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Anion transport through the contraluminal cell membrane of renal proximal tubule. The influence of hydrophobicity and molecular charge distribution on the inhibitory activity of organic anions

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Three different mechanisms of anion transport have been identified for the contraluminal membrane in the proximal tubule of the rat kidney. These mechanisms are specific for the transport of sulfate, dicarboxylate and *p*-aminohippurate anions. Sulfate transport is inhibited by bivalent organic anions with a distance between the charges of less than 7 Å. The sulfate system acts in two modes: in a planar mode for anions with flat charged residues such as COO^- and a charge separation of 3–4 Å or in a bulky mode for groups such as SO_3H^- and a charge separation of 4–7 Å. Monovalent anions can be accepted if there is a hydrophobic core next to the negative charges. Dicarboxylate transport is inhibited exclusively by anions with two charge centers located within 5 to 9 Å, one of those possibly being a partial charge of −0.5 elementary charges. *p*-Aminohippurate transport is inhibited by monovalent anions, if these have a hydrophobic domain with a minimal length of about 4 Å. Bivalent anions inhibit, if they have a charge distance of 6–10 Å; both charges can be partial charges of about −0.5 elementary charges. Longer bivalent anions can be effective provided they have a sufficiently large hydrophobic domain. For the sulfate and *p*-aminohippurate systems it is found that anions with high acidity yield good inhibition. The overlapping specifities of the three systems with respect to charge distance and hydrophobicity allow them to accept a large variety of organic anions.

Introduction

In previous studies it was established that the fluxes of sulfate, dicarboxylates and *p*-aminohippurate through the contraluminal membrane of the proximal tubule of the rat kidney exhibit different patterns of inhibition: Transport of *p*-aminohippurate is markedly inhibited by a series of monovalent anions with hydrophobic domains [1], while transport of sulfate is less inhibited by those anions [2,3] and transport of dicarboxylate (succinate) is almost insensitive to monovalent inhibitors [1,4]. Sulfate transport is inhibited by short bivalent anions [3] while the succinate transport is inhibited by medium-sized bivalent anions [4]. The *p*-aminohippurate transport system, on the other hand, exhibits a complex relationship between the inhibitory activity and the length of bivalent anions [5]. From these experimental results it is obvious that not only the number of and the distance between the negatively charged residues determines the inhibition but also the amount of the charge, the nature of the neighboring groups, steric features, hydrophobicity and acidity of the applied inhibitors. Since the inhibitory activity of the applied anions is a superposition of all these effects, a systematic inspection of the patterns of inhibition is needed in order to characterize the binding properties of the transporting sites. This has been done for a series of different anion-transporting systems, e.g., in red blood cells [6,7]. Many inhibitors have been applied in order to construct models for the binding and transporting sites. In an early review [8] the significance of inhibition of the renal transport system by *p*-aminohippurate-like anions has been recognized and the binding of inhibitors was thought to be realized by a contact of three points between inhibitor and transporter. Later, it was conjectured that hydrophobicity, hydrogen bonds and aromatic groups also play an important role for the anion transport (for review see Ref. 9). More recently, criteria for three different anion transporting systems have been found [1]. Their identification facilitates the interpretation of these data drastically. In the following, two questions are raised: How can the diversity of the

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experimentally obtained inhibitory patterns be unified to a consistent picture? What conclusions must be drawn with respect to the structural organization of the anion transporting system?

Method

The experimental procedure of the stopped flow capillary perfusion method has been described in detail [2,10]. Briefly, the measurements of fluxes from the interstitium into proximal tubular cells of male Wistar rats have been carried out in situ at 37 °C. By clamping the renal artery and vein, the proximal tubules collapsed and the disappearance of radiolabelled test substances out of the interstitium was observed. The transport parameters have been estimated by fitting families of decay curves to a model of facilitated diffusion [10]. Let S and S' stand for the concentration of the test substance in the interstitium and cell, respectively, and set $\bar{S} = (S + S')/2$. The model of facilitated diffusion assumes a linear relationship between the observed flux, dS/dt, and the concentration difference divided by the averaged concentration, $(S - S')/\bar{S}$. Since the fluxes of sulfate, dicarboxylate and *p*-aminohippurate show saturation for high concentrations, a saturable term must be introduced in the transport equations which read:

$$\frac{dS}{dt} = \frac{V_{max} \cdot S \cdot (S - S')}{(K_m + S) \cdot \bar{S}} \tag{1}$$

(V_{max} and K_m are kinetic constants). If V_{ec} and V_{ic} denote the volume of the interstitium and cell, respectively, and S_0 the injected concentration of the test substance, the mass conservation is expressed as $SV_{ec} + S'V_{ic} = S_0V_{ec}$. Introducing the ratio $r = V_{ec}/V_{ic}$ and regarding the mass conservation, an implicit solution of Eqn. 1 exists:

$$t(S) = k \cdot \ln|(aS + b)| + pS + h \tag{2}$$

where

$$a = -(1 + r);\ b = rS_0;\ p = (1 - r)/(2V_{max}a);$$
$$k = (2K_m + rS_0 - b \cdot (1 - r)/a)/(2V_{max}a),$$

and

$$h = -k \ln|(aS_0 + b)| - pS_0$$

Different concentrations of the applied test substances yield different time-courses of the concentration decay. Eqn. 2 has been fitted to families of decay curves for sulfate, succinate and *p*-aminohippurate, respectively. For this purpose, a least-squares curve-fitting routine employing a steepest-descent method was utilized [11]. The unstable convergence of this method near the minimum was circumvented by invoking an anti-zigzag procedure. For determining the errors of the estimated parameters the mean residuals were calculated and the law of error propagation was applied.

