The Role of Cl^- in Organic Acid Active Transport in Renal Proximal Tubules of Rat

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Abstract. The influence of substitution of Cl^- in the bath medium by other inorganic anions (NO_3^-, SCN^-, $S_2O_3^{2-}$, SO_4^{2-}) on the transport process was studied in order to elucidate the role of Cl^- in the organic acid transport in the renal proximal tubules in vitro. The substitutions were made to inhibit both Na-dependent and Na-independent uptake of an organic acid, fluorescein, in the tubules. The inhibition was due to the increase in K_m with V_{max} being unchanged or even increased. The K_m value increased in the following sequence: $SO_4^{2-} < S_2O_3^{2-} < SCN^- < NO_3^-$. A competition between the organic and inorganic anions seems to be absent because fluorescein does not affect the distributions of $S^{14}CN^-$ and $^{35}SO_4^{2-}$ between the renal cortical tissue and the bath medium. In addition, the substitution of Cl^- by SO_4^{2-} results in the diminution of the fluorescein uptake merely due to the omission of Cl^- from the medium. The substitution of Na_2SO_4 or NaSCN for NaCl inhibited the uphill uptake of fluorescein in the metabolically inactive tubules when the uptake was enhanced by an artificial gradient of Na^+ while the rate of dissipation of the Na^+ gradient was practically the same in all these media. Based on the obtained data it is suggested that Cl^- is directly involved into the formation of a transport complex in the basolateral membrane.

Key words: Proximal tubules — Organic acid active transport — Role of Cl^-

Introduction

The transport system for weak organic acids in the renal proximal tubules, studied mainly in vitro, excretes in vivo exogenous substances from the peritubular capillaries into the intratubular fluid (the primary urine). All the compartments of the tubular wall — the basolateral and apical membranes and the cytoplasm are involved in the secretory process, with the basolateral membrane being the rate-limiting step of the transport (for review see Bresler and Nikiforov 1981). The main moving force of the organic acid active transport across the basolateral membrane is the electrochemical gradient of Na^+ (medium/cell) created by Na,

K-ATPase (Hoshi and Hayashi 1970; Bresler and Nikiforov 1977, 1978, 1981; Nikiforov and Bresler 1977; Podevin et al. 1978; Hayashi and Hoshi 1979). That is why the operation of the transport system for the organic acids in the proximal tubules depends intimately on the concentration of Na^+ and K^+.

Taggart et al. (1953) found that the replacement of Cl^- in the bath medium by other inorganic anions (PO_4^{3-}, SO_4^{2-}, Br^-, NO_3^-, I^-, SCN^-) resulted in an inhibition of uptake of p-aminohippurate(PAH) in the rabbit renal cortex.

Recently it has been found (Goldinger et al. 1980) that the substitution of NO_3^- and SCN^- for Cl^- gave rise to the inhibition of the PAH uptake in the rabbit renal cortex due to an augmentation of K_m at unchanged V_{max}, which means the lowering of the affinity of the carrier to the organic acid. At least four explanations for the effect of the Cl^- substitution on the transport system might be suggested.

First, the inorganic anions could compete with the organic ones for the transport system. Since the replacement of Cl^- by other anions results in an augmentation of K_m, this suggestion seems to be very likely (Goldinger et al. 1980).

Second, the substitution of Cl^- gives rise to an alteration of the electrical potential difference on the basolateral membrane (Anagnostopoulos 1977). It should be noted that more permeant anions (NO_3^-, SCN^-) hyperpolarise the membrane while the less permeant ones ($S_2O_3^{2-}$, SO_4^{2-}) depolarise it. If the transport complex in the membrane was affected by the intramembrane electrical field, the more and less permeant anions should have opposite effects on the rate of its translocation. Such influence of the anionic substitutions was observed in the case of the Na-dependent transport of bile acids in the vesicles of the apical membrane of enterocytes (Lücke et al. 1978).

