Biochimica et Biophysica Acta, 556 (1979) 354—365
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BBA 78484

EFFECTS OF CHANGES IN SODIUM ELECTROCHEMICAL POTENTIAL GRADIENT ON *p*-AMINOHIPPURATE TRANSPORT IN NEWT KIDNEY

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(Received February 9th, 1979)

Key words: p-Aminohippurate transport; K^+; Na^+ gradient; Ouabain; (Newt kidney)

Summary

1. The relation between *p*-aminohippurate uptake and the electrochemical potential gradient of Na^+ ($\Delta\bar{\mu}_{Na^+}$) across the peritubular membrane was examined in newt (*Triturus pyrrhogaster*) kidney. The $\Delta\bar{\mu}_{Na^+}$ was modified by changing cellular Na^+ concentration and/or lowering the electrical potential difference across the peritubular membrane (peritubular membrane potential).

2. Elevation of external K^+ concentration or addition of alanine at 40 mM to the medium decreased the $\Delta\bar{\mu}_{Na^+}$ mainly through the depolarization of the cells. Addition of 1 mM ouabain resulted in a decrease in the peritubular membrane potential and increase in cellular Na^+ concentration, thus decrease in the $\Delta\bar{\mu}_{Na^+}$.

3. *p*-Aminohippurate uptake decreased in proportion to the decrease in the $\Delta\bar{\mu}_{Na^+}$ under all experimental conditions, indicating that the maintenance of the $\Delta\bar{\mu}_{Na^+}$ is required for *p*-aminohippurate transport.

4. All three different experimental conditions, high medium K^+ concentration, 40 mM alanine or 1 mM ouabain, increased the apparent Michaelis constant, K_t, without affecting the maximal uptake rate, V, for *p*-aminohippurate. These results suggests that the $\Delta\bar{\mu}_{Na^+}$, largely the peritubular membrane potential, may affect the association and/or dissociation of *p*-aminohippurate and Na^+ at both interfaces of the peritubular membrane of the proximal tubular cells.

Introduction

It is now widely accepted that active transport of organic solutes, such as sugars and amino acids, into the cells is Na^+ coupled (cotransport) and the

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direct driving force for the uphill transport is Na^+ gradient across the membrane (Na^+ gradient hypothesis) [1—5]. Recently the important contribution of the electrical potential difference across the membrane to active solute transport has been pointed out [4—11]. Heinz and coworkers [8,9] have suggested that, in case in which the chemical potential gradient of Na^+ ($\Delta\mu_{Na^+}$) falls short as a driving force for active α-aminoisobutyric acid transport in Ehrlich tumor cells, it is sufficiently supplemented by an increased electrical potential difference due to the stimulation of an electrogenic Na^+ pump. Furthermore, they have shown that, using K^+ ionophore valinomycin, the electrical potential difference has special effects on kinetics of cotransport, which are different from those of an equivalent chemical potential difference.

In our previous paper [12], we have shown that active *p*-aminohippurate transport into the proximal tubular cells of newt kidney is possibly mediated by a *p*-aminohippurate-Na^+ cotransport system and the maintenance of the electrochemical potential gradient of Na^+ ($\Delta\bar{\mu}_{Na^+}$) across the peritubular membrane appears to be very important in *p*-aminohippurate transport. The present study was undertaken to further investigate the possible contribution of the $\Delta\bar{\mu}_{Na^+}$, especially the electrical potential term, to *p*-aminohippurate uptake. For this purpose, we modified the $\Delta\bar{\mu}_{Na^+}$ by changing cellular Na^+ concentration and/or lowering the electrical potential difference across the peritubular membrane (peritubular membrane potential) of the proximal tubule by elevating external K^+ concentration or adding depolarizing substances such as L-alanine and ouabain.

Methods

Preparation of tissues and media. Experiments were performed on newts (*Triturus pyrrhogaster*) of both sexes, weighing 3—10 g. The isolated whole kidneys, 10—30 mg in weight, were used as described previously [12,13]. 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. All experiments were carried out at 25° C under aerobic conditions.