The inhibitory activity of a many organic anions has been analysed with respect to the three test substances. Since different concentrations of the test substances as well as of the inhibitors have been applied, a quantitative measure for the inhibitory activity as a function of these concentrations must be found. For this purpose, an apparent constant is substituted for K_m in Eqn. 2: $K_{app} = K_m\ (1 + S_i/2K_i)$. S_i is the concentration of the inhibitor, K_i is the inhibitor constant, the factor 1/2 accounts for the assumption that the mean of the concentration of the test substance outside of and inside the cell is effective in Eqn. 1 and this approaches $S_i/2$ for concentrations of S_i in the millimolar range that has been applied here. If q_i denotes the fraction of S_0 that is measured in the presence of the inhibitor at time t_i, substitution of K_{app} for K_m in Eqn. 2 leads – after further rearrangements – to

$$K_i = \frac{K_m \cdot S_i}{\dfrac{(1 - r)(1 - q_i)S_0 - 2V_{max}(1 + r)t_i}{\ln|r - (1 + r)q_i|} - \dfrac{rS_0}{2(1 + r)} - \dfrac{K_m}{2}} \tag{3}$$

(K_m, V_{max} and r are the parameters of the test substances sulfate, succinate and *p*-aminohippurate, respectively). Eqn. 3 is applicable outside the linear initial rates of the time-courses and allows the determination of K_i at an arbitrary time, t_i. This is essential, as the favorable times of measurement are different for the three test anions. The experimental errors of q_i amount to 10–20%. Since the fitted values of K_m, V_{max} and r have similar errors, in some cases the obtained values for K_i are merely estimates with errors of more than 50%. Ranging from 0 to infinity, the scale for K_i is inconvenient for graphic representations. Therefore the following measure for the inhibitory activity is introduced: $A_i = K_m/(K_m + K_i)$. The value of A_i equals 0 for no inhibition ($K_i = \infty$); $A_i = 1/2$ for $K_i = K_m$; and A_i approaches 1 for $K_i = 0$, i.e., for strong inhibition.

The inhibition patterns were analysed by comparing the inhibitory activity of selected organic anions with their acidity, hydrophobicity, negative charge distribution and steric arrangement. The hydrophobic domains of the molecules have been determined by measuring the longest extents of the apolar segments of the molecules such as CH_2 and CH_3 groups or of unsubstituted ring structures. The hydrophobic properties for aromatic substituents have been taken from Hansch et al. [12]. The molecular charge distribution is a complex function for each considered anion. In many cases it suffices to consider the distance between the negative charge centers, this has been done by measuring those distances with stick-and-ball models. In Fig. 1 the de-

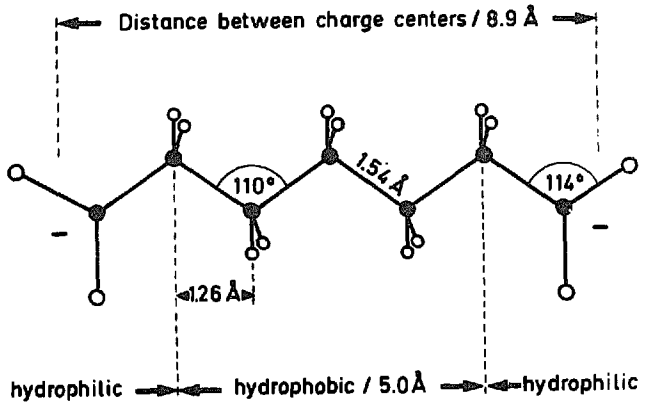

Fig. 1. The molecular geometry of aliphatic anions and the subdivision of the molecules in hydrophilic and hydrophobic moieties. Example: Pimelate. Aromatic anions are treated in an analogous manner.

termination of distances between charges and of 'hydrophobic lengths' is demonstrated. In other cases, however, the distribution of all partial charges must be worked out more carefully. This has been achieved by quantum chemical calculations carried out in the Fachbereich Physikochemie und Informatik, Fa. Schering, Berlin (Dr. Hoyer). By means of a graphic package (SYBYL Release 3.2, TRIPOS Associates), the corresponding molecules have been constructed to have the highest degree of planarity. The calculation of charge distribution has been based on the semiempirical MNDO method (AMPAC) that considers all valence electrons.

