Biochimica et Biophysica Acta, 553 (1979) 404—416

BBA 78377

ROLE OF SODIUM IONS IN *p*-AMINOHIPPURATE TRANSPORT BY NEWT KIDNEY

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(Received September 29th, 1978)
(Revised manuscript received November 24th, 1978)

Key words: p-Aminohippurate; Renal proximal tubule; Na^+-coupled transport kinetics; Na^+ gradient; (Newt kidney)

Summary

1. The effect of external Na^+ concentration on *p*-amonohippurate uptake by isolated kidneys of newt (*Triturus pyrrhogaster*) was studied kinetically and electrophysiologically.

2. *p*-Aminohippurate uptake conformed to Michaelis-Menten type kinetics in regard to both *p*-aminohippurate and Na^+ concentrations in the incubation medium. Kinetic studies revealed that reduction of Na^+ concentration increased the values of K_t without altering the maximal rate (V) of *p*-aminohippurate uptake. The values of K_t were a linear function of the reciprocal of Na^+ concentration. These results suggest the presence of interaction between *p*-aminohippurate and Na^+ at the carrier level, i.e. Na^+-coupled cotransport.

3. *p*-Aminohippurate had no effect on the electrical potential difference across the peritubular membrane in both 10 and 100 mM Na^+ solutions, suggesting that *p*-aminohippurate is transported across the peritubular membrane in a form of electrically neutral carrier complex. This is consistent with the results of the kinetic studies.

4. *p*-Aminohippurate uptake was proportional to the electrochemical potential gradient of Na^+ ($\Delta\bar{\mu}_{Na}$) across the peritubular membrane. This result indicates that the maintenance of sufficient $\Delta\bar{\mu}_{Na}$ appears to be necessary for the accumulation of *p*-aminohippurate against its electrochemical potential gradient, supporting Na^+ gradient hypothesis.

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Introduction

Some organic acids, such as *p*-aminohippurate, are known to be actively transported by the proximal tubular cells across the peritubular membrane through a specific carrier-mediated process [1—4]. Also, it has been shown that active organic acid transport is influenced by external Na^+ concentration [5—14]. However, the specific role of Na^+ in organic acid transport is still disputed. Since Vogel and Kröger [5] showed Na^+ dependence of *p*-aminohippurate transport in artificially perfused frog kidney, many investigators intended to clarify the role of Na^+ in organic acid transport. Hoshi and Hayashi [6] and Bresler and coworkers [7—9] showed a possibility that organic acids were cotransported with Na^+. Some authors [7—10] stressed the importance of the Na^+ gradient in organic acid transport. On the other hand, Gerencser and Hong [13], Berner and Kinne [15] and Ullrich et al. [16] suggested that *p*-aminohippurate transport was not Na^+ coupled and not driven by a Na^+ gradient.

The present study was designed to gain some insight into the specific role of Na^+ in *p*-aminohippurate transport by renal tubules. For this purpose, investigations were made of the effects of chainging external Na^+ concentration on *p*-aminohippurate uptake. Both kinetic properties of the uptake and electrical events associated with *p*-aminohippurate transport were studied in order to elucidate the characteristics of the transport mechanism. Electrolyte contents and the electrical potential difference across the peritubular membrane (peritubular membrane potential) were also determined in order to discuss the relation of *p*-aminohippurate transport to the electrochemical potential gradient of Na^+ ($\Delta\bar{\mu}_{Na}$) across the peritubular membrane.

Methods

Preparation of tissues and media. Experiments were performed on newts (*Triturus pyrrhogaster*) of both sexes, weighing 3—10 g. They were kept unfed in tanks of tap water at about 4°C. The animal was decapitated and the abdominal wall was opened. After being trimmed of excess tissues and mesonephric ducts, the kidneys were removed and transferred into an aerated ice-cold Ringer's solution. The pelvic part of the kidney, 10—30 mg in weight, was used. The basic Ringer's solution had following composition (in mM): NaCl, 90; sodium acetate, 10; $KHCO_3$, 1.8; KH_2PO_4, 0.6; $CaCl_2$, 1.5; $MgSO_4$, 1.0; Tris-HCl, 5. The pH of the solution was 7.4 and the osmolality 210 mosM/kg H_2O. The Na^+-depleted solutions were prepared by replacing NaCl by equimolar choline chloride. Throughout the experimental periods, the solutions were bubbled with air and kept at 25°C.

