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Topical Review

Renal Transporters for Organic Anions and Organic Cations. Structural Requirements for Substrates

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Introduction

Recently multisubstrate specificity became popular through studies of multidrug resistance in tumor cells [16] and bacteria [43]. The phenomenon of multisubstrate specificity, however, was recognized long ago in studies of the transport of organic anions and organic cations in the kidney [11, 21, 48, 83] and the liver [36]. Actually all xenobiotics (chemicals and drugs), which are taken up by animals are finally excreted by these organs, either directly or after metabolic transformation. Thereby only few transporters are involved. The questions discussed in this review are: (i) what renal transporters participate in the transport of these substances which can be subsumed under organic anions and organic cations? (ii) What are the molecular features of substrates which are recognized by the respective transporters? In Fig. 1 the main transport systems for organic anions and organic cations on either cell side of the proximal renal tubule are depicted. Anion transporters: 1. The contraluminal para-aminohippurate (PAH)/α-ketoglutarate exchange system, the main transport system for the secretion of organic anions, not potential sensitive, cloned, sequence not yet published [38, 84]. 2. The luminal anion/anion exchange system(s); for x^- can stand: PAH, urate, Cl^-, OH^-, HCO_3^- and others, facultatively rheogenic, not yet cloned. 3. The luminal Na^+-sulfate cotransport system, rheogenic, cloned, eight putative transmembrane segments [27]. 4. The contraluminal sulfate/oxalate exchange system, not potential sensitive, cloned, twelve putative transmembrane segments [28]. 5. The luminal Na^+/dicarboxylate cotransport system, rheogenic, cloned, eight putative transmembrane segments [34]. 6. The contraluminal Na^+/dicarboxylate cotransport system, rheogenic, clone not yet identified. Cation transporters: 1. Contraluminal organic cation transport system, the main transport system for the secretion of organic cations, exchange possible, facultatively rheogenic, cloned, eleven putative transmembrane segments [15, 26, 33]. 2. The luminal H^+/organic cation exchange system, not potential sensitive, clone not yet identified. 3. The luminal choline transport system, exchange possible, potential sensitive, clone not yet identified. Other transport systems, found in the proximal tubule, but functional role not assessed and not considered in this review are: the ATP driven multidrug transport system for cationic substrates mdr 1, cloned, twelve putative transmembrane segments, located at the luminal cell side [16, 18]; the ATP driven multidrug related transport protein MRP for anionic conjugates, cloned, twelve putative transmembrane segments [7], located at the contraluminal cell side [13]; the transport system OATP, cloned in the liver [19] and kidney, located in the S3 segment of the proximal tubule at the luminal cell side, twelve putative transmembrane segments [3, 42]; a transporter for urate in the brush border of the S1 segment, cloned, one putative transmembrane segment [22, 23].

To give an overview about the specificity of the different transporters in this review, mainly transport data will be discussed that were obtained on the rat proximal tubule *in situ* with the same micropuncture technique

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Key words: Sulfate — Dicarboxylyte — Para-aminohippurate (PAH) — N^1-Methylnicotinamide (NMeN) — N-Methyl-phenyl-pyridinium (MPP) — Choline

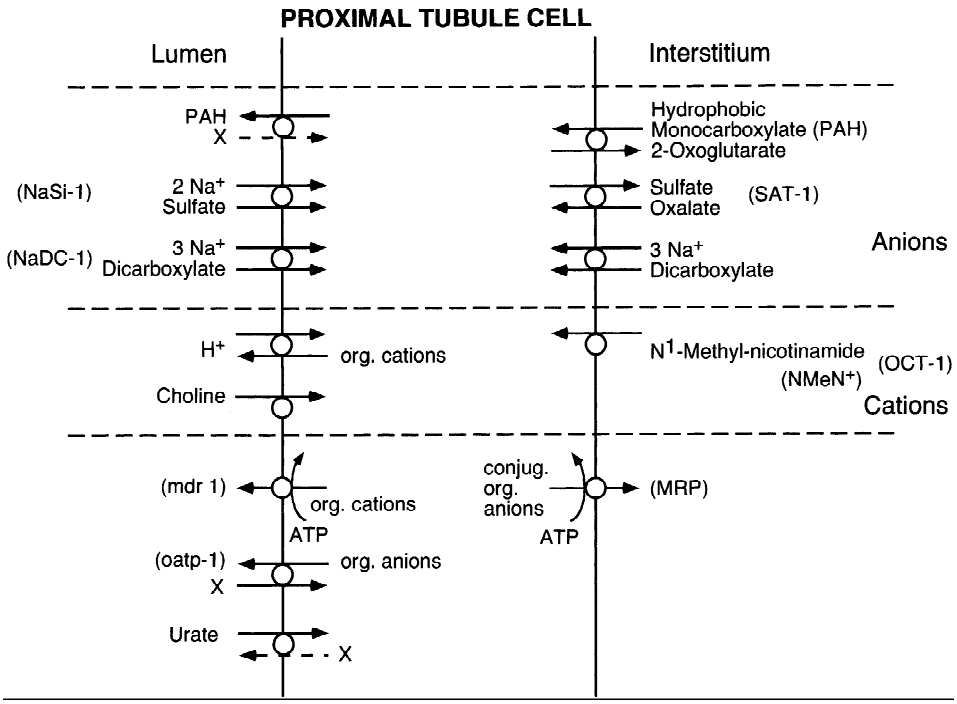

Fig. 1. Sidedness of the main transporters for organic anions and organic cations in the proximal renal tubule. NaSi-1 and NaDC-1 are related proteins (34). SAT-1 is related to sulfate transporters cloned from chondrocytes and colon cells (16a). OCT 2 is supposed to be a luminal transporter for organic cations (15a).

for the luminal and for the contraluminal cell side. For the extensive transport studies appropriate test substances were chosen [50]; para-aminohippurate (PAH), sulfate, and succinate as representative anions; N^1-methyl-nicotinamide ($NMeN^+$), tetraethyl-ammonium (TEA^+), methyl-phenyl-pyridinium (MPP^+) and $choline^+$ as representative cations. Furthermore, many series of interfering substances have been tested on either the luminal or contraluminal cell side. By applying inhibitory kinetics the apparent K_i values of a large amount of interfering substrates have been determined. Since the apparent K_i values vary with the conditions, the experimental techniques were kept constant for each test substance and each cell side. Since short term fluxes (1–4 sec) were measured and the interfering substances were applied only simultaneously with the test substances, disturbing parallel fluxes through the cell membrane and the occurrence of trans-effects were minimized. Thus, the reciprocal apparent K_i values, as determined by these procedures, are considered a reliable measure for the relative affinities of the interfering substrates for the respective transporters under in situ conditions. To reveal molecular features important for interaction with the transporters, we correlated app. affinities with the following parameters of the interfering molecules: size, ionic strength (acidity, basicity), electric charges, hydrophobicity, ability to form hydrogen bonds [20] and π-electron-NH_2 interaction [29, 44]. Furthermore, emphasis will be given on the specificity of the different transporters, while their role on the overall transport in the kidney and their mutual interactions as well as a short description of the applied methods were compiled previously [50].