Results

The values for K_m estimated for the model of facilitated diffusion and the specific inhibitors for the transport of sulfate, succinate and *p*-aminohippurate are given in Table I. The effect of the inhibitors for each transport mechanism is analysed according to the effect of acidity, charge distribution, hydrophobic domains and steric characteristics. Most of the experimental data have been published previously (in terms of K_i instead of A_i); this is indicated in the text.

Sulfate transport

This transport can be inhibited by two types of bivalent anion. First, planar anions like oxalate or maleate yield a significant inhibition for distances between the charges from 3 to 4 Å (Fig. 2) [3]. The insertion of a bulky CH_2 group between the planar charges of COO^-, however, prevents inhibition. Examples of this include malonate, succinate and analogue dicarboxylates [2]. Among 150 applied substrates, no inhibitor was found where planar COO^- charges have been combined with bulky CH_2 or CH_3 residues [3,13]. Second, anions with bulky outer charges, such as SO_3H^-, yield good inhibition for charge distances up to 7 Å,

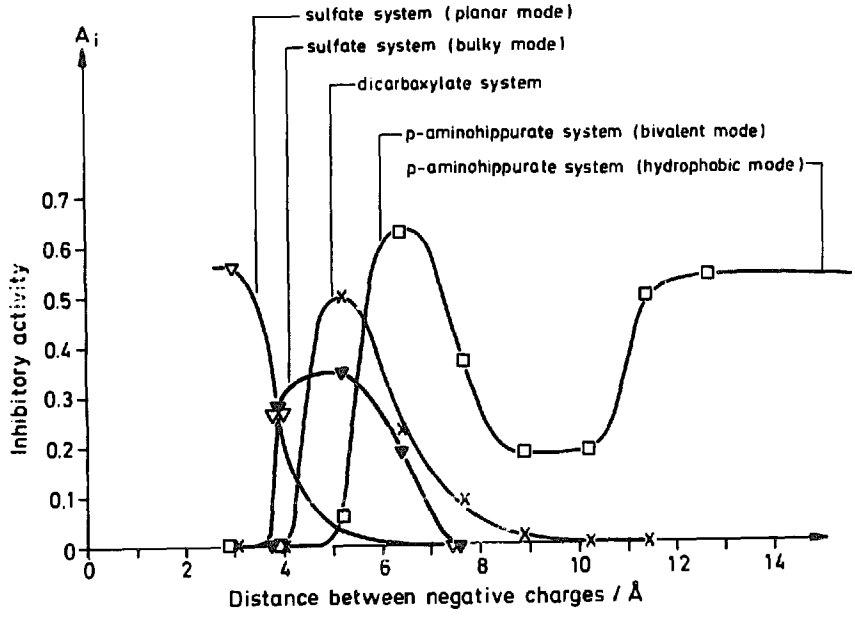

Fig. 2. The inhibitory effect of bivalent anions as function of their charge distance. The transport of sulfate is inhibited by anions with short charge distances, it behaves in two modes according the planarity of the anions (▽, A_i for sulfate inhibited by oxalate; phthalate; maleate; terephthalate; ▼, A_i for sulfate inhibited by 1,2-benzenedisulfonate; methanedisulfonate; ethanedisulfonate; 1,3-benzenedisulfonate; 1,4-benzenedisulfonate). The transport of dicarboxylate responds to medium-sized distances (×, A_i for succinate inhibited by dicarboxylates from oxalate to azelate (methylsuccinate instead of succinate)) whereas the transport of *p*-aminohippurate shows a more complicated relationship due to the additional hydrophobic effect of long bivalent anions (□, A_i for *p*-aminohippurate inhibited by dicarboxylates from oxalate to sebacate). Note the overlapping effect of the three anion-transporting mechanisms: Bivalent anions of any distance between the charges are accepted at least by one of the three systems.

TABLE I

K_m values and specific properties of the transport of sulfate, succinate and p-aminohippurate

	K_m (mM)	Examples of specificity
Sulfate	1.4 ±0.3	inhibited by oxalate
Succinate	0.09±0.04	Na^+-dependent
p-Aminohippurate	0.08±0.01	inhibited by monocarboxylates

even when they include bulky groups. Thus, methane- and ethanedisulfonates are as effective as 1,3-benzenedisulfonate (Table II) [2]. Monovalent anions are good inhibitors of the sulfate transport, if there is a hydrophobic domain in the immediate neighborhood of the negative charge. Thus, 2-chlorobenzoate is an effective inhibitor, whereas 3- and 4-chlorobenzoate are ineffective (unpublished data). With a series of polysubstituted benzene rings it is shown that hydrophobic groups in the neighborhood of the negative charge significantly increase the inhibitory activity [2,3]. In the same way, the inhibition of the sulfate transport by naphthalene-1- and naphthalene-2-sulfonate and by anthracene-1- and pyrene-3-sulfonate [2] must be attributed to the hydrophobic ring structures next to the negative charges.