Third, the anionic substitutions could influence the organic acid transport indirectly through the cellular energy metabolism.

And, fourth, Cl^- could be directly involved in the formation of the transport complex. Such mechanism was suggested for the glycine transport system in the pigeon red cells (Vidaver et al. 1980).

In the present paper we attempted to test these suggestions. The experiments were carried out at two temperatures of the incubating medium: 30°C for the Na-dependent fluorescein transport and at 20°C when for the Na^+ independent transport (Bresler and Nikiforov 1978).

Material and Methods

Kidneys of male rats (body weight 180—200 g) were used. The animals were quickly decapitated and kidneys were removed and decapsulated. Thin slices (0.5—0.8 mm) were prepared by hand of the outer cortex with the essential surface being left intact. The fresh slices were incubated in vitro in aerated media containing a marker organic acid, fluorescein. The temperature of the bath media was either 20° or 30°C. The basic medium contained (mmol/l): NaCl 120; KCl 6; $NaHCO_3$ 3.6; Na_2HPO_4 4.8; NaH_2PO_4 3.3; $CaCl_2$ 1.5; pH 7.0—7.3. When needed NaCl was completely or partially replaced by

$NaNO_3$, NaSCN, Na_2SO_4 or $Na_2S_2O_3$. Complete Cl^- substitution was obtained by replacing KCl by KNO_3, KSCN, K_2SO_4 or $K_2S_2O_3$. When substituting Na_2SO_4 and $Na_2S_2O_3$ for NaCl the Na^+ and K^+ concentrations in the media were kept unchanged. The osmolarity was adjusted by appropriate amounts of sucrose. In a series of experiments Cl^- was omitted from the incubating medium by substituting sucrose for NaCl. To substitute SCN^- or SO_4^{2-} for Cl^- in the tubular lumen the kidneys were perfused in situ through the aorta with the corresponding solution at a pressure of 1800 mm of water column.

The amount of fluorescein accumulated in the intact superficial proximal tubules was measured using a special microfluorimeter with a contact objective lens (Bresler and Kachman 1975). Details concerning the technique of the measurements and visual control, and the method of calculation of the molar fluorescein concentrations in the tubules have been described previously (Bresler and Nikiforov 1977, 1978). It was found that the substitutions of SO_4^{2-}, $S_2O_3^{2-}$ or NO_3^- for Cl^- practically did not alter the intensity of luminescence of fluorescein solutions in the spectrum range used. The complete substitution of SCN^- for Cl^- gave rise to a 25% decrease in the luminescence. However, since SCN^- concentration in the tubular cells is well below that in the bath medium (see Results), this quenching of the luminescence cannot result in artifacts interfering significantly with the action of the fluorescein concentration.

The intensity of fluorescein luminiscence was measured on the surface of each slice in 40 proximal tubules. The measurements were repeated on kidneys from 3—5 rats, so that each value represents the mean of 120—200 individual measurements. The results of the transport studies are expressed as the tissue (tubules)/medium ratio (T/M). The data are presented as mean ± 2 S.E. The parameters of Michaelis-Menten equation (K_m and V_{max}) were calculated from the Lineweaver-Burk transformation by the method of least squares taking into account the statistical weightings (Bresler and Nikiforov 1977).

To test whether the effect of the Cl^- substitution is mediated through the cellular energy metabolism, special experiments with energy-, sodium- and potassium-depleted tubules in the presence of an artificial Na gradient (Podevin et al. 1978; Mozhayeva et al. 1982) were carried out. For this purpose the cortical slices were cold-preincubated (2—4°C) for 3 h in a solution containing choline-chloride instead of NaCl and KCl with subsequent incubation at 30°C in the anoxic K^+-free medium containing ouabain (0.05 mmol/l). The tissue Na^+ and K^+ content after preincubation and during the incubation was checked by means of flame photometry. The composition of the solutions and the preincubation and incubation techniques were described in detail previously (Mozhayeva et al. 1982). After the cold preincubation for 1.5—2 h under anoxia the renal cortical tissue practically did not contain ATP (Sehr et al. 1979; Maxild et al. 1981). The subsequent incubation was carried out under anoxia in the presence of ouabain. Since the proximal segment of the nephron shows very weak activity of glycolytic enzymes (Cohen and Barac-Nieto 1973), such tubules may in fact be referred to as "metabolically inactive" (Podevin et al. 1978). The only moving force to the uptake of fluorescein in these tubules may be the artificially created gradient of Na^+ (medium/cell) (Podevin et al. 1978; Mozhayeva et al. 1982).