Uptake experiments. *p*-Aminohippurate uptake was studied by incubating the isolated kidneys in the various test media. The excised tissues were first collected in an ice-cold 10 mM Na^+ solution and then four to nine tissues were transferred to one preincubation tube containing 5—10 ml of the 10 mM Na^+ solution. After preincubation for 20 min at 25°C, the tissues were transferred to the test incubation media and then uptake was measured. At the end of incubation, the tissues were picked up from the incubation media, blotted,

weighed and then extracted overnight in 0.4—0.8 ml distilled water at room temperature. Tissue water content was determined from the loss of weight on drying the tissues overnight at 100°C. We determined the inulin space, estimating the extracellular space, by addition of 5 g/l inulin in the media. *p*-Aminohippurate was assayed by the method of Waugh and Beall [17], inulin by the simplified anthrone method of Davidson and Sackner [18], and Na^+ and K^+ by flame photometry. All values for tissue *p*-aminohippurate and inulin contents were corrected for the tissue blank. Assuming that *p*-aminohippurate and electrolytes were distributed uniformly in cell water, intracellular contents of these solutes were calculated by using the tissue water content and the inuline space.

Electrical potential measurements. The methods employed for the measurements of the peritubular membrane potential of the proximal tubular cell were mostly the same as those in the previous study [19]. The tissue was transferred into a small lucite chamber which had a small silicone rubber plate on its bottom. The tissue was fixed on the plate with fine syringe needles. Inside of the chamber was continuously perfused with an aerated Ringer's solution at a rate of 1.0—1.3 ml/min. The fluid volume of the chamber was about 2.5 ml. Identification of the proximal segment was made by observing the location and the size of the tubules under a stereomicroscope and recording the alanine-induced depolarization of the peritubular membrane potential [19]. The peritubular membrane potential was recorded during alternate perfusion with the control and test solutions. The peritubular membrane potentials in the test solutions were observed for more than 20 min, 30 min in most cases, because the incubation period of uptake experiments was 30 min. The recovery of the peritubular membrane potential in the control solution was followed up for more than 20 min. The data obtained from samples which showed the initial peritubular membrane potential lower than 50 mV (cell interior negative) were discarded.

Drugs. Probenecid was supplied by Japan-Merck-Banyu Phc. Ltd. Other chemicals of reagent grade were obtained from commercial sources.

Statistics. Regression lines were calculated by the least-squares method. The values were represented as means ± S.E. Statistical significance was determined by the Student's *t*-test.

Results

Time course of p-aminohippurate uptake

Water content of the tissue was almost constant up to 60 min and not different from 80% of wet weight of the tissue in both groups of samples that were incubated in the Na^+-depleted (10 and 50 mM Na^+) and basic (100 mM Na^+) media. Fig. 1 shows the time courses of *p*-aminohippurate uptake and inulin content. A significant difference in the initial rate of *p*-aminohippurate uptake was observed between in the 10 mM Na^+ and basic media. In the basic medium, the uptake increased almost linearly up to 60 min. In the 10 mM Na^+ medium, the uptake continued to increase with time and tissue-to-medium *p*-aminohippurate concentration ratio exceeded unity after 15 min incubation. However, the rate of increase gradually slowed down. The linear increase observed