Contraluminal Transport System for PAH

Substrate specificity for the contraluminal PAH-transport system was tested by measuring apparent K_i values for contraluminal PAH uptake *in situ* [68] using different groups of substrates: monocarboxylates [74], aliphatic dicarboxylates [67], mono- and polysubstituted benzene analogues [75], phenolphthaleins, sulfonphthaleins, fluoresceines [56], amino acids, di- and oligopeptides (methyl-, acetyl-, benzoylderivatives, glutathione and cystein-conjugates) [81], cyclic nucleotides, eicosanoids [80], corticosteroids [79], β-lactam antibiotics [76], sulfamoyl-compounds [54, 76] and sulfonylurea-derivatives [54], oxazaphosphorines [63], analgesics: salicylates, aniline derivatives, weak organic acid derivatives [64], mercapto-compounds and their Hg-complexes [69], and several other chemicals and drugs [8, 62, 63], which act as bisubstrates i.e., they interact with both the contraluminal PAH- and $NMeN^+$-transporter. From the large body of data available several rules for the substrate specificity of the contraluminal PAH-transporter can be established (Fig. 2).

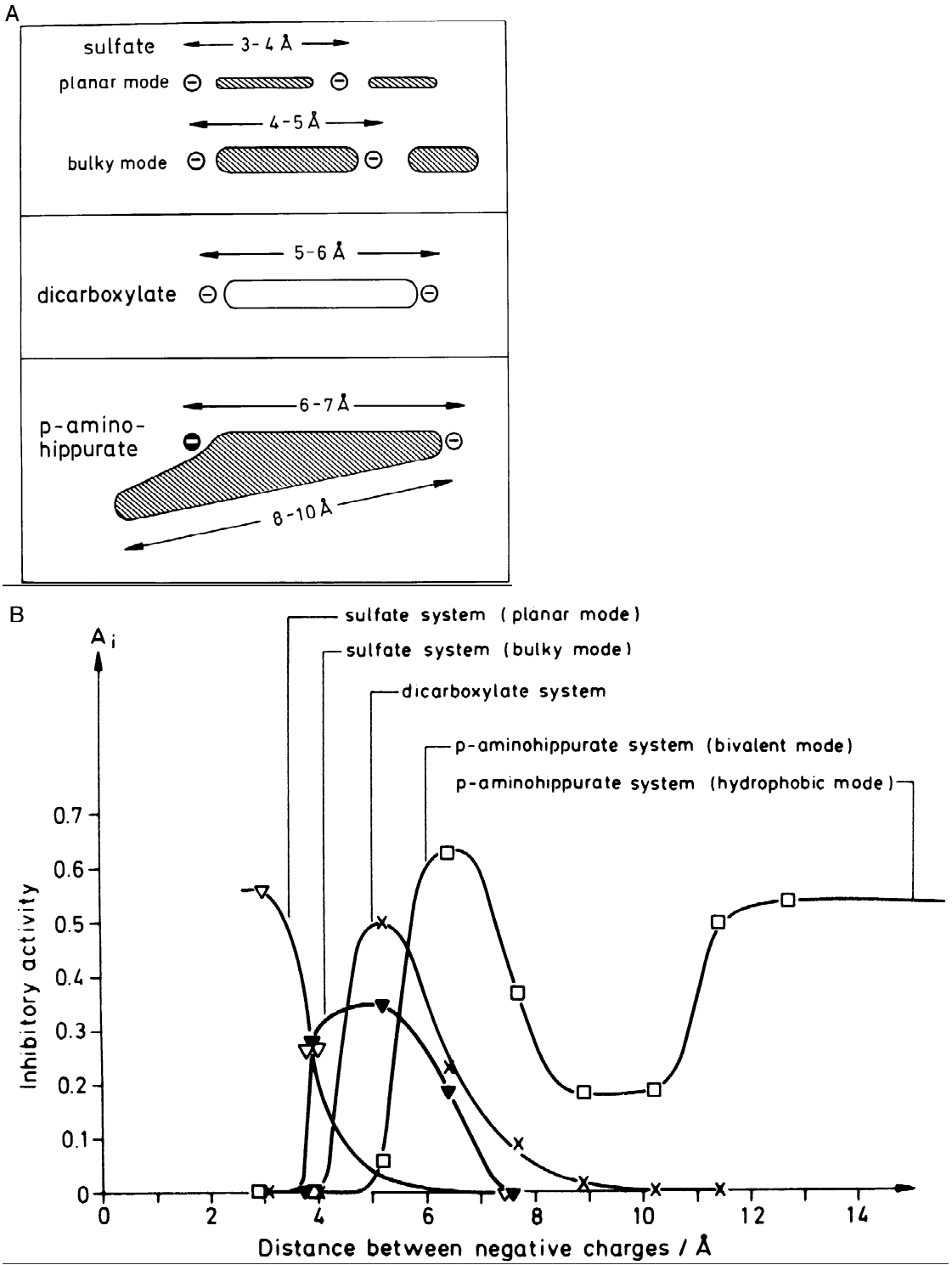

Fig. 2. Structural requirements of substrates of the three contraluminal anion transporters for sulfate/oxalate, dicarboxylates, and hydrophobic organic anions (PAH). The *sulfate/oxalate system* accepts short mono- or bivalent anions: planar molecules with COO^- residues, and flat hydrophobic domains, and a second ionic or partial negative charge at 3–4Å distance and bulky molecules with SO_3^- residues, bulky or flat hydrophobic domain and a charge separation of 4–5Å. The *dicarboxylate system* accepts bivalent anions with a charge distance of 5–6Å, whereby one charge might be a partial electronegative charge. The *PAH system* accepts monovalent anions if they bear a hydrophobic moiety of a minimal length of 4Å and substrates that have only electron attracting site groups. It also accepts bivalent anions with a charge distance of 6–7Å. For some inhibitors the hydrophobic area is longer than the spacing between the two charges. A ▨Hydrophobic domain; ⊖negative ionic charge obligatory; ⬬negative ionic charge favorable, but electron attracting or hydrogen bond forming groups sufficient. (*B*) Inhibitory activity ($K_m/(K_m + K_i)$) against distance of negative charges (From [14]).