$^{35}SO_4^{2-}$ and $S^{14}CN^-$ were used to investigate the distribution of sulfate and thiosyanate between the tissue and the medium after incubation with or without the organic acid. The specific activities of both these labels were equal to 1.85 Bq/ml. Sulfate or thiocyanate were present in the bath medium either alone or with 1; 10; 30 or 60 mmol/l of unlabelled SO_4^{2-}, or 1; 60; or 123 mmol of unlabelled SCN^-, respectively. The amount of the labels in the tissue was determined after 10; 20; 30; 40 and 60 min of incubation. The labels were extracted from the tissue using distilled water. The extract (0.25 ml) was added to the scintillator ZS-8 (Reachim, USSR) (10 ml) and the activity was counted using the SL-4000 counter (France). Data obtained in these experiments are expressed as cpm/1 g wet weight of the tissue: cpm/1 g of the incubating medium (CPM_T/CPM_M) ratio. Individual values are means from 3—6 measurements. Vertical lines indicate standard errors of the mean.

The extracellular volume was estimated using ^{14}C-inulin.

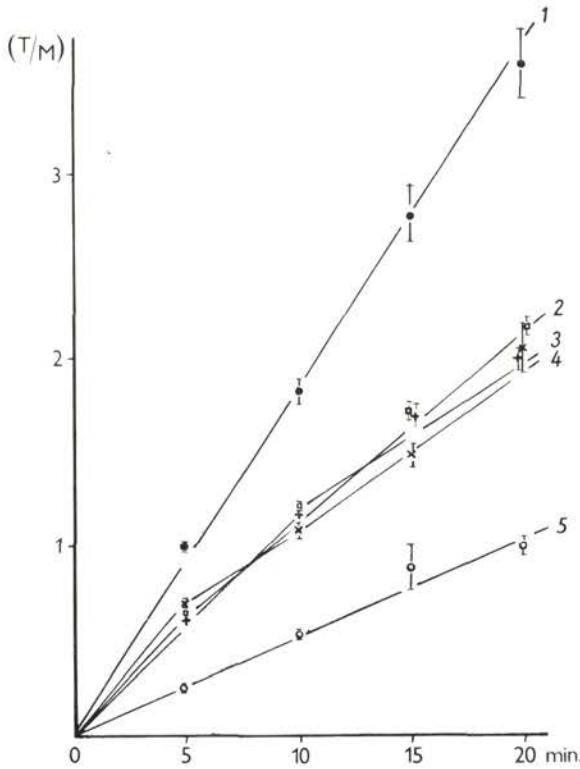

Fig. 1. Influence of the complete replacement of Cl^- in the bath medium by other inorganic anions on the fluorescein uptake in the proximal tubules.
Abscissa — duration of incubation (min); ordinate — T/M (Tubule/Medium) for fluorescein uptake. Media with: 1 — Cl^-; 2 — SO_4^{2-}; 3 — $S_2O_3^{2-}$; 4 — NO_3^-; 5 — SCN^-. The slices were incubated in aerated media with 0.05 mmol/l of fluorescein at 20°C. Vertical lines represent the 95% confidence limits.

Fluorescein (disodium salt, uranin, C.I. 45350) was obtained from Koch Light Laboratories Ltd., ouabain from Calbiochem, Los Angeles, CA., cholinechloride from Chemapol, Praha, Czechoslovakia. All other reagents were of commercial grade.