Experimental procedures. Tissue water content, inulin space, cellular Na^+ and K^+ concentrations, *p*-aminohippurate uptake and the peritubular membrane potential were measured under various conditions. The effects of elevation of K^+ concentration were examined in both 10 and 50 mM Na^+ media. In this experiment, choline chloride was replaced by equimolar KCl. The conditions are outlined in Table I. When the effects of 40 mM L-alanine were tested, mannitol was added to the solution at the same concentration as a control. In that case, NaCl was reduced to 70 mM (80 mM total Na^+) or choline chloride was reduced to 70 mM (10 mM Na^+), respectively. The experimental procedures were mostly the same as described previously [12]. For the uptake experiments, four to nine tissues were preincubated for 20 min in one tube containing 5—10 ml of the Na^+-depleted (10 mM Na^+ and 2.4 mM K^+) solution. After preincubation, the tissues were transferred to the test incubation media and then uptake was measured for 30 min. At the end of incubation, the tissues

were blotted, weighed and then extracted. Inulin space, estimating the extracellular space, by addition of 5 g/l inulin in the media. For the potential measurements, the tissue was transferred into a small lucite chamber and fixed. Inside of the chamber was continuously perfused. The peritubular membrane potential was recorded during alternate perfusion with the control and test solutions by glass microelectrode with 3 M KCl. The peritubular membrane potentials in the test solutions were observed for more than 20 min. The effects of 1 mM ouabain (Merck) were investigated in the basic solution. To observe the time course of the ouabain effects, the tissues were preincubated for 20 min in the absence of ouabain and then uptake was measured in the presence of ouabain up to 90 min. The peritubular membrane potential was recorded for 90 min in the ouabain-containing solution. When the value of the initial peritubular membrane potential was lower than 50 mV, cell interior negative, the data were discarded.

Analytical methods. Tissue water content was determined from the difference in weight between wet and dried tissues. *p*-Aminohippurate was assayed by the method of Waugh and Beall [14], inulin by the simplified anthrone method of Davidson and Sackner [15], and Na^+ and K^+ by flamephotometry. All tissue *p*-aminohippurate and inulin contents were corrected for the tissue blank. Intracellular contents of *p*-aminohippurate, Na^+ and K^+ were calculated by using the tissue water content and the inulin space, assuming that these solutes distributed uniformly in cell water.

Statistics. Regression lines were calculated by the least-squares method. Values were represented as means ± S.E. Data were statistically analysed with the Student's *t*-test.

Results

Effects of medium K^+ concentration

The effects of elevation of external K^+ concentration were examined in both 10 and 50 mM Na^+ media (Table I and Fig. 1). The increase in K^+ concentration caused a swelling of the cells equally when incubated in both Na^+ media. Cellular Na^+ concentration slightly decreased and cellular K^+ concentration increased when medium K^+ concentration was elevated. The intracellular negativity significantly decreased by elevation of K^+ concentration. The effect of high K^+ medium on the peritubular membrane potential was similar to that observed by Sakai et al. [16] in newt kidney and by Giebish [17] in *Necturus* kidney. The increase in K^+ concentration caused a marked decrease in *p*-aminohippurate uptake ($P < 0.05$). Calculation of the $\Delta\bar{\mu}_{Na^+}$ across the peritubular membrane toward cell interior was made by assuming that the activity coefficients for electrolytes are equal between intracellular and extracellular fluids. The $\Delta\bar{\mu}_{Na^+}$ at various K^+ concentrations was shown in Fig. 1. It was found that *p*-aminohippurate uptake decreased in proportion to the decrease of the $\Delta\bar{\mu}_{Na^+}$ (*p*-aminohippurate uptake (mmol/kg cell water per 30 min) = 0.043 + 0.103 $\Delta\mu_{Na^+}$ ($\times 10^3$ J/mol), correlation coefficient, $\gamma = 0.976$).