(i) The substrate must have a certain hydrophobicity, whereby the hydrophobic domain must have a minimal length of 4 Å [14]. Thus, in the fatty acid series interaction with the transporter starts with molecules larger than valerate [74]. The hydrophobic domain can have a size of up to 10 Å. The role of hydrophobicity for interaction with the PAH transporter was impressively shown for substituted benzoates [75], amino-acids and dipeptides [81], and imidazoles [62].

(ii) The strength of the ionic charge is a determinant for the interaction with the contraluminal PAH-transporter. Thus, for substituted phenols and benzene carboxylates a direct relationship exists between pK_a and app. K_i values [75].

(iii) The PAH-transporter also accepted hydrophobic substrates which cannot be ionized: benzene aldehydes [75], steroid hormones [79] and aniline derivative analgesics [64]. Also with ionizable substrates the carrier does not sense the degree of ionization [57].

(iv) Electron attracting side groups (Cl, Br, NO_2) augment the interaction with the PAH transporter, whereby their electron attracting power (Hantsch constant), their number and their location within the molecules play a role [62, 75].

Thus, the available data point to the possibility that the interaction of a substrate with the contraluminal PAH carrier is achieved (*a*) by hydrophobic interaction; (*b*) not or not only by ionic interaction, but rather by formation of possibly multiple H-bonds, whereby the anionic site might also be involved. And indeed a negative correlation was found between the app. $K_{i,PAH,cl}$ values and the number of H-bond acceptors of ''bisubstrate'' molecules (*see below*), i.e., substrates that interact with the contraluminal PAH- and NMeN transporter [62, 63]. Cl^--ions which augment PAH transport are also known to be good gap fillers in H-bond formation [20]. In general, hydrogen bond formation seems to be an advantageous mechanism for the interaction of a substrate with its transporter. The strength of hydrogen bonds, especially when several bonds are involved, seems to be high enough to stabilize the complex of substrates and transporters. On the other hand the interaction is weak enough to allow rapid dissociation from the carrier. In this context it is interesting to note that the carboxylic group of fatty acids is linked to the intestinal fatty acid binding proteins by an electrostatic network that includes seven H-bonds [41].

The knowledge of the primary and secondary structure of the PAH transporter is necessary to understand how the carrier is able to accommodate such a large variety of substrates, among them many drugs. For the latter reason it is also important to know, how the renal PAH transporter is related to the similar transport system(s) in the blood brain barrier (chorioid plexus) [1, 2, 4, 85], in the ciliary body of the eye [2], in liver [36], and in many other cells, where probenecid sensitive anionic fluorophore transport (fluorescein, Fura 2 etc.) was observed [12, 25, 35, 49].

Luminal Transport System(s) for PAH

The luminal transport step of PAH, i.e., transport from the cell into the tubular lumen, was mostly investigated in brush border membrane vesicles under simplified conditions, i.e., usually with no Cl^-, HCO_3^- and Na^+ ions present in the incubation medium [for literature *see* 60]. It was found that luminal PAH and luminal urate transport have much in common despite significant species differences. They are potential sensitive and/or involve exchange with PAH, urate, OH^-, Cl^-, HCO_3^-, 2-oxoglutarate and lactate. The general approach to reveal the specificity of luminal transporters, i.e., to measure fluxes from the lumen into the cell and its variation by interfering substrates, was not successful for PAH *in vivo*. Therefore, another approach was chosen: Loading tubular cells *in situ* by microperfusion of the peritubular capillaries with ^{3}H-PAH and measuring the initial ^{3}H-PAH flux from the cell into the tubular lumen. With this technique only trans-effects could be investigated. Interestingly, no trans-stimulation was seen. However, a semiquantitative pattern of trans-inhibition by different groups of substrates could be demonstrated. Trans-inhibition was seen with 2 OX substituted benzoates: (2-OH-benzoate, 2-methoxy-benzoate, 2-OH-benzoate-acetyl ester, 2-OH-3,5-NO_2-benzoate), and with multi-Cl substituted benzoates (3,5-Cl-benzoate, and 2,3,5-Cl-benzoate). No effect was seen with benzoate, 3-OH-benzoate, 2-Cl-benzoate, 2-NO_2-benzoate, 2,5-NO_2-benzoate, 3-SO_2NH_2-benzoate and 4-SO_2NH_2-benzoate. However, analogues of the latter two compounds possessing two additional side groups such as furosemide and piretanide or a hydrophobic moiety as probenecid were inhibitory. Phenoxyacetate had no effect; it inhibited, however, if it had in addition three chloro-groups as in 2,4,5-Cl-phenoxyacetate or a hydrophobic carbamoyl side group as in mersalylic acid. Benzene-sulfonate trans-inhibited as did phenolsulfonphthalein (phenol red) and sulfofluorescein. However, the trans-inhibitory effect of the corresponding carboxy-compounds was absent (phenolphthalein) or weaker (fluorescein). The trans-inhibitory effect of the uricosurics ethacrynic acid, tienilic acid indacrinon and benzbromarone could be attributed to two chloro or bromo side groups on the benzene ring. Other trans-inhibiting uricosuric substances were indomethacin, sulfinpyrazone, losartan its metabolite EXP 3174, and AA 193. These organic acids with pK_a values between 2.8 and 4.9 possess Cl^-, SO-groups, heterocyclic 5-ring and hydrophobic ring or chain areas.