Results

The replacement of Cl^- in the bath medium by SO_4^{2-}, $S_2O_3^{2-}$, NO_3^- or SCN^- resulted in a significant decrease in the fluorescein uptake by the tubules at 20°C, the greatest inhibition being observed in the SCN^- medium (Fig. 1). Similar data were obtained at 30°, the inhibition being the greatest and practically identical in both the SCN^- and NO_3^- media (not shown).

At 20°C when the fluorescein transport does not depend on Na^+, the influence

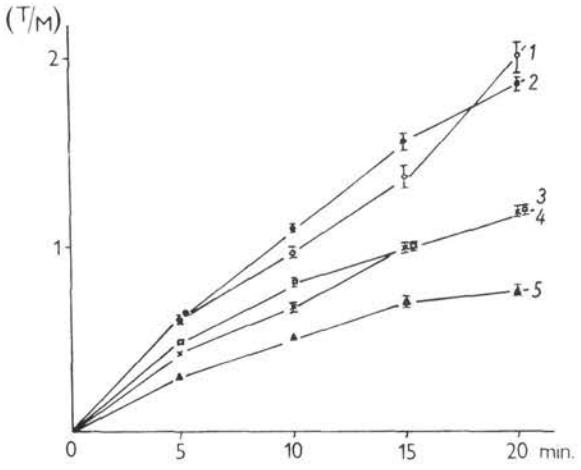

Fig. 2. Influence on the fluorescein uptake of the complete replacement of NaCl (1) in the bath medium by cholinechloride (2), sucrose (3), Na_2SO_4 (4) and NaSCN (5). All other conditions and symbols as in Fig. 1.

of the complete substitution of NaSCN, Na_2SO_4 or cholinechloride for NaCl was investigated and compared with that by sucrose. Fig. 2 shows that the substitution of SO_4^{2-} for Cl^- results in the same effect as the omission of Cl^- from the bath medium (the substitution of sucrose for NaCl does). The substitution of SCN^- for Cl^- inhibits the fluorescein uptake more sharply. This implies that in this case the fluorescein uptake is inhibited due to both the absence of Cl^- from the medium and the presence of SCN^-.

In addition, the fluorescein uptake in media containing different amounts of Cl^- together with SO_4^{2-} or SCN^- was studied (Fig. 3). In the presence of SO_4^{2-} at 30 mmol/l of Cl^- the fluorescein uptake returned near to the control level. At the same time, the fluorescein transport remains markedly inhibited in the presence of SCN^- even at 90 mmol/l of Cl^-.

Preincubation of the renal slices for 5 min in the solution containing SCN^- instead of Cl^- did not alter the fluorescein uptake during the incubation in the chloride medium at 20°. In this case T/M for the fluorescein uptake were 0.87 ± 0.04 and 2.43 ± 0.33 after 5 and 15 min of incubation, respectively, as compared to the control (preincubation for 5 min in the chloride solution) — 0.93 ± 0.04 and 2.45 ± 0.14, respectively. It follows that the inhibitory effect of SCN^- is reversible. Hovewer, it was shown that preceeding replacement of Cl^- in the intratubular fluid by SO_4^{2-} or SCN^- (by means of perfusion through aorta) did not alter the fluorescein uptake in the tubules during the incubation in the chloride medium (not shown).