Effects of alanine

Addition of L-alanine to the peritubular fluid caused a depolarization of the

TABLE I

EFFECTS OF EXTERNAL K^+ CONCENTRATION ON TISSUE WATER CONTENT, INULIN SPACE, CELLULAR Na^+ AND K^+ CONCENTRATIONS AND PERITUBULAR MEMBRANE POTENTIAL

For ion concentrations, the subscripts o and i denote extracellular and intracellular concentrations at 30 min incubation period. Corrected peritubular membrane potential ($\Delta\Psi$), cell interior negative, was obtained from the mean value of the total observed control values and the test-to-control ratios of the observed peritubular membrane potentials. Numbers is parentheses indicate number of observations.

$[Na^+]_o$ (mM)	$[K^+]_o$ (mM)	Tissue water content (% of tissue wet wt.)	Inulin space (% of tissue water)	$[Na^+]_i$ (mmol/kg cell water)	$[K^+]_i$ (mmol/kg cell water)	Corrected $\Delta\Psi$ (mV)
	control					
10	2.4	80.1 ± 0.8 (16)	30.7 ± 1.8 (8)	16.0 ± 0.6 (8)	88.7 ± 3.9 (8)	56.2 (50)
	test					
	12.4	81.0 ± 0.3 (9)	27.9 ± 2.2 (8)	14.4 ± 0.8 (8)	87.5 ± 3.0 (8)	39.2 * (9)
	27.4	82.4 ± 0.5 (9)	24.9 ± 1.9 * (8)	12.6 ± 0.9 * (8)	89.6 ± 2.7 (8)	27.7 * (9)
	52.4	84.7 ± 0.7 * (9)	22.3 ± 1.0 * (8)	10.5 ± 0.5 * (8)	90.8 ± 1.6 (8)	16.9 * (9)
	92.4	86.9 ± 0.5 * (9)	19.7 ± 1.4 * (8)	10.4 ± 0.3 * (8)	109.3 ± 1.6 * (8)	5.8 * (10)
	control					
50	2.4	80.1 ± 0.5 (9)	30.2 ± 1.1 (9)	26.9 ± 1.4 (9)	88.0 ± 3.5 (9)	59.4 (44)
	test					
	12.4	80.9 ± 0.2 (9)	27.5 ± 0.9 (9)	26.3 ± 1.4 (9)	93.1 ± 3.2 (9)	42.3 * (10)
	27.4	82.4 ± 0.2 * (9)	25.0 ± 0.9 * (9)	23.2 ± 1.1 (9)	100.4 ± 2.2 * (9)	28.4 * (10)
	52.4	84.5 ± 0.4 * (9)	22.2 ± 0.7 * (9)	21.2 ± 0.9 * (9)	104.8 ± 1.8 * (9)	17.0 * (9)

* Statistically significant ($P < 0.05$) compared to the control.

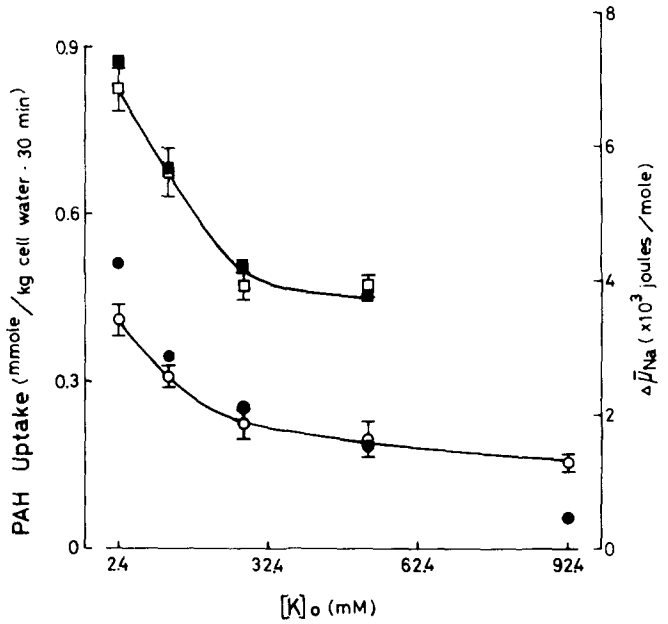

Fig. 1. Effects of external K^+ concentration on *p*-aminohippurate (PAH) uptake and the $\Delta\bar{\mu}_{Na^+}$. *p*-Aminohippurate uptake was studied in 50 mM (n = 9 for each K^+ concentration, □) and 10 mM (n = 8, ○) Na^+ media with 0.2 mM *p*-aminohippurate. The $\Delta\bar{\mu}_{Na^+}$ was calculated using the corrected peritubular membrane potential and mean intracellular Na^+ concentration (■ and ● for 50 and 10 mM Na^+ media). Vertical bars indicate the S.E.

