transporters of the ATP-binding cassette (*ABC*) family,[1] the cation/anion/zwitterion transporter (*SLC22*) family,[2] and the family of multidrug and toxin extrusion (MATE) transporters[3] increased our understanding of the molecular mechanisms of how organic cations are transported in the small intestine, liver, and kidney. Organic cation transporters of these transporter families are expressed in renal proximal tubules and in other nephron segments.[2] Figure 1a shows polyspecific cation transporters that are expressed in the renal proximal tubules of rodents. These are the electrogenic organic cation transporters OCT1 (*Slc22a1*) and OCT2 (*Slc22a2*) in the basolateral membrane, and the proton–cation exchangers OCTN1 (*Slc22a4*) and MATE1, the carnitine–cation exchanger OCTN2 (*Slc22a5*), and the multidrug resistance proteins MDR1a and MDR1b in the brush border membrane. With the use of various expression systems, the functional properties, substrate and inhibitor specificities, and regulation of these transporters have been investigated.[1–3] Some cations are translocated by most of the transporters depicted in Figure 1a, whereas others appear to be more specific for individual transporters. Inhibitors were identified that specifically block individual transporters. Significant differences concerning the regulation of different transporters and the regulation of individual transporters in different expression systems have been observed. Whereas measurements with expressed transporters are useful to characterize functional mechanisms and regulatory pathways of individual transporters, such studies cannot answer to what extent a specific transporter participates in the excretion of drugs. Experiments with animals in which genes of individual transporters are disrupted may help to answer these questions; however, compensatory up- or downregulation of other transporters may obscure the picture.

In pioneering studies, Steinhausen and co-workers used fluorescent anions to investigate renal tubular secretion by using light microscopic intravital fluorescence microscopy.[4] This method was limited by large out-of-focus fluorescence. In 1990, two-photon laser scanning fluorescence microscopy was developed in which fluorescence excitation of fluorescent dye occurs only at the focus of excitation.[5] This method enabled the researchers to collect fluorescence that is generated within a distinct optical section below the kidney surface.[6] Moreover, different locations where filtered, non-filtered, and endocytosed fluorescent dyes accumulated along the kidney nephron could be distinguished.[6] Recently a method was described to quantify the fluorescence intensities measured by *in vivo* two-photon microscopy in distinct compartments of kidney cortex.[7] Tanner *et al.*[8] introduced *in vivo* two-photon microscopy for transport measurements in the kidney and was able to dissolve different steps during renal excretion of sulfonefluorescein. Sulfonefluorescein is poorly filtered and is mainly secreted into the proximal tubule. The first step in secretion is mediated by a probenecid-inhibitable anion–dicarboxylate exchanger in the basolateral membrane. Tanner *et al.*[8] showed that probenecid decreased the ratio of sulfonefluorescein in proximal tubular cells to sulfonefluorescein in plasma of peritubular capillaries.

In 1995, Pietruck and Ullrich reported that the cationic fluorescent dye 4-(4-dimethylaminostyryl)-*N*-methylpyridinium (ASP^+) is one of the