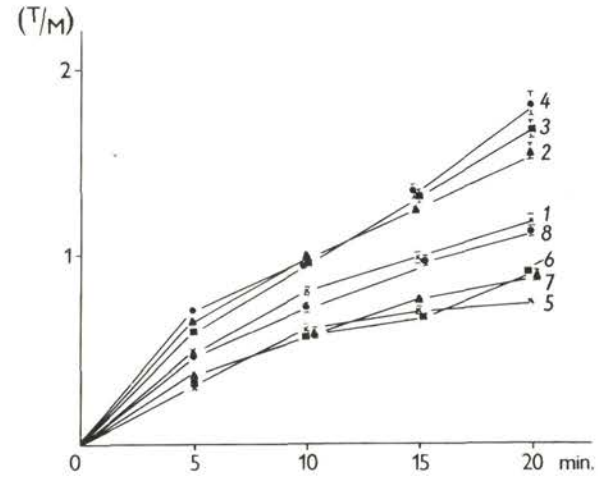

Fig. 3. Influence of partial replacement of Cl^- by SO_4^{2-} and SCN^- on the fluorescein uptake. Set up — as in Fig. 1. Media with (mmol/l): 1 — SO_4^{2-} 60; 2 — SO_4^{2-} 45 and Cl^- 30; 3 — SO_4^{2-} 30 and Cl^- 60; 4 — SO_4^{2-} 15 and Cl^- 90; 5 — SCN^- 120; 6 — SCN^- 90 and Cl^- 30; 7 — SCN^- 60 and Cl^- 60; 8 — SCN^- 30 and Cl^- 90. All other conditions and symbols as in Fig. 1.

The values of K_m and V_{max} for the fluorescein transport in the media with the various inorganic anions are shown in the Table. As a matter of convenience, the ratios of K_m and V_{max} for the transport after the anionic substitutions to those for the transport in the chloride medium have also been calculated. As can be seen the inhibition of the fluorescein transport after the substitutions of Cl^- is the result of

Table 1. Values of K_m and V_{max} for the fluorescein transport in the tubules in media with various inorganic anions

Anion	K_m^* 10^{-4} mol/l		V_{max}^{**} 10^{-6} mol/l.min		$K_m^{(A)}/K_m^{(Cl)}$		$V_{max}^{(A)}/V_{max}^{(Cl)}$	
	20°	30°	20°	30°	20°	30°	20°	30°
Cl^-	1.1 (0.8—1.8)	0.8 (0.6—1.3)	45±9	53±9	1	1	1	1
NO_3^-	7.0 (4.8—13.5)	4.3 (3.3—6.2)	82±33	88±19	6.4	5.4	1.8	1.7
SO_4^{2-}	1.6 (1.3—2.0)	1.3 (0.9—1.9)	37±5	46±10	1.4	1.6	0.8	0.9
$S_2O_3^{2-}$	3.4 (2.5—5.7)	2.0 (1.4—3.0)	66±19	68±14	3.1	2.5	1.5	1.3
SCN^-	5.8 (2.7—∞)	3.7 (2.0—25.6)	53±43	57±20	5.3	4.6	1.2	1.1

* In the brackets −95% confidence limits for K_m

** V_{max} ±95% confidence limits

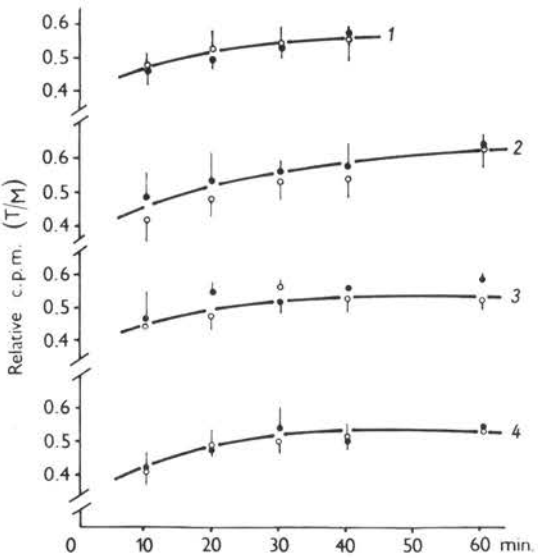

Fig. 4. Uptake of $S^{14}CN^-$ in the renal cortical tissue under various conditions at 20°C. Abscissa — duration of incubation (min); ordinate — CPM_T/CPM_M for $S^{14}CN^-$ uptake. The aerated media contained 0.002 mmol/l of $S^{14}CN^-$; they contained in addition, unlabelled SCN^- (mmol/l): 1 — 0; 2 — 1; 3 = 60; 4 — 126. Open circles — control, in the absence of fluorescein. Closed circles — in the presence of 0.2 mmol/l of fluorescein in the bath medium. Vertical lines represent SEM.