Most of these uricosuric agents were tested by F. Roch-Ramel et al., in human brush border membrane vesicles [40] and by Dan and Koga [8] in rat brush border membrane vesicles for their ability to inhibit urate transport. It was found that they cis-inhibit with high affinity all modes of urate/anion exchange (urate/urate exchange, urate/lactate exchange, urate/Cl^- exchange and urate/OH^- exchange) as well as potential driven urate transport. These data suggest that those substrates which have a high affinity to the transporter exert trans-inhibition, while substances with lower affinity, such as PAH itself, show no trans-effect. Anyhow, the luminal PAH transporter(s) is/are highly unspecific, whereby a 2-hydroxy-/2-oxo-group, several Cl groups on a benzene ring, a SO_3^- group, hydrophobic ring moiety or a heterocyclic 5-ring structure (π-electron/NH_2-protein interaction) favors interaction.

Contraluminal Transport System for Sulfate

The specificity of the contraluminal sulfate transporters was tested for tetrahedral molecules [70], sulfonates, amino-sulfonates [71] disulfonates, di- and tricarboxylates [72], salicylate analogues [73], phenolphthaleins, sulfonphthaleins, sulfamoyl compounds, diphenylamine-2-carboxylates [77], substituted benzene analogues [75], and sulfate esters [65, 71]. The available data allow a chemical structure/sulfate transporter interaction relationship with regard to the size and charge distribution of the interacting molecules [14, 77]. With regard to the latter, it should be kept in mind that 6 or 5 member ring structure can form negative π-electron/NH_2-protein interaction [29, 44].

Concerning the rough structure of the molecules inhibiting contraluminal sulfate influx, five categories can be discriminated [77]: to category Ia belong substances which have a molecular structure similar to sulfate, i.e., thiosulfate, selenate, molybdate [70], to Ib oxalate, disulfonates (as SITS and disulfonic dyes), dicarboxylates, sulfo-carboxylates [72], and diphosphonates [61] which have two negative ionic charges in the proper distance. To category II belong sulfate-monoesters [71]. To category III belong compounds with a SO_3^- group and a nearby located OH^- or NH group: for example 2-hydroxyethanesulfonate, benzene-aminosulfonate, NAP-taurine, 8-anilinonaphthalene-1-sulfonate [71] and diphenylamine-2-aminosulfonate [77]. To category IV belong sulfonates with a hydrophobic moiety as naphthalene-1-sulfonate [71], naphthalene-phosphonates [61] pyrene-3-sulfonate [71], phenol-red and analogues [77]. To category V belong carboxylates with a nearby located OH^- or NH group, but in addition with an electronegative side group or hydrophobic region on the benzene ring: substituted sulfamoyl-benzoates and diphenylaminecarboxylates [77]. The existence of at least five categories of substances interacting with the contraluminal sulfate transport system leads to the assumption that a complex interaction involving at least 4 reaction sites must occur.

A detailed analysis of inhibitors of contraluminal sulfate transport reveals that two types, planar mode and bulky mode, can be discriminated (Fig. 2) [14]: Planar anions like oxalate or maleate yield a significant inhibition for charge distances from 3 to 4 Å [72]. The insertion of a CH_2 group between the planar charges of the COO^- makes the molecule ''bulky'' and prevents inhibition. Among 150 tested substrates, no inhibitor was found where planar COO^- charges have been combined with bulky CH_2 or CH_3 residues [72, 77]. Anions with bulky outer charges, such as SO_3^- yield good inhibition for charge distances up to 7 Å, even when they include other bulky groups. Thus, methane- and methane-disulfonates are as effective as 1,3-benzene-disulfonate [72]. In this category also belong he diphosphonates [61]. Monovalent anions are good inhibitors of the sulfate transport, if there is an electrophilic side group in the immediate neighborhood of the negative ionic charge. Thus, 2-chlorobenzoate is an effective inhibitor, whereas 3- and 4-chlorobenzoate are ineffective [75]. Similar is the situation with Cl, F-methyl phosphonate while ethyl-phosphonate does not interact [61]. With a series of polysubstituted benzene analogue it was shown that electronegative groups in the neighborhood of the negative charge significantly increase the inhibitory activity [71, 72]. On the contrary, the inhibition of the sulfate transport by naphthalene-1- and naphthalene-2-sulfonate and by anthracene-1- and pyrene-3-sulfonate [71] as well as with naphthyl-methyl-phosphonate and analogues [61] might be attributed to the π-electron situation of the multiring structure besides the negative ionic charge.

The influence of acidity on inhibitory activity was studied with monosubstituted benzoates, but these anions are weak inhibitors of the sulfate transport. There is a general tendency that acid dicarboxylates yield strong inhibition, but this effect is paralleled by the short distances between the charges. Thus, although acidity seems to enhance the inhibition, an unambiguous relation is difficult to establish. Considering the structure of the extremely strong inhibitor, bromophenol blue, supports this conjecture: Bromophenol blue is a planar molecule with a bulky charged SO_3^- group. It contains four Br residues with strong hydrophobicity and strong electron accepting effect. One of the bromines is located next to the SO_3^- group. The insertion of a bulky CH_3 group between the bromine and the SO_3^- decreases the inhibitory potency, indicating that the methyl group hinders interaction, even in the presence of a bulky SO_3^- group. The common features of the most effective sulfate inhibitors are: (i) at least one SO_3^- group, and (ii) electronegative domains in the neighborhood of the SO_3^- group.

Luminal Transport System for Sulfate

The specificity for substrate interaction with the luminal Na^+ dependent sulfate transporter was tested for the tetrahedral molecules sulfate, thiosulfate, molybdate, selenate [9], for benzenecarboxylates, benzenesulfonate and their hydroxy analogues [9], for fluorescein dyes and phenolsulfonphthaleins [9] as well as for some diuretics [9]. The tetrahedral sulfate-like molecules as well as oxalate and phosphate exert approximately the same inhibitory potency against luminal sulfate transport as against the contraluminal sulfate transport: thiosulfate and sulfate somewhat more luminally, oxalate somewhat more contraluminally. Some of the dicarboxylates and disulfonates tested show the same affinity to the luminal Na^+-dependent sulfate transporter and the contraluminal sulfate exchange system, whereas most of the benzenecarboxylate and benzene-sulfonate derivatives tested exhibit higher luminal than contraluminal K_i values. The inhibitory potency increases with rising numbers of substituents on the benzene ring. This effect is more pronounced for the contraluminal sulfate transporter. In general, only disulfonates and analogues as well as similarly structured compounds (5-sulfosalicylate, 2-hydroxy-5-nitrobenzenesulfonate, eosine-5-isothiocyanate) have a good inhibitory potency toward the luminal sulfate transporter (apparent K_i 0.9–3.1 mmol/l). All the tested sulphamoyl- and phenoxy diuretics, fluorescein- and phenolsulfonphthalein dyes showed no inhibition or a smaller inhibitory potency at the luminal sulfate transport system than at the contraluminal system.