The influence of acidity on inhibitory activity was studied with monosubstituted benzoates, but these anions are bad 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 determine. Consideration of the structure of the extremely strong inhibitor, bromphenol blue, ($A_i = 0.93$), supposes this conjecture. It is a planar molecule with a bulky charged SO_3H^- group [13]. It

TABLE II

The influence of molecular charge distances and planarity on the inhibition of the sulfate transport

	Charge distance (Å)	Shape		Inhibitory activity
		charged residue	apolar moiety	
Oxalate	3.0	planar	planar	0.57
Phthalate	3.8	planar	planar	0.26
1,2-Benzenedisulfonate	3.8	bulky	planar	0
Malonate	3.9	planar	bulky	0
Methanedisulfonate	3.9	bulky	bulky	0.28
Maleate	4.0	planar	planar	0.27
Succinate	5.2	planar	bulky	0
Ethanedisulfonate	5.2	bulky	bulky	0.35
1,3-Benzenedisulfonate	6.4	bulky	planar	0.19
1,4-Benzenedisulfonate	7.5	bulky	planar	0
Terephthalate	7.5	planar	planar	0

TABLE III

The inhibitory activity, A_i, as function of charge distance

	Charge distance (Å)	A_i for	
		dicarboxylate	*p*-amino-hippurate
Oxalate	3.0	0	0
Malonate	3.9	0	0
Succinate	5.2	0.5	0.06
Glutarate	6.4	0.28	0.63
Adipate	7.7	0.09	0.37
Pimelate	8.9	0.02	0.19
Suberate	10.2	0	0.19
Azelate	11.4	0	0.50
Sebacate	12.7	0	0.54

contains four Br residues with strong hydrophobicity and strong electron accepting effect. One of the bromines is located next to the SO_3H^- group. The insertion of a bulky CH_3 group between the bromine and the SO_3H^- decreases the inhibitory activity from 0.93 to 0.64, indicating that the bulky methyl group hinders good inhibition, even in the presence of a bulky SO_3H^- group. The common features of the most effective sulfate inhibitors are: (1) at least one SO_3H^- group; (2) planar shape of the rest of the molecule; (3) hydrophobic and/or electron-accepting domains next to the SO_3H^- group.

Dicarboxylate transport

The transport of succinate, which we term dicarboxylate transport, is Na-dependent and is inhibited exclusively by bivalent or pseudo-bivalent anions [4,5]. The term 'pseudo-bivalent' describes monovalent anions which possess a high negative partial charge of a non-dissociated electron accepting residue. The characteristic relationship between charge distance and inhibitory activity is easily studied with the aliphatic dicarboxylates [5]. Oxalate and malonate are ineffective, medium-sized dicarboxylates with 4, 5, 6 or 7 C atoms (methylsuccinate, glutarate, adipate and pimelate) inhibit significantly (Table III and Fig. 2). These molecules have distances between the negative charges of about 5–9 Å. Maximal inhibition occurs at a distance of 5–6 Å. This distance-dependent inhibition is confirmed by substituted aliphatic and by aromatic anions. In contrast to sulfate transport, no restriction of inhibition is observed for bulky groups like CH_2 or CH_3. Monocarboxylic acids of the homologous series and benzoate do not inhibit the succinate transport [1]. Four monovalent anions, however, are found to be significant inhibitors: the monomethylesters of succinate and glutarate ($A_i = 0.02$ and $A_i = 0.06$, respectively), 3,5-diiodo-2-hydroxybenzoate ($A_i = 0.04$) and 9-carboxyphenanthrene ($A_i = 0.06$). In Fig. 3 the distribution of partial charges is represented for these molecules in order to study the possible effects of partial charges. Both the esters have a