an augmentation of K_m. This implies that in this case (when V_{max} does not significantly decrease) the inhibition of the transport is due to the lowered affinity of the carrier to the organic acid. The K_m increases in the following sequence: $SO_4^{2-} < S_2O_3^{2-} < SCN^- < NO_3^-$.

The effect of fluorescein on the uptake of labelled sulfate and thiocyanate in the cortical tissue was studied in order to test the possibility of competitive relationship between the transport of organic and inorganic anions in the proximal tubules. The experiments were carried out at 20°C. Media with fluorescein (0.2 mmol/l) or without it (control) were used. Fig. 4 shows that in media with different concentrations of unlabelled SCN^- the maximal extent of uptake of labelled thiocyanate was not changed. Fluorescein did not affect the distribution of the label.

$^{35}SO_4^{2-}$ accumulated in the tissue against its electrochemical gradient so that after 60 min of incubation the value of CPM_T/CPM_M for its uptake exceeded the value of 2 (Fig. 5). The increase in the medium containing unlabelled SO_4^{2-} diminshed this ratio and at 30 mmol/l of SO_4^{2-} the value of CPM_T/CPM_M for $^{35}SO_4^{2-}$

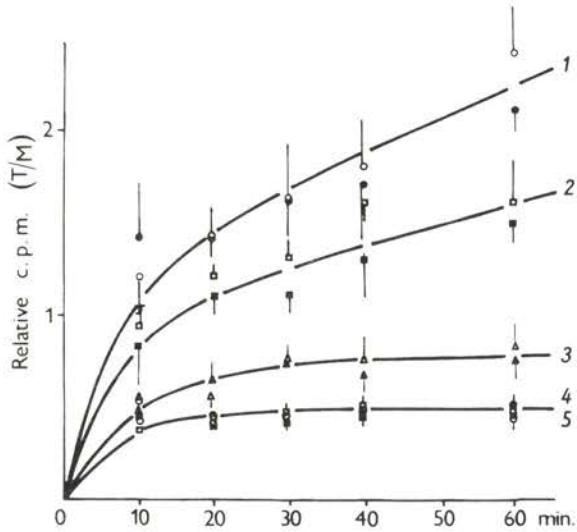

Fig. 5. Uptake of $^{35}SO_4^{2-}$ by renal cortical slices under various conditions at 20°C.
Abscissa — duration of incubation (min); ordinate — CPM_T/CPM_M for $^{35}SO_4^{2-}$ uptake. The aerated media contained 0.002 mmol/l of $^{35}SO_4^{2-}$; they contained in addition, unlabelled SO_4^{2-} (mmol/l): 1 — 0; 2 — 1; 3 — 10; 4 — 30; 5 — 60. All other conditions and symbols as in Fig. 4.

uptake was equal to 0.4. As in the case of SCN^-, the $^{35}SO_4^{2-}$ uptake was not influenced by fluorescein.

Based on the experiments with the labelled anions it may be assumed that thiocyanate is distributed between the tissue and the medium passively, without involvement of any carriers. Sulfate accumulates against its electrochemical gradient, with its uptake reaching saturation. However, fluorescein does not affect the sulfate uptake. The uphill uptake of sulfate is likely to proceed via the transport system in the apical membrane where the sulfate is being reabsorbed in the proximal tubules (Deyrup 1964).