proximal tubular cells [13]. The alanine-induced depolarization could be ascribed to the luminal membrane transport of alanine which entered the lumen through the nephrostomes. Fig. 2 shows a typical example of change in the peritubular membrane potential induced by 40 mM alanine in the perfusion fluid. Alanine always evoked a depolarization and the depolarized level of the

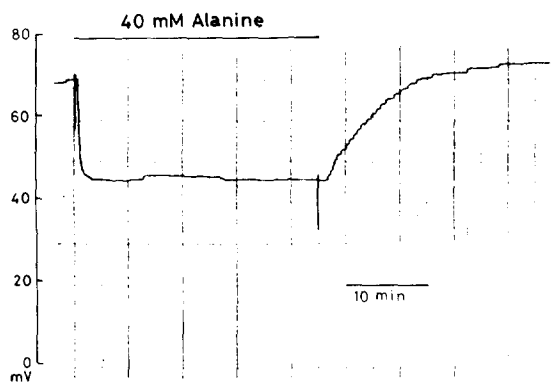

Fig. 2. Response of the peritubular membrane potential to 40 mM L-alanine added to the perfusion fluid (80 mM Na^+). Alanine was perfused during periods indicated by horizontal bar. During the other periods, control solution was perfused. Figures on the left side of the panel indicate the levels of the peritubular membrane potential, cell interior negative.

membrane potential was sustained as long as the perfusion was continued. On switching to the control solution, the peritubular membrane potential returned (repolarized) to the initial level. Table II summarizes the results of the effects of 40 mM alanine. Alanine caused a slight swelling of the cells in the 80 mM Na^+ medium. This is probably due to the osmotic effect of intracellular accumulated alanine. Cellular Na^+ concentrations were not significantly altered by alanine in both 10 ($P > 0.5$) and 80 mM ($0.4 < P < 0.5$) Na^+ media. Cellular K^+ concentration was also not significantly altered ($0.05 < P < 0.01$). These findings are consistent with the observations by Wheeler and Christensen [18] and Koopman and Schultz [19], in which alanine did not affect cellular Na^+ and K^+ concentrations. The alanine-induced depolarizations were observed in both 10 and 80 mM Na^+ media. When the $\Delta\bar{\mu}_{Na^+}$ decreased to 69% of the control in the 1C mM Na^+ medium and 72% in the 80 mM Na^+ medium, *p*-aminohippurate uptake decreased to 77% and 61% of the control, respectively (*p*-aminohippurate uptake = $-0.446 + 0.182\ \Delta\bar{\mu}_{Na^+}$, $\gamma = 0.966$).