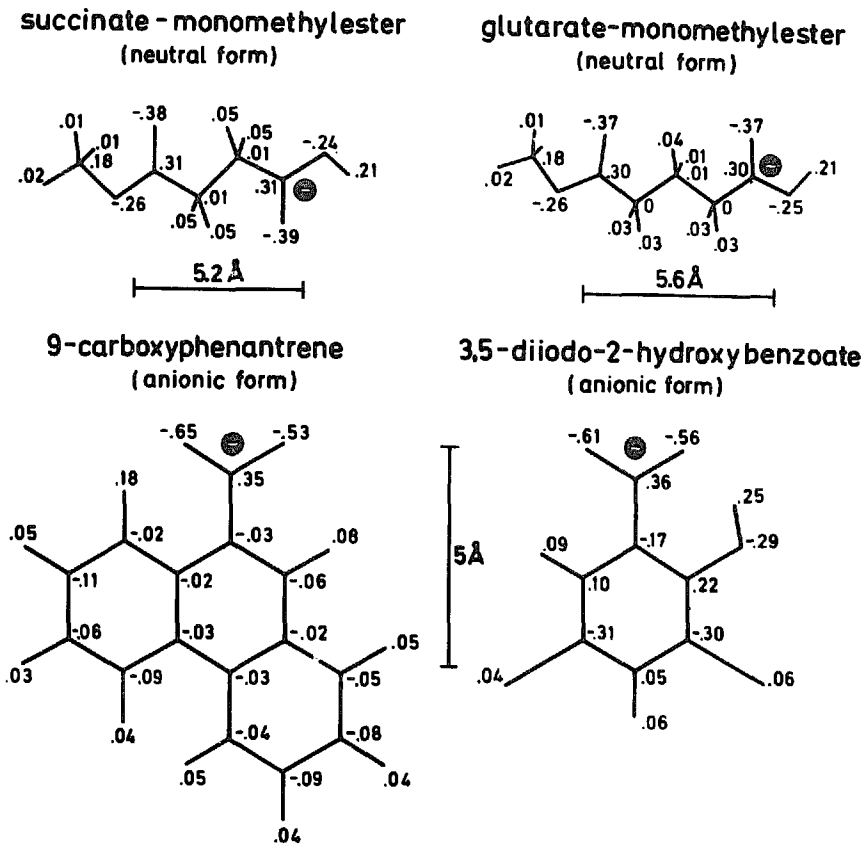

Fig. 3. The distribution of partial charges (as calculated by the MNDO method) of the monovalent or 'pseudo-bivalent' anions which show measurable inhibition of the of the dicarboxylate transport. For the two esters above, the partial charges have been calculated only for the non-dissociated (neutral) form. These charges, however, remain almost the same in the dissociated form. In these forms, the centers of entire negative charges are located as indicated. Note the accumulation of partial negative charges at the distance of 5–6 Å from the COO^- residues, i.e., in the 'dicarboxylate sensitive' distance. (The scales in the two parts of the figure differ slightly.)

center of a negative partial charge of −0.5 elementary charges about 5.5 Å from the carboxyl group, i.e., in the optimal distance for the dicarboxylate transport mentioned before. Thus, the binding of these esters can be attributed to an electrostatic interaction of an entire and a partial charge of the esters with the transport system rather than to a hydrophobic interaction. With 3,5-diiodo-2-hydroxybenzoate, the C-atoms in the 3- and 5-positions accumulate negative partial charges of a total more than −0.5 elementary charges in a distance of about 5.2 Å. More complicated, however, is the situation with 9-carboxyphenanthrene, where no significant partial charges occur. Here, a circular line in a distance of about 5 Å from the negative charge center of the COO^- residue is covered by exclusively negative partial charges of small amount (Fig. 3). These charges in the phenanthrene ring can easily be dislocated and they yield a total of −0.5 elementary charges as in the case of the previously mentioned methyl esters. In this context, it is reasonable to call these kinds of monovalent anion 'pseudo-bivalent'. Consequently, the dicarboxylate transport system can recognize anions with one (entire) negative charge and a second entire or partial negative charge at qa distance of 5–6 Å, regardless of whether the partial charge is concentrated at one point or distributed over a properly located arc in a ring structure. Experiments with dialdehydes (two partial charges) do not show inhibitory activity. There are no experimental indications that the acidity or hydrophobic interactions play any role in the dicarboxylate transport.

p-Aminohippurate transport

Like the transport of sulfate and dicarboxylate, the transport of *p*-aminohippurate is also strongly inhibited by bivalent anions (Fig. 2 and Table III) [1,5]. For medium-sized dicarboxylates with 4–8 C atoms, the inhibition pattern is similar to that of the dicarboxylate system with the slight difference that the maximal inhibition occurs with a charge separation of 6–7 Å, as opposed to 5–6 Å for the dicarboxylate system. In general, the inhibitory activity is greater for the *p*-aminohippurate transport than for the transport of succinate, e.g., A_i equals 0.62 for glutarate whereas it is not larger than 0.5 for the dicarboxylate system. The inhibition becomes weaker for longer molecules upto 8 C atoms (suberate). Then, however, the inhibitory activity increases again [5]. This phenomenon is analysed below

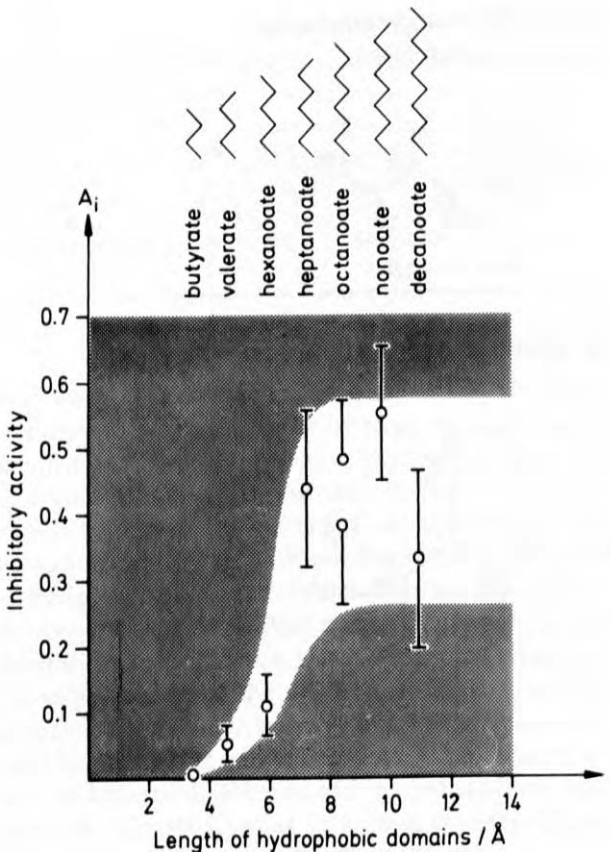

Fig. 4. The inhibitory activity, A_i, of the *p*-aminohippurate system as function of the length of the hydrophobic domains in monocarboxylates. Here, the hydrophobic length is defined as the distance between the C2 atom and the most distant H atoms of the methyl group. The A_i of octanoate has been observed for two different concentrations (2 and 10 mM). The majority of more than 100 tested aliphatic and aromatic monovalent anions fall in the unhatched area.