Substrates ordered according to their inhibitory potency against contraluminal and luminal sulfate transport (Table 2 in [50]) gives the following picture: Poor inhibitors for both sulfate transporters, but with lower affinity to the luminal transporter, are probenecid and ethacrynic acid, moderate inhibitors H_2-DIDS and eosine-isothiocyanate, good inhibitors 8-anilinonaphthalene-1-sulfonate (8-ANS) and orange G. It should, however, be considered that none of these compounds are specific inhibitors of sulfate transport as all inhibit also contraluminal PAH transport very well [51].

Luminal Transport System for Dicarboxylates

The specificity of the luminal dicarboxylate transport system was studied on the perfused rat proximal tubules *in situ* [45] as well as with rabbit brush border membrane vesicles [86] by determining the relative inhibitory constants of ~40 dicarboxylates. The results with both preparations are very similar. At the luminal cell side dicarboxylates with a chain length of 4 and 5 carbon atoms have a high affinity, while molecules with a chain length of 3 and 6 carbon atoms as well as cyclic compounds interact with much lower affinity. Thus, the size of the molecule and the distance between the two carboxylic groups are very important for the interaction with the transporting protein (Fig. 2) [14]. Introduction of a keto group in the C2-atom (glutarate → 2-oxoglutarate, succinate → acetoacetate) does hardly change the high affinity. Other substituents on C2 lead to an affinity sequence $H > OH = CH_3 > SH = NH{-}CO{-}CH_3$ suggesting that the size of the side group plays an important role. Side groups on both C2 and C3 atoms, prevent high affinity interaction, although low affinity interaction is still present for the meso form: again in the sequence $OH > CH_3 > SH$. Further information about molecule structure and interaction with the transporting protein is given by the tartrate (2,3-OH-succinate) stereoisomers. They interact in the sequence D(−)-tartrate > meso-tartrate > L(+)-tartrate. Thus, the transporting protein prefers the D(−)-form, and as indicated by introduction of one OH into succinate or glutarate, the change of the position of one OH to meso-tartrate is tolerated. The change of two OH groups, however, to the mirror-like L(+)-tartrate highly reduces the interaction with the transporting protein. This example provides a key information of the stereospecificity of that system.

Introduction of a double bond (succinate → fumarate, methylsuccinate → mesaconate) does not change the high affinity for the dicarboxylate transport system as long as the molecule stays in the trans-configuration. In the cis-configuration (maleate, citraconate), however, only a low affinity remains. Interestingly, with tricarboxylic aconitate the inhibitory pattern was reversed, i.e., the trans-isomer inhibited only slightly, while the cis-isomer inhibited to a larger extent. This can be explained by the assumption that both isomers interacted with the carrier by the two carboxylic groups having largest distance from each other [67].

Among the aromatic dicarboxylic acids without additional side group, terephthalate (1,4-benzene-dicarboxylate) and 1,2-benzene-diacetate show lowest apparent K_i values for dicarboxylate transport. This seems to indicate that these compounds fit to the recognition of transport site as well as the aliphatic dicarboxylates with 4 or 5 C-atoms, because the distance between the two carboxyl groups is almost identical (~6Å). All deviations to a smaller distance reduce the affinity for the dicarboxylate transport system. If a benzene ring bears two carboxy groups in meta- or para-position (isophthalate, terephthalate, pyridine-2,4-dicarboxylate, pyridine-2,5-dicarboxylate, pyrazol-3,5-dicarboxylate) a moderate affinity was found. Even insertion of a N-atom into the benzene ring or two N-atoms into the five-membered pyrazol ring is without influence on the interaction with the dicarboxylate transport system. If, however, the electronegative ring nitrogen is symmetrically located to the meta-positioned carboxy groups as in pyridine-2,6-dicarboxylate or pyridine-3,5-dicarboxylate, interaction

is reduced as compared to pyridine-2,5-dicarboxylate. This result demonstrates that the location of the pyridine nitrogen relative to the carboxy groups is important for the affinity. If an additional carboxy group is introduced into a benzene dicarboxylate, the inhibition of dicarboxylate transport depends on the orientation of the three negatively charged carboxy groups. Thus, a high affinity for the dicarboxylate transport system is retained in the case of 1,2,4-benzene-tricarboxylate, whereby the carboxyl groups in 1 and 4 position are in optimal distance to each other. However, when the three carboxy groups are close together as in 1,2,3-benzene-tricarboxylate or orientated symmetrically as in 1,3,5-benzene-tricarboxylate the inhibition of the dicarboxylic acid transport by these compounds is abolished. Sulfonate can replace carboxylate in the aromatic dicarboxylates although the affinity toward the dicarboxylate transport system is somewhat reduced [75]. Similarly a phosphonate group can replace a carboxylate group in aliphatic phosphono-carboxylates [61].

Contraluminal Transport System for Dicarboxylates

The specificity for the contraluminal dicarboxylate transport system [53] is very similar to that of the luminal one [53, 86]. The affinity of dicarboxylic acids toward the transport system is primarily determined by the distance between the two carboxylic groups (Fig. 2). Thus highest affinity was observed with succinate, glutarate, mesaconate, and terephthalate. Trans-configuration is preferred over the cis-configuration. One substitution (O, OH, SH, CH_3) hardly influences the affinity (2-oxoglutarate, malate, mercapto- and methylsuccinate) while two substitutions or a NH_3^+ group reduce the affinity significantly (tartrate, dimercaptosuccinate, aspartate). There are, however, also differences between the contraluminal and luminal dicarboxylate transport systems. Thus, dimethylsuccinate inhibits the contraluminal, but not the luminal dicarboxylic transport system. The situation is similar with meso-2,3-dimercaptosuccinate, cis-aconitate, benzene-1,3-disulfonate, pyridine-3,4- and pyridine-2,6-dicarboxylate. These substances therefore might be particularly useful for differentiating luminal and contraluminal dicarboxylate transport systems. On the other hand, pyruvate and DL-isocitrate had luminally much higher inhibitory potency than contraluminally. Disregarding these differences, there exists a significant agreement in the measured values of the luminal and contraluminal K_i values for the majority of dicarboxylic acids [82]. There exists, however, an interesting difference between luminal and contraluminal dicarboxylate transport during starvation [82]. In the rat, after 3 days starvation, luminal dicarboxylate transport was augmented, while contraluminal dicarboxylate transport remained unchanged. In agreement with this, overall active secretory transport rate of methylsuccinate was found to be reduced, as judged by the transtubular static head concentration difference.