Effects of ouabain

Fig. 3 shows the time course of ouabain (1 mM) effects on tissue water content, inulin content, cellular Na^+ and K^+ concentrations and *p*-aminohippurate uptake. Significant swelling of the cells was not observed up to 60 min and then the cells slightly swelled with time. Addition of ouabain resulted in a decrease in cellular K^+ concentration and increase in cellular Na^+ concentration and finally abolished the difference between intracellular and extracellular Na^+ concentrations after 60 min incubation. Cellular Na^+ plus K^+ concentrations remained constant during exposure to ouabain. After 40 min incubation, further accumulation of *p*-aminohippurate practically seased. Fig. 4 shows a typical example of tracings of the peritubular membrane potential in the presence of ouabain. Ouabain depolarized the cell gradually. On switching to the basic solution, the peritubular membrane potential did not repolarized to the initial level. 1 mM ouabain could not abolish the cell negativity completely during 90 min perfusion. The peritubular membrane potentials were 27.4 ± 6.7 mV at 60 min and 22.6 ± 1.9 mV at 90 min, respectively ($n = 9$, cell interior negative). These values of the peritubular membrane potential were lower than that caused by 0.14 mM ouabain in *Necturus* kidney, in which 36 mV was observed [17]. Giebish [17] showed that the reduction of intracellular negativity by ouabain was in parallel with the loss of intracellular K^+. Such a parallelism was confirmed in the present observations (Figs. 3 and 4). To investigate *p*-aminohippurate uptake under ouabain-poisoned condition, the uptake was measured for 30 min in the presence of ouabain after preincubation with ouabain for 60 min. Control experiments were done in the same manner without ouabain. The results are shown in Table III. The $\Delta\mu_{Na^+}$ was completely abolished by ouabain but cell-to-medium ratio of *p*-aminohippurate concentrations exceeded unity. However, the remaining electrical potential gradient might account for this accumulation of *p*-aminohippurate. *p*-Aminohippurate uptake by ouabain-treated tissues decreased to 27% of the control with concomitant decrease in the $\Delta\bar{\mu}_{Na^+}$ to 27% of the control.

TABLE II

EFFECTS OF ALANINE (40 mM) ON TISSUE WATER CONTENT, INULIN SPACE, *p*-AMINOHIPPURATE UPTAKE, CELLULAR Na^+ AND K^+ CONCENTRATIONS, PERITUBULAR MEMBRANE POTENTIAL AND $\Delta\bar{\mu}_{Na^+}$

p-Aminohippurate concentration in the medium was 0.2 mM. Control peritubular membrane potentials included initial and recovery peritubular membrane potentials. Numbers in parentheses indicate number of observations.

	$[Na^+]_0$ (mM)			
	10		80	
	+40 mM Mannitol (control)	+40 mM Alanine	+40 mM Mannitol (control)	+40 mM Alanine
Tissue water content (% of tissue wet weight)	79.9 ± 0.4 (9)	80.0 ± 0.7 (9)	80.6 ± 0.3 (8)	82.4 ± 0.3 ** (8)
Inulin space (% of tissue water)	*	29.9 ± 1.3 (9)	*	24.9 ± 2.7 (8)
p-Aminohippurate uptake (mmol/kg cell water per 30 min)	0.407 ± 0.023 (18)	0.314 ± 0.025 ** (18)	1.126 ± 0.048 (26)	0.687 ± 0.022 ** (26)
$[Na^+]_i$ (mmol/kg cell water)	12.3 ± 0.6 (9)	12.4 ± 0.6 (9)	35.1 ± 1.5 (8)	36.6 ± 1.3 (8)
$[K^+]_i$ (mmol/kg cell water)	—	—	78.3 ± 2.3 (8)	71.3 ± 2.6 (8)
Peritubular membrane potential (mV)	61.6 ± 0.9 (18)	44.5 ± 1.4 ** (12)	66.2 ± 1.3 (21)	42.9 ± 0.9 ** (12)
$\Delta\bar{\mu}_{Na^+}$ ($\times 10^3$ J/mol)	5.43	3.76 **	8.42	6.07 **

* Inulin space in the control media was not measured in this experiment, since mannitol interferes with the color development of inulin [15]. So that inulin space was estimated 30% of total tissue water.