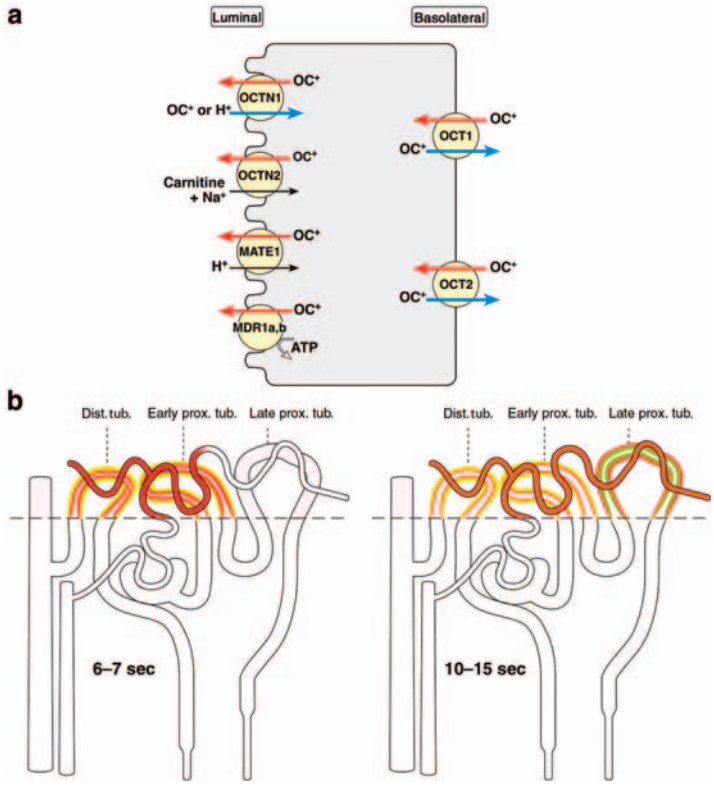

Figure 1 | Excretion and reabsorption of organic cations in rat kidney. (**a**) Transporters in the basolateral and luminal membrane of proximal tubular cells that mediate secretion (red arrows) and reabsorption (blue arrows) of organic cations. The individual transporters are described in the text. (**b**) Visualization of secretion and reabsorption of intravenously injected ASP^+ in rat kidney. *In vivo* fluorescence in the outer cortex is shown that was observed 6–7 seconds or 10–15 seconds after injection of ASP^+. Fluorescence in blood vessels and tubular cells is shown in red. Very weak fluorescence in tubular lumina is shown in light pink. Strong fluorescence on basolateral membranes of early proximal tubules and distal tubules is shown in yellow, whereas strong fluorescence at the luminal membrane of late proximal tubules is shown in green. The dashed line indicates the border of fluorescence excitation.

organic cations that are secreted in the kidney.[9] Later it was shown that ASP^+ is a substrate of OCT1 and OCT2.[2] ASP^+ was also used to study the regulation of individual organic cation transporters expressed in epithelial cell lines and to characterize organic cation uptake into isolated human proximal tubules.[2] Hörbelt *et al.*[10] (this issue) used ASP^+ to study the organic cation transport in rat kidney by *in vivo* two-photon microscopy. Six to seven seconds after injection of the dye into the jugular vein, moderate ASP^+ fluorescence appeared in some peritubular capillaries, strong staining was observed at basolateral membranes of early convoluted proximal tubules, and less-intense staining was observed within the cytosol of the proximal tubular cells (Figure 1b). Basolateral membranes of some distal tubules were also stained. Ten to fifteen seconds after ASP^+ injection, more peritubular capillaries showed ASP^+ fluorescence. The intensity of capillary staining was reduced; however, strong staining at basolateral membranes of early proximal tubular convolutes was still observed (Figure 1b). During this period of time, strong fluorescence of brush border membranes of late convoluted proximal tubules became apparent. In addition, strong staining of basolateral membranes and of distal tubular cells was observed. The contribution of organic cation transporters in ASP^+ uptake was indicated by the demonstration that ASP^+ fluorescence in proximal tubular cells was reduced by cimetidine.

The data of Hörbelt *et al.*[10] indicate heterogeneity between different parts of the nephron concerning the excretion and/or reabsorption of organic cations. They show that ASP^+ is mainly secreted within early and intermediate proximal tubular segments and that some secretion may also occur in the distal tubules. On the other hand, ASP^+ appears to be also reabsorbed in later proximal tubular segments. Does it make physiological sense that the same compound is excreted as well as reabsorbed within the nephron? Yes, if one takes into account that organic cations are translocated by polyspecific transporters with overlapping substrate specificities. Cationic substrates of organic cation transporters may be divided into three groups. One group of cations may be mainly accepted by excretion transporters, another group may be mainly accepted by transporters that are involved in reabsorption, and a third group, including ASP^+ and choline,[2] may be excreted and reabsorbed. In combination with specific inhibitors of individual cation transporters, quantitative *in vivo* two-photon fluorescence microscopy has the potential to determine the contribution of individual transporters to renal secretion and absorption of cations.

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