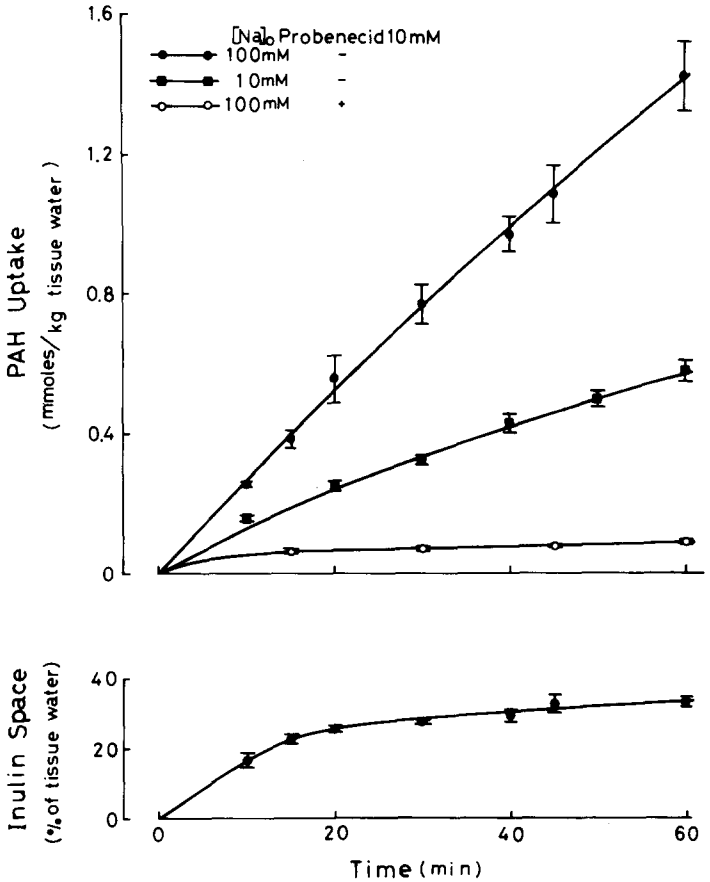

Fig. 1. Time courses of *p*-aminohippurate (PAH) uptake and inulin space in the basic medium (●), basic medium with 10 mM probenecid (○) and Na^+-depleted medium (■). Medium *p*-aminohippurate and inulin concentrations were 0.2 mM and 5 g/l, respectively. Each point with a bar indicates mean ± S.E. (n = 4—8).

up to 30 min in the present preparations suggests that efflux from the cells is negligible at least up to 30 min. Thus, *p*-aminohippurate uptake during initial 30 min could be regarded as initial influx. The inulin space attained a quasi-steady level at 20 min, thereafter a slight increase with time continued. This slow increase is probably due to the continued entry of inulin into the tubular lumen through the nephrostomes and to the concentration of its intraluminal fluid at the lower nephron segment. At 30 min, the inulin space was 30% of total tissue water in the Na^+-depleted and basic media. In the following uptake experiments, 30 min incubation period was chosen for determination of the rate of *p*-aminohippurate uptake.

Effects of probenecid on p-aminohippurate uptake

Simultaneous presence of probenecid, a competitive inhibitor of organic acid transport [1,3,15], at relatively high concentration (10 mM), caused a marked reduction in *p*-aminohippurate uptake (Fig. 1). In the basic medium, addition

of 10 mM probenecid depressed the uptake from the media containing *p*-aminohippurate at 0.2 and 0.4 mM to 2 and 6% of that from the probenecid-free media (control), respectively. In the 10 mM Na^+ medium containing 0.2 mM *p*-aminohippurate and 10 mM probenecid, the uptake was depressed to 3% of the control. In the basic medium, *p*-aminohippurate uptake was competitively inhibited by probenecid and the inhibitory constant for probenecid was 0.032 mM. These results suggest that *p*-aminohippurate accumulation in the absence of probenecid is due to a carrier-mediated process which is locating at the peritubular membrane of the proximal tubule [15].

Effects of external p-aminohippurate and Na^+ concentration on p-aminohippurate uptake: kinetic studies

p-Aminohippurate uptake increased with increase in *p*-aminohippurate concentration and showed a tendency toward saturation. At a constant Na^+ concentration, the uptake followed Michaelis-Menten kinetics. Preliminary, the uptake was examined up to 2 mM *p*-aminohippurate in the 10 and 100 mM Na^+ media. It was found that the double-reciprocal plots were linear up to 1 mM, however, at the concentrations more than 1 mM, the points shifted upward from the linearity. Thus, for the kinetical analyses, the concentration range from 0.05 to 0.4 mM was used. The relationships at various Na^+ concentrations (10, 25, 50 and 100 mM) were shown in Fig. 2, where the data were plotted double recip-