There are, however, a number of monocarboxylates which interact also with the dicarboxylate transport systems: pyruvate [5, 30, 32, 45, 53], monomethylester of succinate and glutarate [14] poly-Cl and poly-NO_2 substituted benzoates [75], phenol and some Cl^- and NO_2^- substituted phenols [75]. These data indicate that a partial electronegative charge or electron attracting groups at a proper distance to the negative ionic charge might be sufficient for interaction with the dicarboxylate carriers (Fig. 2) [14]. However, dimer formation as in the case of phenol must also be taken in consideration.

As already discussed above there exists an overlapping substrate specificity of the contraluminal dicarboxylate transporter with the contraluminal PAH transporter in regard to succinate, glutarate, adipate [6, 56, 67], benzene-dicarboxylate and -sulfonate [6, 56, 75], polysubstituted benzencarboxylates [75], and polysubstituted phenolphthaleins [6, 56]. The overlapping specificity of the contraluminal PAH transporter for 2-oxoglurate is the prerequisite that in the kidney in situ intracellular α-ketoglurate provides the driving force for contraluminal uptake of PAH and other substrates of the PAH transporter [37, 46].

Contraluminal Transport System for $NMeN^+$/TEA^+

Substrate specificity for contraluminal organic cation transport was tested with N^1-methylnicotinamide ($NMeN^+$) and tetraethylammonium (TEA^+) by measuring apparent K_i values of different groups of substrates: primary, secondary, tertiary amines, mono- and bisquaternary compounds [55], anilines, phenylalkylamines (catecholamines), pyridines, quinolines, acridines [62, 78], imidazoles, guanidines, hydrazines, piperidines, piperazines, azepines [62], dipeptides, cephalosporines, quinolone-carboxylate gyrase inhibitors, steroid hormones, cyclophosphamides [63], sulfamoyl-, sulfonylurea-, thiazide-, and diphenylamine-carboxylate-analogue [54]. The specificity requirements for substrates of the contraluminal NMeN/TEA transporter are astonishingly similar to that of the contraluminal PAH transporter; but in place of acidic, electron-attracting, electrophilic, stand now basic, electron-donating, nucleophilic. Thus, the rules are:

(i) Interaction with the contraluminal NMeN/TEA transporter increases with increasing hydrophobicity (log oct > −1,0) (Fig. 3), [55] whereby ionization does not play a role (Fig. 6 in [57]). Ionization, on the other hand prevents penetration through the lipid bilayer [62]. With other words: permanently ionized quaternary ammonium compounds penetrate the cell membrane only by a transporter (except they contain extremely hydrophobic groups) while ionizable compounds of compa-

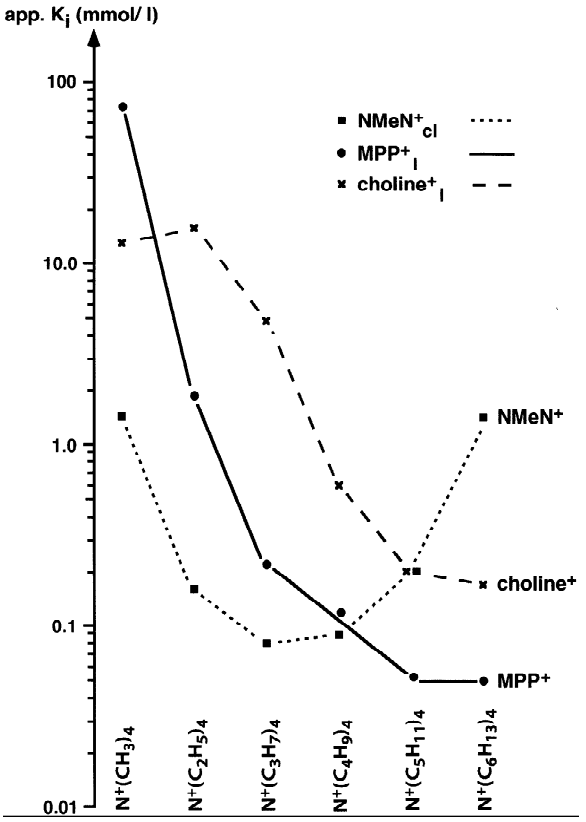

Fig. 3. Effect of hydrophobicity and size of tetralkyl-ammonium compounds against their app. K_i values on contraluminal organic anion ($NMeN^+$), luminal H^+/organic cation (MPP^+) and luminal choline$^+$ transport. (From [59]).

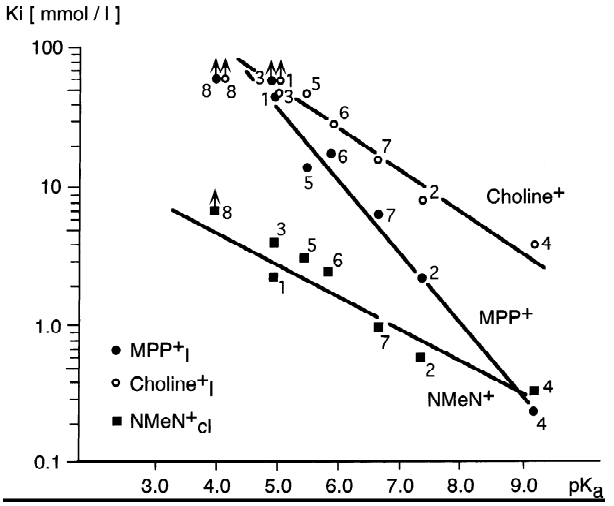

Fig. 4. Plot of the pK_a values of the different aminoquinoline analogues against their app. K_i values on contraluminal organic anion ($NMeN^+$), luminal H^+/organic cation (MPP^+) and luminal choline$^+$ transport. (From [59]).

rable hydrophobicity penetrate the cell membrane through the lipid bilayer by nonionic diffusion. There are, however, compounds such as guanidine, where hydrophobicity does not seem to play a role [62].