The effects of the anionic substitutions on the fluorescein uptake in the metabolically inactive tubules were investigated. The tubules depleted of Na^+ and K^+ were capable of the uphill fluorescein uptake in the anoxic medium containing ouabain at the presence of 145 mmol/l of NaCl (Fig. 6a). A comparison of the time course of the fluorescein uptake (Fig. 6a) and the dissipation of the artificial Na^+ gradient (Fig. 6b) shows that the uphill uptake of fluorescein can occur untill a significant gradient of Na^+ is present. After complete dissipation of the Na^+ gradient, fluorescein begins to efflux from the tubules. When Na^+ is absent from the bath medium (NaCl replaced by cholinechloride) the uphill uptake of fluores-

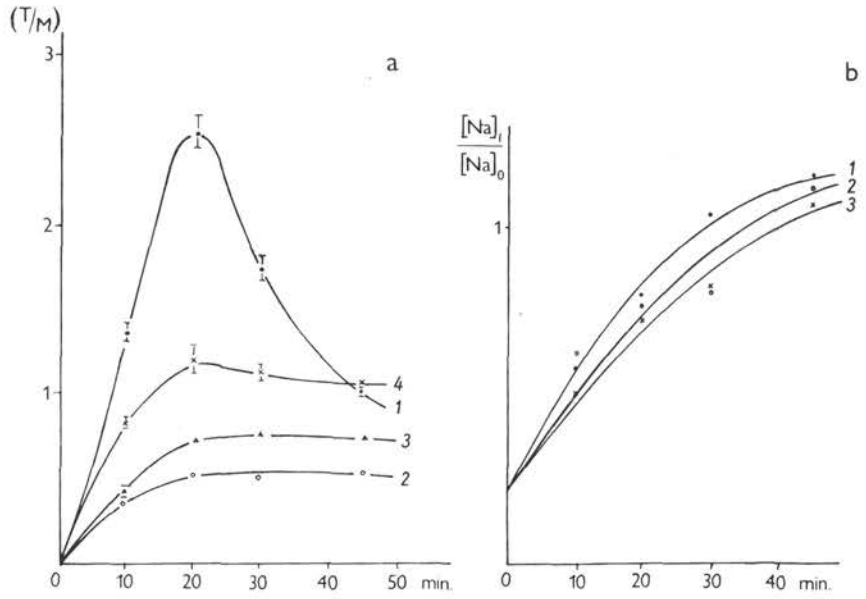

Fig. 6. Time courses of the fluorescein uptake(a) and degree of the Na^+ gradient(b) in the metabolically inactive tubules depleted of Na^+ and K^+. (a) Set up as in Fig. 1. Media with: 1 — 145 mmol/l of NaCl; 2 — 145 mmol/l of cholinechloride; 3 — 145 mmol/l of NaSCN; 4 — 73 mmol/l of Na_2SO_4. The concentration of fluorescein in the media was 0.05 mmol/l. (b) Abscissa — duration of incubation (min); ordinate — ratio of Na^+ concentration in the cellular water $[Na]_i$ to that in the extracellular medium $[Na]_o$. Media with: 1 — 145 mmol/l of NaCl; 2 — 145 mmol/l of NaSCN; 3 — 73 mmol/l of Na_2SO_4. Cold-preincubated slices for 3 h (see Methods) were incubated under anoxia at 30°C in media containing 0.05 mmol/l of ouabain. Both experimental series, (a) and (b), were carried out on slices from the same animals.

cein does not occur. Also the uphill uptake of fluorescein is not observed if Cl^- in the bath medium is substituted by SCN^- or SO_4^{2-} with the Na^+ concentration remaining unchanged (Fig. 6a), although the rate of dissipation of the Na^+ gradient is practically the same in all these media (Fig. 6b).