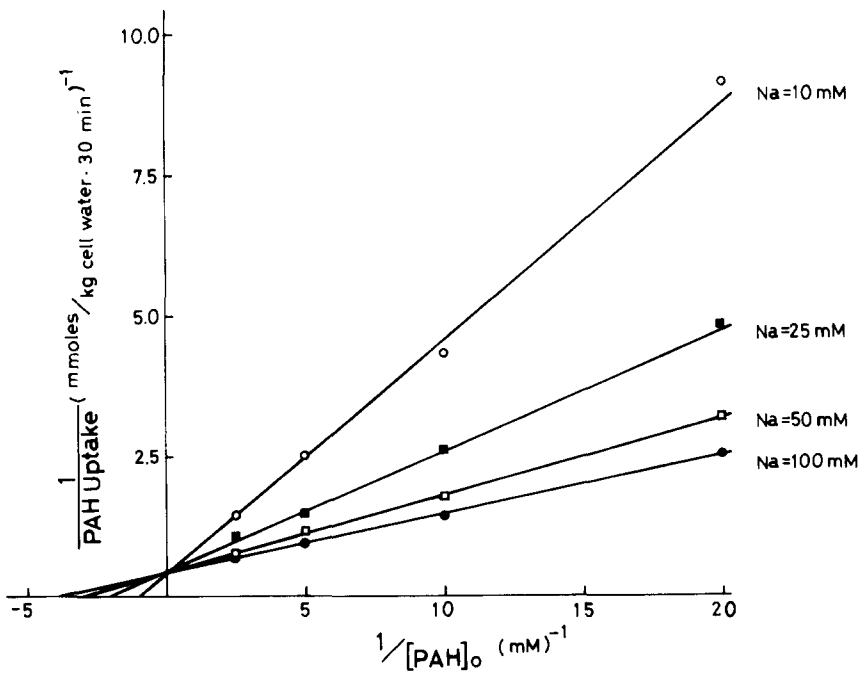

Fig. 2. Double-reciprocal plots of *p*-aminohippurate (PAH) uptake against *p*-aminohippurate concentration ($[PAH]_o$) in the media at various external Na^+ concentrations. Each point represents mean of eight kidneys.

rocally. From the linearity of the plots, the rate of *p*-aminohippurate uptake, v, can be described by an equation

$$v = \frac{V[S]_o}{K_t + [S]_o} \quad (1)$$

in which $[S]_o$ is medium *p*-aminohippurate concentration, V the maximal rate of the uptake and K_t the apparent Michaelis constant for *p*-aminohippurate. The values of V and K_t at various Na^+ concentrations were summarized in Table I. The data show that the values of V are not dependent on Na^+ concentration, while K_t increases with reduction in Na^+ concentration. The values of K_t are a linear function of the reciprocal of Na^+ concentration (K_t (mM) = $0.168 + 8.97/[Na]_o$ (mM), correlation coefficient, $r = 0.999$). The same data were used to examine the relationship between the rate of *p*-aminohippurate uptake and external Na^+ concentration at constant *p*-aminohippurate concentration. 100 mM Na^+ gave nearly maximum *p*-aminohippurate uptake at each *p*-aminohippurate concentration. The double-reciprocal plots also gave straight lines. The rate of the uptake, v, can be given by

$$v = \frac{V'[Na]_o}{K_t' + [Na]_o} \quad (2)$$

in which $[Na]_o$ is medium Na^+ concentration, V' the maximal rate of *p*-aminohippurate uptake and K_t' the apparent Michaelis constant for Na^+. The values of these kinetic parameters at different *p*-aminohippurate concentrations were shown in Table II. the reciprocal of V' is a linear function of the reciprocal of *p*-aminohippurate concentration. This relation is almost the same as the lower line of Fig. 2 (100 mM Na^+), thus this result also shows that the 100 mM Na^+ gave nearly maximum *p*-aminohippurate uptake. The reciprocal of K_t' is a linear function of *p*-aminohippurate concentration. The data also show external Na^+ concentration needed for half-maximal *p*-aminohippurate uptake and show that this value decreases when external *p*-aminohippurate concentration increases.

Effects of p-aminohippurate on peritubular membrane potential

Fig. 3 shows the typical recordings of the peritubular membrane potential in

TABLE I

KINETIC PARAMETERS FOR *p*-AMINOHIPPURATE AT VARIOUS Na^+ CONCENTRATIONS

Three experiments were done to obtain the kinetic parameters. In one experiment, each eight or nine kidneys were examined at concentrations of *p*-aminohippurate, 0.05, 0.1, 0.2 and 0.4 mM in the media at constant Na^+ concentrations (10, 25, 50 and 100 mM), respectively. Each V and K_t was obtained by the equation, $[S]/v = K_t/V + [S]/V$, modified from Eq. 1. Each value represents mean ± S.E. of three experiments.

$[Na]_o$ (mM)	V (mmol/kg cell water per 30 min)	K_t (mM)
10	2.45 ± 0.04	1.069 ± 0.013
25	2.20 ± 0.10	0.514 ± 0.009
50	2.38 ± 0.04	0.352 ± 0.005
100	2.38 ± 0.04	0.263 ± 0.005

TABLE II

KINETIC PARAMETERS FOR Na^+ AT VARIOUS *p*-AMINOHIPPURATE CONCENTRATIONS

In one experiment, each eight or nine kidneys were examined at concentrations of Na^+, 10, 25, 50 and 100 mM, in the media. Each value represents mean ± S.E. of three experiments. PAH, *p*-aminohippurate.