(ii) The strength of the ionic charge is a determinant for the interaction with the contraluminal NMeN/TEA transporter. Thus, for the heterocyclic pyridine-, quinoline-, isoquinoline- and acridine analogues an inverse relationship between pK_a values and app. K_i values was seen (Fig. 4), [55]. OH- or NH_2-side groups seem to influence the affinity by changing the pK_a values.

(iii) Substances which have the amino group directly on the benzene ring (aminobenzenes, aminonaphthalenes) do not interact with the contraluminal NMeN/TEA transporter even when they are quite hydrophobic.

(iv) The contraluminal NMeN/TEA transporter also accepts hydrophobic substrates which cannot be ionized: steroid hormones and cyclophosphamides [63]. Furthermore, the transporter does not sense the degree of ionization of a substrate [57].

(v) Introduction of electrophilic C^-, Br^-, NO_2 groups might increase interaction with the NMeN/TEA transporter (phenylhydrazines in [62], sulfamoyl compounds in [54]), might decrease it (aminothiazoles and aminopyridines in [62]) or might be without influence (phenylguanidines in [62]). Thus, the electrophilic and H-bond forming side groups might not only act by influencing the pK_a values, but also by direct interaction with the transporter.

The available data indicate that most N-containing substrates might serve as substrates for the contraluminal NMeN/TEA transporter whereby their pK_a values can be quite low (Fig. 4). Ionic interaction is not necessary. Since also steroid hormones, which do not contain a N-atom but several H-bond forming OH groups, interact with the NMeN/TEA carrier, also H-bond formation, besides hydrophobic interaction, seems to be a crucial event.

That certain OH-groups are important for the interaction with the contraluminal organic cation ($NMeN^+$,TEA^+) transporter was convincingly demonstrated with morphine analogues [58]. Here those compounds which carry on C atom 6 an oxo-group have a three times higher affinity than the analogues with an OH-group in the same position. The low specificity of the contraluminal organic cation ($NMeN^+$,TEA^+) transporter is well documented by the finding that amongst 10 pairs of cationic enantiomers/diastomers only three show stereospecificity but only with affinity differences by not more than a factor of three [47].

Luminal Transport System for H^+/Organic Cation (MPP^+) Exchange

While in the contraluminal cell membrane one common transport system for organic cations seems to exist [39]

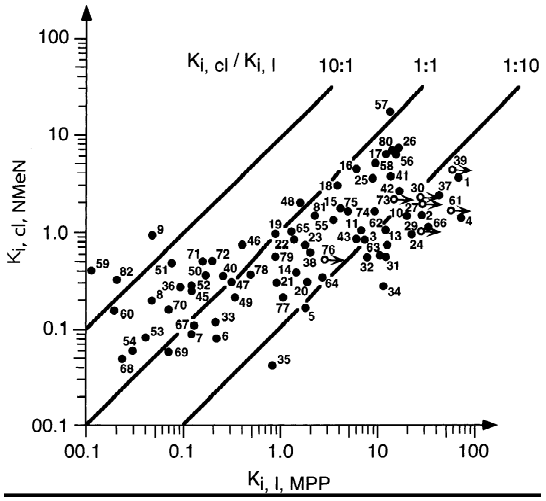

Fig. 5. Comparison of the app. K_i values of different substrates on luminal H^+/organic cation (MPP^+) and contraluminal organic anion ($NMeN^+$) transport. (For the code of the numbers *see* [10]).

the luminal brush border membrane contains more than one transport system for organic cations [24]: en electroneutral H^+/organic cation exchanger and a rheogenic choline transporter (for literature *see* [10]). Using the luminal stop flow microperfusion technique the 2 sec efflux of N-methyl-4-phenyl-pyridinium$^+$ (MPP^+) from the tubular lumen into proximal tubular cells via the H^+/organic cation exchanger was measured and with competitive inhibition kinetics the app. $K_{i,l,NMeN^+}$ values of the following cationic substrates were determined [10]: Aliphatic amines, tetraacyl-ammonium compounds, benzylamine derivatives, piperidines, piperazines, pyridines-pyridinium compounds, quinolines, acridines-acridinium compounds, benzodiazepines, cyanine dyes, pseudoisocyanines, imidazoles, guanidines, cephalosporines, quinolones, corticosteroids, aminoindoles [10], morphine analogues [58], ephedrine analogues and other cationic stereoisomers [47], N-containing diuretics [52]. It was found that for the luminal organic cation MMP^+/H^+ exchanger: (i) limitation by molecular size occurs at larger size than for the contraluminal organic cation ($NMeN^+$) transporter and at smaller size than for the luminal choline transporter (Fig. 3), [59]; (ii) affinity increases with hydrophobicity (Fig. 3) — (see also BBM vesicle data of Wright et al. [88] — with a similar slope than for the other two organic cation transporters; (iii) affinity increases with basicity (14- pK_a values) with a steeper slope than for the contraluminal organic cation ($NMeN^+$) transporter and the luminal choline transporter (Fig. 4), [59]). Considering all tested organic cations there exists a relationship between the affinity to the luminal H^+/organic cation (MPP^+) exchanger and that to the contraluminal organic cation ($NMeN^+$) transporter with a slope <1 (Fig. 5). This means that a substance with a relative high (low) affinity to one transporter has also a relative high (low) affinity to the other transporter. Furthermore, a slope <1 says that the affinity range for the contraluminal organic cation ($NMeN^+$) transporter is smaller than for the luminal H^+/organic cation (MPP^+) exchanger. Thus, it follows that at high absolute affinity to the luminal H^+/organic cation (MPP^+) exchanger the absolute affinity to the contraluminal organic cation ($NMeN^+$) transporter is lower and vice versa that at low absolute affinity for the luminal H^+/organic cation (MPP^+) exchanger the absolute affinity to the contraluminal organic cation ($NMeN^+$) transporter is higher (Fig. 5) [10]). Furthermore, a relationship exists between the affinity to the luminal H^+/organic cation (MPP^+) exchanger and the luminal choline transporter (Fig. 8 in [59]) whereby the affinity to the exchanger is higher than to the choline transporter but becomes equal for substrates with low affinities. It was seen in experiments with morphine analogues [58] that certain side groups are important for the interaction with the luminal H^+/organic cation (MPP^+) exchanger. Here those analogues with methoxy- and/or methylamine groups have a lower affinity than those with corresponding OH-/NH-groups. The unspecificity of the luminal H^+/organic cation (MPP^+) exchanger is well documented by the finding that amongst 10 pairs of enantiomers/diastomers only three, i.e., ephedrine, norephedrine and norpseudo-ephedrine show stereospecificity with $K_{i,l,MPP}$+ values varying by not more than a factor of 3.6 [47].