after the discussion of monovalent anions. Aromatic anions such as the different configurations of benzenedicarboxylates and benzenedisulfonates fit into the relationship shown in Fig. 2. Significant differences in the effect of COO^- and SO_3H^- groups are not detectable. If one of the negative charges is a partial charge, the inhibition is still good, e.g., the three configurations of carboxybenzenealdehyde have a full negative charge with the COO^- residue and a partial negative charge with the aldehyde residue. They have inhibitory activities of about 0.2 (unpublished). In contrast to the sulfate and dicarboxylate mechanisms, the *p*-aminohippurate system can even accept two partial negative charges: 1,4-dibenzaldehyde has an inhibitory activity of 0.1 and 4-nitrobenzaldehyde shows a slight but detectable inhibition (unpublished ata). In contrast to the dicarboxylate system, the *p*-aminohippurate system can be strongly inhibited by purely monovalent anions provided they have a hydrophobic domain. In Fig. 4 the inhibition of the *p*-aminohippurate transport by monocarboxylates is shown [1]. If there is a hydrophobic domain at least 4 Å long in the neighborhood of a negative charge, then a measurable inhibition occurs. Thus, anions with one negative charge plus a hydrophobic domain interact with the *p*-aminohippurate system. A saturation effect is observed for 'hydrophobic lengths' of 8 Å or longer, i.e., from heptanoate on. If double bonds are introduced into aliphatic monocarboxylates, the inhibitory activity is unchanged or slightly decreased [1]. This indicates that the *p*-aminohippurate mechanism accepts the aliphatic monocarboxylates essentially in their straight conformation. The inhibition by monovalent aromatic anions with hydrophobic moieties fits into this picture with the difference that these anions show a stronger inhibition. The knowledge about the hydrophobic interaction of the *p*-aminohippurate system with monovalent anion is necessary for an understanding of the strong inhibitory effect of dicarboxylic acids with a charge separation of more than 11 Å (Fig. 2). The longer these anions are, the longer the hydrophobic core is between the negative charges; the electrostatic effect of the two charges is superimposed on a hydrophobic interaction. For those molecules the *p*-aminohippurate system seems to ignore one of the negative charges and the binding probably involves both the hydrophobic interaction due to the C-chain and the electrostatic interaction of one negative COO^- group. Thus, the length of the hydrophobic domain of the dicarboxylate azelate (nine C atoms) is 7.6 Å, the inhibitory activity is $A_i = 0.50$, whereas these values for the monocarboxylate heptanoate are 7.2 Å and 0.44, respectively. The influence of acidity on the inhibition of the *p*-aminohippurate system has been

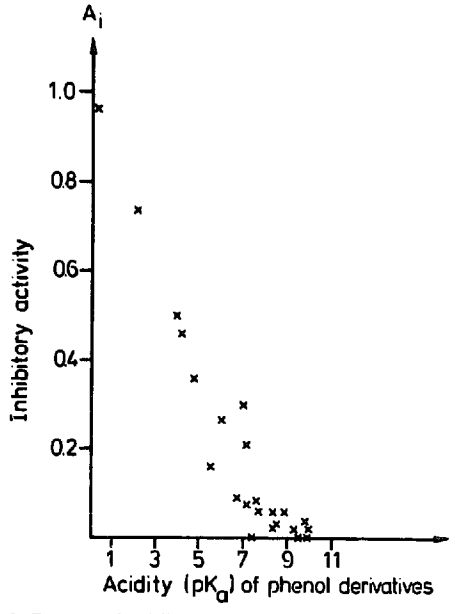

Fig. 5. The influence of acidity on the inhibition of *p*-aminohippurate transport as studied with phenol derivatives. Although influences other than acidity (hydrophobicity and charge distribution) cannot be excluded with these measurements, there is a significant dependence of the inhibitory activity on the pK_a value of the inhibitors. For pK_a values greater than 8 the inhibition is negligible.