$[PAH]_o$ (mM)	V' (mmol/kg cell water per 30 min)	K'_t (mM)
0.05	0.54 ± 0.01	42.8 ± 2.2
0.1	0.86 ± 0.03	32.3 ± 2.5
0.2	1.25 ± 0.04	21.3 ± 1.9
0.4	1.58 ± 0.03	14.3 ± 0.1

the basic solution where the perfusion fluid was switched to the solution containing 0.2 or 1 mM *p*-aminohippurate. No detectable change was seen in the peritubular membrane potential with the 0.2 mM *p*-aminohippurate solution. A very small depolarization was observed after switching to the 1 mM *p*-aminohippurate solution. At 10 mM Na^+, the minimal level of the peritubular membrane potential in the 1 mM *p*-aminohippurate solution was 97.2% (n = 7) of the peritubular membrane potential in the *p*-aminohippurate-free solution (control). At 100 mM Na^+, the minimal levels of the peritubular membrane potential in the 0.2, 1 and 5 mM *p*-aminohippurate solutions were 98.5 (n = 9), 95.4 (n = 9) and 95.8% (n = 9) of the control peritubular membrane potential, respectively. The hyperpolarization was never seen in the *p*-aminohippurate solution. Though the depolarization was small, the cause was studied further. Addition of 10 mM mannitol did not cause any changes in the peritubular membrane potential, therefore, the osmotic effect of the *p*-aminohippurate

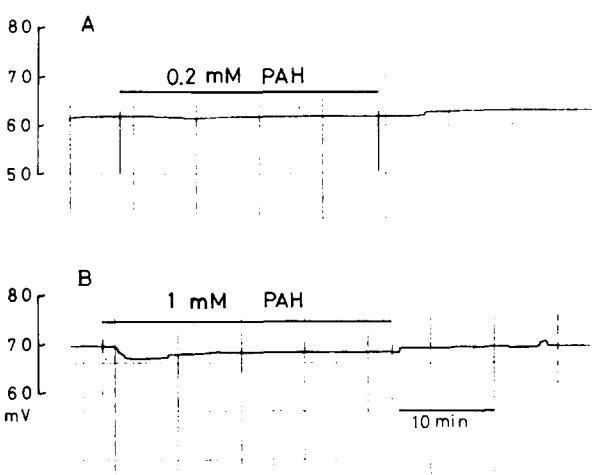

Fig. 3. Typical examples of tracings of the effects of the *p*-aminohippurate (PAH) perfusion in the basic solution on the peritubular membrane potential. 0.2 (A) and 1 mM (B) *p*-aminohippurate solutions were perfused during periods indicated by horizontal bars. Figures on the left side indicate the levels of peritubular membrane potential in mV, cell interior negative.

solution on the peritubular membrane potential was ruled out. The pH of the *p*-aminohippurate solution was adjusted to 7.4 by adding Tris, so that the effects of excess Tris on the peritubular membrane potential were studied. Such slight depolarization as seen in the *p*-aminohippurate solution were also observed when 2.5 mM Tris-HCl (pH = 7.4) was applied, where the minimal level of the peritubular membrane potential was 97.1% ($n = 7$) of the peritubular membrane potential in the basic solution. Thus the excess of Tris could account for at least a part of the slight depolarization produced by the *p*-aminohippurate solution. Therefore, it is concluded that *p*-aminohippurate seems to have no effect on the peritubular membrane potential.

p-Aminohippurate uptake and Na^+ gradient

The relationship between *p*-aminohippurate uptake and the $\Delta\bar{\mu}_{Na}$ was analysed. Intracellular concentrations of Na^+ and K^+ measured at various external Na^+ concentrations after 30 min incubation period were summarized in Table III. 0.2 mM *p*-aminohippurate in the basic medium produced no

TABLE III

EFFECTS OF EXTERNAL Na^+ CONCENTRATION ON INTRACELLULAR ELECTROLYTES CONCENTRATIONS, PERITUBULAR MEMBRANE POTENTIAL AND $\Delta\bar{\mu}_{Na}$

For ion concentrations, the subscripts o and i denote extracellular and intracellular concentrations at 30 min incubation period. Values of *p*-aminohippurate (PAH) uptake were obtained from the kinetic studies. Peritubular membrane potential ($\Delta\psi$), cell interior negative, was measured without *p*-aminohippurate. Control peritubular membrane potential values included initial and recovery peritubular membrane potential. Corrected peritubular membrane potential was derived from the mean value of the total observed control values in all series of experiments and the test-to-control ratios of the observed peritubular membrane potential. $\Delta\bar{\mu}_{Na}$ was calculated by using mean values of $[Na]_i$ and corrected peritubular membrane potential. Numbers in parentheses indicate the number of observations.