Discussion

The data obtained in the present work strongly confirm the results of other investigators (Taggart et al. 1953; Goldinger et al. 1980) concerning the reversible inhibitory effects of the replacement of Cl^- in the bath medium by other inorganic

anions on the transport of weak organic acids in renal proximal tubules. It should be emphasized that the inhibition of the transport is only observed when Cl^- is replaced in the peritubular fluid. Goldinger et al. (1980) have recently found that the inhibition of the transport after the substitution of NO_3^- and SCN^- for Cl^- is the result of an augmentation of K_m. In the present work such a mode of inhibition is observed for SO_4^{2-} and $S_2O_3^{2-}$ as well. Taking into account the competitive type of the alteration of the transport parameters K_m and V_{max}, Goldinger et al. (1980) proposed that the decrease in the affinity of the carrier to the organic acid may be due to the competition between the organic acid and NO_3^- or SCN^- for the binding site on the carrier. In the experiments with labelled sulfate and thiocyanate we did not find any influence of the organic acid, fluorescein, on the uptake of these inorganic anions by the tissue. It is conceivable that the inorganic anions substituted for Cl^- compete with the organic anions for the binding site on the carrier but are not transported by it. However, this assumption does not account for the inhibition of the fluorescein transport when NaCl is replaced by sucrose.

The substitution of other anions for Cl^- may hardly inhibit the fluorescein transport through an alteration of the electrical potential difference on the basolateral membrane. As a matter of fact, the substitution of NO_3^- and SCN^- for Cl^- leads to hyperpolarisation of the lateral membrane (Anagnostopoulos 1977), while substitution of SO_4^{2-} and $S_2O_3^{2-}$ leads to its depolarisation, but the organic acid transport itself becomes inhibited after all these substitutions only.

The possibility that the anionic substitutions affect the organic acid transport through the influence on the cellular energy metabolism may also be ruled out. First, the substitution of other inorganic anions for Cl^- did not alter the rate of O_2 consumption by the renal tissue (Taggart et al. 1953; Cunarro and Weiner 1978; Goldinger et al. 1980). Second, in the present work the substitution of SO_4^{2-} and SCN^- for Cl^- resulted in the inhibition of the fluorescein uptake by the metabolically inactive tubules. The uphill uptake of organic acids in such a case is possible owing to the presence of the artificial Na^+ gradient (Podevin et al. 1978; Mozhayeva et al. 1982). The effect of the Cl^- substitutions might be a result of the influence of SO_4^{2-} and SCN^- on the rate of dissipation of the Na^+ gradient. However, our data show that this was not the case. Consequently, the anionic substitutions affect the carrier for organic acids in a direct way.

According to the gradient hypothesis of Crane (1965, 1977) the carrier of the Na^+-dependent transport system has a specific site that binds Na^+ on the outer side of the plasma membrane; this is the cause for an increase in affinity of the carrier to the transported solutes. Inorganic anions could affect the Na^+-binding site on the carrier for organic acids. However, this assumption is not valid because the effects of the anionic substitutions are practically the same for both Na^+-dependent and Na^+-independent components of the fluorescein transport.

The experiments with NaCl replaced by sucrose give evidence that the absence

of Cl^- from the bath medium results in a significant inhibition of the fluorescein transport. All things considered, it may be suggested that the carrier for organic acids, has a binding site for Cl^- in addition to the binding sites for organic anions and for Na^+. Similar suggestion has been introduced earlier for the glycine carrier in the pigeon red cells (Vidaver et al. 1978). Cl^- bound to the carrier is likely to stabilize the transport complex (seen as an increase in the affinity of the carrier to the organic acid), as it does in the case of the folate-binding protein in the apical membrane of the rat proximal tubules (Selhub et al. 1979). Other inorganic anions are probably ineffective as imitators of Cl^- in the formation of the transport complex of the carrier and organic acid. Moreover, SCN^- per se has an additional inhibitory effect on the carrier. The mechanism of this effect is yet unclear.

Thus the operation of the transport system for weak organic acids in the renal tubules in vitro depends essentially on both the cationic and anionic composition of the incubating medium. These relations should be taken into account to avoid artifacts whenever modified salt media are used.

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Received July 16, 1982 / Accepted September 24, 1982