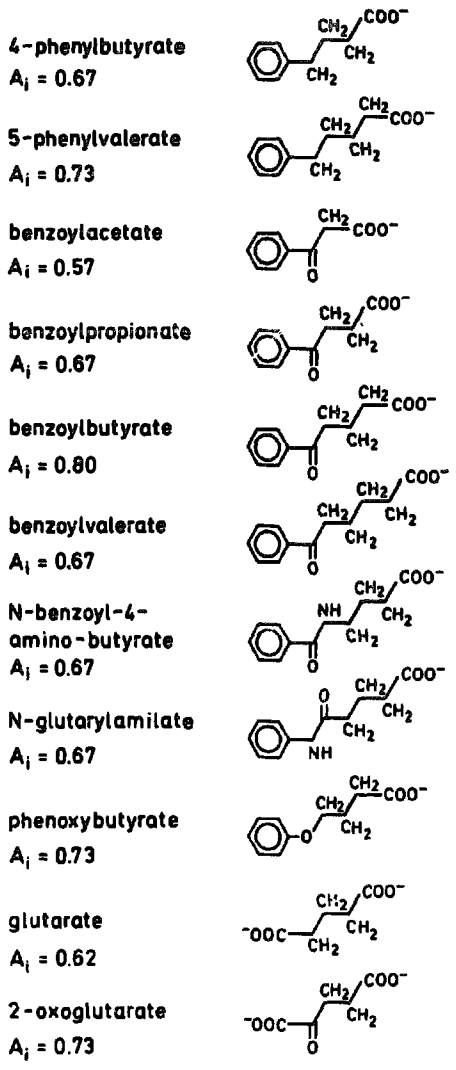

Fig. 6. The structures and activities of the most effective inhibitors of the *p*-aminohippurate transport. In all of them a hydrophobic moiety is located next to the negatively charged residue. The inhibition is augmented if a full negative charge as COO^- or a partial negative charge as $=C=O$ appears in a distance of about 6–7 Å from the charge center of the carboxyl residue.

analysed with substituted benzoates and phenolates [14]. The relationship represented in Fig. 5 shows the effect of acidity on the inhibitory activity of phenolates. Here, it must be taken into account that some of the substituents are electron accepting residues and bear partial negative charges at a distance from the dissociated COO^- group that is sensitive for the *p*-aminohippurate system. As an example, *o*-nitrobenzoate has a $pK = 2.2$ and an inhibitory activity of $A_i = 0.5$, whereas the more basic *o*-aminobenzoate with a $pK = 4.8$ yields an $A_i = 0.05$.

Special attention must be drawn to anions with the following structure: a negative COO^- group is followed by three or more unbranched C or N atoms and then by a ring (Fig. 6). Regardless of whether phenylcarboxylates, benozylcarboxylates, benzoylaminocarboxylates or phenoxycarboxylates are considered, they all cause a stronger inhibition than equally long monocarboxylates. One reason for this strong interaction is the hydrophobicity of the phenyl residue [12]. But, in the cases of benzoyl- and benzoylaminocarboxylates, the partial negative charge of the carbonyl group next to the ring gives rise to a further increase of the inhibitory activity. Interestingly, *p*-aminohippurate itself has this structure. Note that the effective hydrohobic length is 8 Å or longer, whereas the effective distance between the negative charges is 6–7 Å, i.e., for good *p*-aminohippurate inhibitors the hydrophobic domain does not fit into the space between the negative charges.

Discussion

The transport of organic anions through the contraluminal membrane of the rat kidney is mediated by at least three mechanisms. They are termed sulfate, dicarboxylate and *p*-aminohippurate transport. These mechanisms are overlapping, so that the majority of organic anions are recognized by one, two or all of the transporting units. By means of the described inhibition experiments it cannot be concluded whether the applied anions are definitively transported by or only bound to the transporting unit. It is supposed that the three test substances and the inhibitors work competitively [10,15]. The explicit expression for this is Eqn. 3. In order to analyse the inhibitory activity of special anions it is rational to group them by physicochemical properties like electronic structure rather by purely chemical criteria [16]. The strength of inhibition depends on the distribution of charges, on hydrophobic moieties and on acidity as well as on steric properties of the anions. The superposition of many effects complicates the interpretation of inhibitory activities drastically, since few inhibitors exhibit only one effect. Thus, a quantitative analysis of inhibition patterns is needed for the characterization of the transporting systems.

The sulfate system accepts short bivalent anions up to a distance between the charges of 7 Å. It prefers planar molecules, but with the help of bulky negative charges, e.g., as SO_3H^- groups, this system can also accept bulky apolar domains. This indicates that the sulfate system probably consists of a slit-shaped opening that can be broadened provided that bulky charged residues interact with this system. It is assumed that the sulfate system has two positive charges with a distance less than about 5 Å from each other. A small hydrophobic moiety in this system seems to be located in the neighborhood of one (or both) of its positive charges. These findings are similar to those found for the anion transport in red blood cells [6,7].

The dicarboxylate system is Na-dependent and it accepts medium-sized bivalent anions (4–7 aliphatic C atoms). It is probably an energetic link between the Na^+/K^+ pump and the Na^+-independent sulfate and

p-aminohippurate system (Burckhardt, G. and Ullrich, K.J., unpublished results). The assumed positive charges at the dicarboxylate system are located about 5–6 Å apart. This system is able to interact with monovalent anions only if a partial charge of at least −0.5 elementary charges is present at a distance of 5–6 Å from the dissociated group. The 9-carboxyphenanthrene is the only example of a monovalent anion without a significant partial negative charge. Its inhibition has obviously to do with the ability of the dicarboxylate system to accumulate negative partial charges of apolar ring structures of organic anions provided these charges are located about 5 Å from the negative charge center of the COO^- residue. It is concluded that the dicarboxylate system has no hydrophobic moiety.