$[Na]_o$ (mM)	PAH (0.2 mM)	$[Na]_i$ (mmol/kg cell water)	$[K]_i$ (mmol/kg cell water)	PAH uptake (mmol/kg cell water per 30 min)
100	—	37.9 ± 2.1 (9)	79.1 ± 2.8 (9)	
100	+	37.8 ± 1.9 (9)	79.0 ± 2.2 (9)	1.053 ± 0.028 (26)
50	+	24.7 ± 0.9 (8)	92.2 ± 4.6 (8)	0.874 ± 0.024 (25)
25	+	20.2 ± 1.3 (8)	95.3 ± 4.5 (8)	0.665 ± 0.025 (25)
10	+	15.7 ± 0.9 (8)	93.2 ± 4.4 (8)	0.396 ± 0.012 (25)

	Observed $\Delta\psi$ (mV)			Corrected $\Delta\psi$ (mV)	$\Delta\bar{\mu}_{Na}$ ($\times 10^3$ joules/mol)
	Series 1	Series 2	Series 3		
Control					
100	61.7 ± 1.2 (18)	63.1 ± 1.2 (19)	60.5 ± 1.0 (19)	62.0 (49)	8.39
Test					
50	57.1 ± 1.7 (10)	—	—	57.4	7.28
25	—	57.6 ± 1.4 (10)	—	56.5	5.99
10	—	—	54.1 ± 1.9 (11)	55.4	4.23
Test/control	0.926	0.913	0.894		

significant change in intracellular Na^+ and K^+ concentrations ($P > 0.5$). This is probably because the maximal *p*-aminohippurate uptake rate is not so high and Na^+ entered the cells is extruded immediately by Na^+ pump at the peritubular membrane. When the tissues were incubated in the Na^+-depleted solutions, the cells lost a part of their Na^+ ($P < 0.001$ compared to the basic medium) and gained K^+ ($P < 0.02$). Intracellular Na^+ concentration decreased in parallel with decreasing external Na^+ concentration ($[Na]_i$ (mM) = 13.4 + 0.242 $[Na]_o$ (mM), $r = 0.917$). In *Necturus* kidney, intracellular Na^+ concentration was reported to be a saturable function of extracellular Na^+ concentration when tetramethylammonium was used as the substitute for Na^+ [20].

When we assume that the activity coefficients of the electrolytes are equal inside and outside the cell, the electrochemical potential gradient of monovalent cation ($\Delta\bar{\mu}_C$) across the peritubular membrane toward cell interior was calculated by the equation,

$$\Delta\bar{\mu}_C = RT \ln \frac{[C]_o}{[C]_i} - F\Delta\psi \qquad (3)$$

where $[C]_o$ and $[C]_i$ are extracellular and intracellular cation concentrations, R is the gas constant, T the absolute temperature, F the Faraday's constant and $\Delta\psi$ the peritubular membrane potential (cell interior negative). The chemical potential gradient of Na^+ ($\Delta\mu_{Na}$) alone did not appear to provide a sufficient driving force for *p*-aminohippurate transport, because in the 10 mM Na^+ medium the $\Delta\mu_{Na}$ was reversed but unphill *p*-aminohippurate transport occurred. Amino acid transport in ascites tumor cells was related to the both electrochemical potential gradients of Na^+ and K^+ ($\Delta\bar{\mu}_K$), i.e. related to $RT\ln([Na]_o[K]_i/[Na]_i[K]_o)$ [21]. However, such a relation was not the case in the present preparations (Table III). The peritubular membrane potential tended to fall slightly but significantly ($P < 0.05$ compared to the control) with reduction

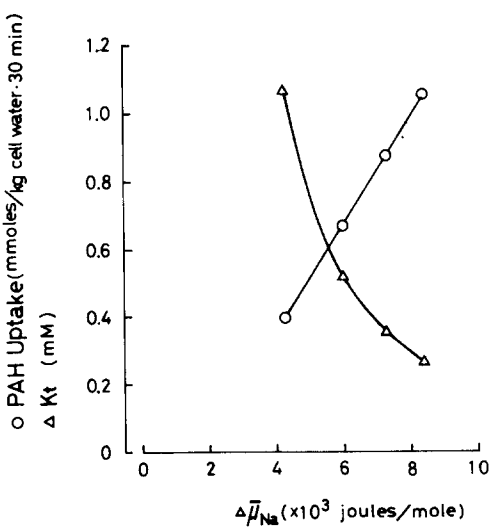

Fig. 4. Relationships between *p*-aminohippurate (PAH) uptake and $\Delta\bar{\mu}_{Na}$ (○) and between K_t and $\Delta\bar{\mu}_{Na}$ (△). Each point represents the mean value of the data from Tables I and III.