Luminal Choline Transporter

Using the luminal stop flow microperfusion technique 2s efflux of choline$^+$ from the tubular lumen into proximal tubular cells was measured [59]. In competitive inhibition studies the app. $K_{i,l,choline}$+ values of the following cationic substrates were determined: alkyl-, and arylamines; NH_2 and OH substituted quinolines; methylphenylpyridinium (MPP^+), methylamino-styryl- and rhodamine compounds; acridine- phenanthrene and cyanine compounds; aminoethanol- and aminopropanol analogues; OH- SH- and NH_2 containing ethylamine analogues; choline esters. With most of the tested compounds K_i values against luminal H^+/organic cation (MPP^+) exchanger and the contraluminal organic cation ($NMeN^+$) transporter were measured in parallel. Thus the affinity of many substrates to the three organic cation transporters in the proximal tubule can be compared with each other. For the interaction with the luminal choline transporter the following substrate parameters are important [59]: hydrophobicity (size) and basicity (14-pK_a values) (Fig. 4). The effect of hydrophobicity was seen at larger size, i.e., larger hydrophobic moiety, than for the contraluminal organic cation ($NMeN^+$) transporter and the luminal H^+/organic cation (MPP^+) exchanger (Fig. 3), [59]. As tested with aminoquinoline

analogues the negative slope of app. $K_{i,l,choline^+}$ values against their pKa values is for the choline transporter actually the same as for the contraluminal organic cation ($NMeN^+$) transporter, although at a higher K_i level (Fig. 4), [59]. The negative slope of the relationship between app K_i values and the pK_a values of substrates for the luminal H^+/organic cation (MPP^+) exchanger is steeper, so that at low pK_a values the K_i values for the choline$^+$ and the MPP^+ transporter are identical and at high pK_a values those of the MPP^+ and $NMeN^+$ transporter. These data indicate that the affinity of the aminoquinolines toward all three transporters increases with the basicity of the substrates, whereby the choline$^+$ and $NMeN^+$ transporter show equal sensitivity to pK_a while the H^+/organic cation (MPP^+) exchanger shows a higher sensitivity. By comparing the $K_{i_{l,choline^+}}$ values of a large group of compounds with their K_{i,l,MPP^+} and $K_{i,cl,NMeN^+}$ values, a direct correlation was observed (Figs. 5 to 8 in [59]). However, two groups of compounds can be discriminated. First, the group of aminoethanol, choline and choline esters which have a higher affinity to the choline transporter than to the luminal H^+/organic cation (MPP^+) exchanger and to the contraluminal organic cation ($NMeN^+$) transporter (*see also* BBM vesicle data of Wright et al. [87]. To this group belong also dimethyl-amiostyryl-pyridinium-, rhodamine-, and cyanine dyes (Figs. 5 and 6 in [59]). Second, all other tested compounds which have an equal or lower affinity to the choline transporter than to the other two transporters (Figs. 7 and 8 in [59]). Probably hydrogen bond formation of the OH group in choline and analogues is involved in binding to the choline transporter. The OH group can apparently be functionally replaced by an NH_2 group in the same position, but not by an SH or a Cl group. Also a carboxyether-, thioether-, or phosphoether group in the same position can serve this function. In the polycyclic dyes which have high affinity to the choline transporter amino-aromatic hydrogen bonds [29, 44] might be involved. It is interesting that the luminal choline transporter has not only the same slope of K_i/pK_a values as the contraluminal organic cation ($NMeN^+$) transporter [59] but also a similar interaction pattern for morphine analogues [58], although both times at a lower affinity level. Thus the luminal choline transporter and the contraluminal organic cation ($NMeN^+$) transporter, which are both rheogenic, might have similar molecular structure. The low specificity of the luminal choline transporter is documented by the fact, that among 10 pairs of enantiomers/diastomers only one, namely verapamil shows stereospecificity but only with a K_i difference by a factor of 2.6 [47].

Perspective

Consecutive steps in carrier mediated transport through cell membranes are: (i) Binding of the substrate to the respective binding site. (ii) Subsequent conformation change of the carrier molecule. (iii) Release of the substrate at the other side of the membrane. The structure of the substrates, as discussed in this review, and the structure of their binding sites must match each other. Thus, as depicted in Fig. 2 for organic anions, the structure of the substrates must fit mirrorlike to the structure of their binding sites at the carriers, whereby an electronegative site at the substrate must match with an electropositive site at the transporter, both connected by electrostatic forces. Hydrophobic sites must match with hydrophobic areas. By analogy with other better known transporters the binding site is supposed to be located in the transmembrane region of a pore formed by four to six membrane spanning α-helices [31, 89, E. Wright, *personal communication*], or an antiparallel β-barrel comprised of sixteen or eighteen strands [17]. As mentioned in the Introduction only for some, not all, renal transporters for organic anions and organic cations the primary structure inclusively hydropathy plot is known. It is hoped that in the future clone expression, mutagenesis, in concert with selective affinity labeling may help to identify the substrate binding sites at the different transporters. (For interaction of a variety of affinity labels with the contraluminal anion transporters see [66]).

It is astonishing that each of the few transporters for organic anions and organic cations interact with a huge variety of chemically unrelated substances. In most cases, an ionic or electronegative or electropositive partial charge and a hydrophobic moiety are satisfactory for their interaction with the transporters. Furthermore, it became evident that multiple hydrogen bond formation favors the interaction between substrate and transporter. While we know now a lot about substrates for the different transporters, we ought to learn in the future more about the structure of the transporters and the mode they translocate substrates through cell membranes.

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