The *p*-aminohippurate transporter accepts preferentially bivalent anions with a charge separation of about 6–7 Å. This indicates that the distance between the two assumed positive charges of this system is 1 Å longer than for the dicarboxylate system. The *p*-aminohippurate system accepts very well hydrophobic anions even if they are monovalent. The minimal length of an effective hydrophobic domain is 4 Å. Since the hydrophobic binding domain of the *p*-aminohippurate system cannot fit between the two charges, it is concluded that hydrophobic pockets are integrated in the *p*-aminohippurate system, located at least partially outside the connection line of the two charges. This finding is supported by the detected high affinity of the *p*-aminohippurate like structures in Fig. 6. The strong effect of acidity has probably to do with the easy dislocation of negative changes in acid molecules. In Fig. 7, rough schemes of the supposed shapes of all three anion binding sites are represented.

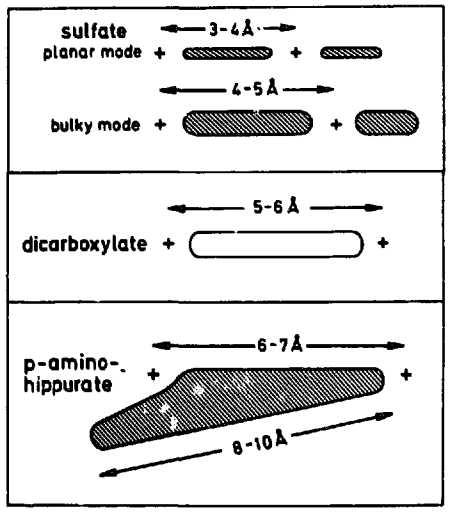

Fig. 7. The resting structural models of the anion binding sites for the three types of anion-transporting systems in the kidney. The sulfate system accepts short bivalent anions. It operates in two modes: planar (COO^--residues and flat hydrophobic domains) for charge distances of 3–4 Å; and bulky (SO_3H residues and bulky or flat hydrophobic domains) for charge distances of 4–7 Å. Anions with flat charged residues and bulky apolar groups are not accepted. The dicarboxylate system is inhibited preferentially by bivalent anions with a charge distance of 5–6 Å ('pseudo-bivalent' anions). The *p*-aminohippurate system interacts with 1 Å longer anions, preferentially with a charge distance of 6–7 Å. It accepts easily monovalent anions if they bear a hydrophobic moiety of a minimal length of 4 Å. There is evidence that the area of hydrophobic interaction is longer than the spacing between the two charges. These three systems have overlapping specificities.

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References

1 Ullrich, K.J., Rumrich, G. and Klöss, S. (1987) Pflügers Arch. 409, 547–554.
2 Ullrich, K.J., Rumrich, G. and Klöss, S. (1985) Pflügers Arch. 404, 293–299.
3 Ullrich, K.J., Rumrich, G. and Klöss, S. (1985) Pflügers Arch. 404, 300–306.
4 Ullrich, K.J., Fasold, H., Rumrich, G. and Klöss, S. (1984) Pflügers Arch. 400, 241–249.
5 Ullrich, K.J., Rumrich, G., Fritzsch, G. and Klöss, S. (1987) Pflügers Arch. 408, 38–45.
6 Cabantchik, Z.I., Knauf, P.A. and Rothstein, A. (1978) Biochim. Biophys. Acta 515, 239–302.
7 Passow, H. (1986) Rev. Physiol. Biochem. Pharmacol. 103, 61–203.
8 Despopoulos, A. (1965) J. Theor. Biol. 8, 163–192.
9 Møller, J.V. and Scheik, M.I. (1983) Pharmacol. Rev. 34, 315–358.
10 Fritzsch, G., Haase, W., Rumrich, G., Fasold, H. and Ullrich, K.J. (1984) Pflügers Arch. 400, 250–256.
11 Fritzsch, G. (1985) J. Theor. Biol. 117, 397–415.
12 Hansch, C., Leo, A., Unger, S.H., Kim, K.H., Nikaitani, D. and Lien, E.J. (1973) J. Med. Chem. 16, 1207–1216.
13 Ullrich, K.J., Rumrich, G., Klöss, S. and Lang, H.J. (1985) Pflügers Arch. 404, 311–318.
14 Ullrich, K.J., Rumrich, G. and Klöss, S. (1988) Pflügers Arch. 413, 134–146.
15 Ullrich, K.J., Rumrich, G., Fritzsch, G. und Klöss, S. (1987) Pflügers Arch. 409, 229–235.
16 Münter, K., Mayer, D. and Faulstich, H. (1986) Biochim. Biophys. Acta 860, 91–98.