of Na^+ concentration in the perfusion fluid. When the Na^+-depleted and basic solutions were compared, the observed values of the peritubular membrane potential did not differ significantly from that in the sucrose-substituted Ringer's solution [22]. However, choline chloride substitution depolarized the tubular cells in *Necturus* kidney [20]. The relationship between *p*-aminohippurate uptake and the estimated $\Delta\bar{\mu}_{Na}$ are shown in Fig. 4, in which *p*-aminohippurate uptake is linearly related to the calculated $\Delta\bar{\mu}_{Na}$ at least in the range of Na^+ concentration used (*p*-aminohippurate uptake (mmol/kg cell water per 30 min) $=-0.276 + 0.158\ \Delta\bar{\mu}_{Na}$ ($\times 10^3$ joules/mol), $r = 1.00$). It was found also that the electrical potential term was larger than the chemical potential term in the $\Delta\bar{\mu}_{Na}$. The relation between K_t and the $\Delta\bar{\mu}_{Na}$ was not linear but tended to be hyperbolic.

Discussion

Active transport of various organic solutes, such as sugars and amino acids, in a variety of animal cells, is known to be Na^+ dependent, and the driving force of active solute transport is a Na^+ gradient [16,23–27]. The present study showed that *p*-aminohippurate uptake was dependent on the presence of Na^+ in the external medium and simply obeyed Michaelis-Menten kinetics with respect to both *p*-aminohippurate and Na^+ concentrations (Fig. 2 and Tables II and III). It was found also that K_t was a linear function of the reciprocal of Na^+ concentration, while V was not altered by Na^+ concentration. The time course of *p*-aminohippurate uptake (Fig. 1) and the effect of probenecid on *p*-aminohippurate uptake indicated that the uptake appeared to reflect the influx into the cells through a carrier-mediated process at the peritubular membrane. According to the review of Schultz and Curran [24], the data obtained in the present kinetic studies seem sufficient to warrant consideration of a possible model which could describe the *p*-aminohippurate-Na^+ interaction at the peritubular membrane of the proximal tubule. It is assumed that the carrier (X) combines with Na^+ to form a binary complex (XNa) first, then combines with *p*-aminohippurate (S) to form a ternary complex (XNaS). *p*-Aminohippurate is supposed to be transported across the membrane in a form of XNaS. Then, we obtain the following expression

$$v = k'[\mathrm{XNaS}] = \frac{k' X_t [\mathrm{S}]_o}{\frac{K_1 K_2}{[\mathrm{Na}]_o} + K_2 + [\mathrm{S}]_o} \tag{4}$$

where k' is the rate constant of translocation of XNaS, XNaS the concentration of the ternary complex, X_t the concentration of total carrier, K_1 and K_2 are the dissociation constants for the binary and ternary complexes. The values of dissociation constants for Na^+ (K_1) and *p*-aminohippurate (K_2), determined from the Eqns. 1 and 4, i.e. the relation between K_t and Na^+ concentration, were 53.2 mM and 0.168 mM, respectively. There was no change in the peritubular membrane potential during perfusion of the *p*-aminohippurate solution (Fig. 3), suggesting that the transport process at the peritubular membrane is non-electrogenic. Since pK_a of *p*-aminohippurate is 3.8, the substance is

present almost entirely in an anionic form at physiologic pH, therefore, no net charge transfer would occur on cotransport of *p*-aminohippurate with Na^+, if the carrier is electrically neutral. Thus, the electrophysiological findings support this model. Similar interactions of Na^+ and organic acid molecule at the carrier level have been reported by Hoshi and Hayashi [6] and Bresler and coworkers [7—9]. Hoshi and Hayashi [6] demonstrated that, in goldfish kidney, one molecule of phenol red was cotransported with two Na^+ across the peritubular membrane. Bresler and coworkers [7—9] also showed that, in surviving frog kidney, two Na^+ were necessary for transport of one fluorescein anion whereas one Na^+ was required for active transfer of one uranin anion. They have suggested from their findings that one Na^+ may be necessary for active transport of *p*-aminohippurate in frog kidney. This suggestion is consistent with the present results in newt kidney.

Bresler and coworkers [7—9] postulated that a Na^+ gradient in the peritubular microenvironment (membrane-side Na^+ gradient) was a driving force for organic acid transport. The present study showed that the $\Delta\mu_{Na}$ alone could not account for *p*-aminohippurate accumulation and *p*-aminohippurate uptake was not related to the both $\Delta\bar{\mu}_{Na}$ and $\Delta\bar{\mu}_K$. It was observed that when external K^+ concentration was changed, *p*-aminohippurate uptake was related only to the $\Delta\bar{\mu}_{Na}$ (unpublished observation). So that the $\Delta\bar{\mu}_K$ seems not to be related directly to *p*-aminohippurate transport in newt kidney. On the other hand, *p*-aminohippurate uptake decreased linearly with decrease in the $\Delta\bar{\mu}_{Na}$ (Table III and Fig. 4). These results suggest that the simultaneous presence of both Na^+ and the electrical potential difference, i.e. the presence of the $\Delta\bar{\mu}_{Na}$ across the membrane are necessary for *p*-aminohippurate transport in newt kidney. The assumptions of uniform distribution of cellular electrolytes and equal activity coefficients for the extracellular and intracellular electrolytes may be limitted as pointed out by Heinz [21] and Schultz and Curran [24]. However, it is thought that these assumptions do not largely influence the above conclusion, because the electrical potential term is larger than the chemical potential term in the $\Delta\bar{\mu}_{Na}$ (Table III). Also, if the nuclear sequestration of Na^+ increases with increasing external Na^+ concentration as observed in ascites tumor cells [21], the slope of the line of Fig. 4 may be only gentler. However, it is difficult to think that the electrical potential gradient acts directly on the translocation of the ternary complex since the transport is non-electrogenic.

Gerencser and coworkers [12,13,28] have shown that, in rabbit kidney, Na^+ gradient hypothesis does not adequately explain the transport of organic acids and that Na^+ possibly has an intracellular role through its stimulation of (Na^+ + K^+)-ATPase, which channels energy into the *p*-aminohippurate accumulative mechanism. The authors supported the model proposed by Kimmich [29], who suggested a direct participation of energized intermediates generated by (Na^+ + K^+)-ATPase for intestinal sugar transport. However, such ($Na^+ + K^+$)-ATPase dependence was denied by an observation in purified ATP-free brush border membrane vesicles [25]. Podevin and Boumendil-Podevin [10] have reported, by using rabbit kidney cortex slices, that *p*-aminohippurate has a similar kinetic response to Na^+ as that observed by Gerencser et al. [12], namely, increasing external Na^+ increased V without affecting K_t. However, Podevin and Boumendil-Podevin [20] found that *p*-aminohippurate uptake was specifically

enhanced by a Na^+ gradient in the absence of active electrolyte transport. They concluded that their results were more consistent with Na^+ gradient hypothesis rather than with the model of Kimmich. Furthermore, recently, Kimmich et al. [27] themselves showed that Na^+ concentration gradient, as well as the electrical potential gradient, could provide energy for intestinal Na^+-dependent transport systems.

Berner and Kinne [15] showed that, in baso-lateral membrane vesicles isolated from rat kidney cortex, *p*-aminohippurate uptake increased when inside of the membrane became more positive, suggesting that the transfer of *p*-aminohippurate across the membrane was accompanied by a transfer of negative charge. They also could not provide an evidence for the existence of Na^+-*p*-aminohippurate cotransport system. On the other hand, in newt kidney, no hyperpolarization was observed in the *p*-aminohippurate solution (Fig. 3) and also *p*-aminohippurate uptake decreased when the cells depolarized (thus the $\Delta\bar{\mu}_{Na}$ decreased) (unpublished observations). A possible explanation for the absence of Na^+ dependence in their vesicle preparations may be the alteration in the cation permeability from the intact cell membranes. Ullrich et al. [16] have shown that *p*-aminohippurate transport was only slightly affected by ouabain in the kidney of golden hamster and concluded that *p*-aminohippurate transport did not seem to be driven by a Na^+ gradient. However, in newt kidney, we observed that *p*-aminohyppurate uptake by the ouabtain-treated tissue decreased to 27% of that by the untreated tissue (control) with concomitant decrease in the $\Delta\bar{\mu}_{Na}$ to 27% of the control (unpublished observation).

It is concluded, therefore, that the peritubular process of *p*-aminohippurate transport in newt kidney is mediated by a Na^+-coupled transport mechanism and the maintenance of the $\Delta\bar{\mu}_{Na}$ across the peritubular membrane is required for *p*-aminohippurate transport.

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