** Statistically significant ($P < 0.01$) compared to the control.

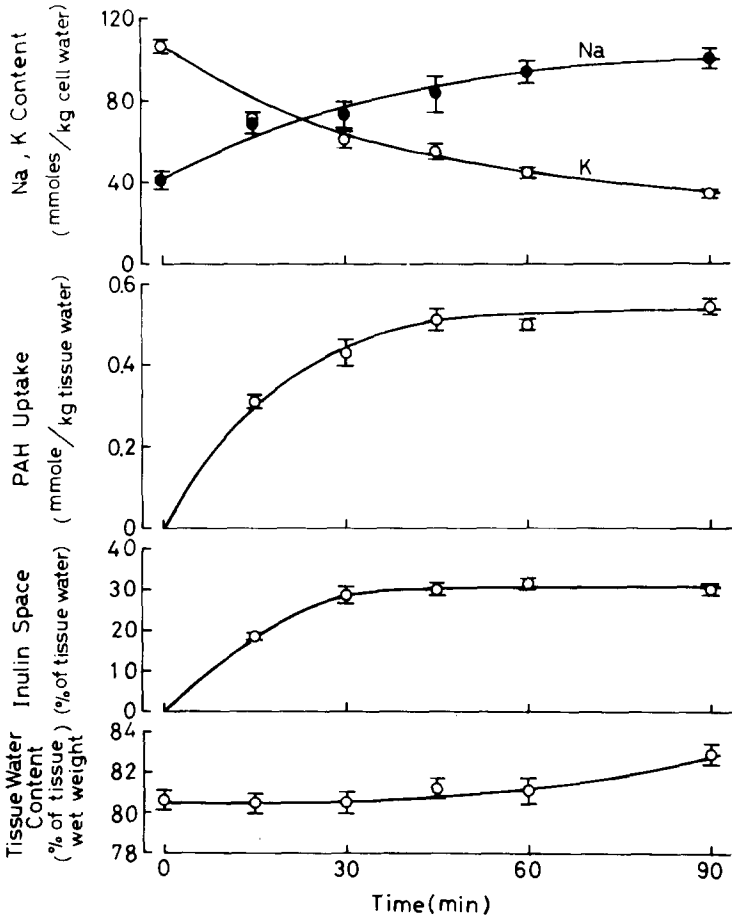

Fig. 3. Time course of ouabain (1 mM) effect. *p*-Aminohippurate (PAH) and Na^+ concentrations in the medium were 0.2 and 100 mM. *p*-Aminohippurate uptake and cellular Na^+ and K^+ concentration were calculated estimating that tissue water content was 80% and inulin space was 30%. Each point represents the mean of four to five observations with bars indicating S.E.

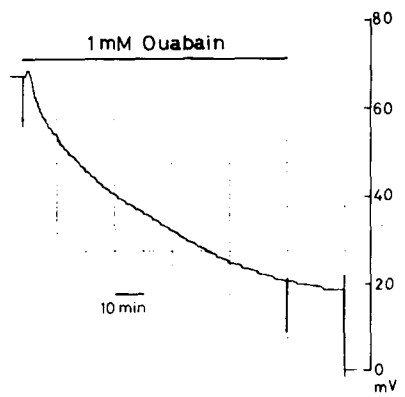

Fig. 4. Time course of the peritubular membrane potential after perfusion of 1 mM ouabain (100 mM Na^+). Ouabain was perfused during period indicated by horizontal bar.

TABLE III

EFFECTS OF OUABAIN (1 mM) ON TISSUE WATER CONTENT, INULIN SPACE, *p*-AMINOHIPPURATE UPTAKE, CELLULAR Na^+ AND K^+ CONCENTRATIONS, PERITUBULAR MEMBRANE POTENTIAL AND $\Delta\bar{\mu}_{Na^+}$

p-Aminohippurate and Na^+ concentrations in the medium were 0.2 and 100 mM.

	n	Ouabain-free (control)	Ouabain (1 mM)
Tissue water content (% of tissue wet weight)	9	80.3 ± 0.5	80.5 ± 0.3
Inulin space (% of tissue water)	9	30.1 ± 0.6	24.1 ± 0.6 *
p-Aminohippurate uptake (mmol/kg cell water per 30 min)	27	1.000 ± 0.031	0.270 ± 0.011*
$[Na^+]_i$ (mmol/kg cell water)	9	49.8 ± 2.4	99.8 ± 2.6*
$[K^+]_i$ (mmol/kg cell water)	9	81.8 ± 1.8	27.7 ± 1.5 *
Peritubular membrane potential (mV)	9	66.1 ± 2.4	22.6 ± 1.9 *
$\Delta\bar{\mu}_{Na^+}$ ($\times 10^3$ J/mol)		8.11	2.19 *

* Statistically significant ($P < 0.001$) compared to the control.

TABLE IV

EFFECTS OF K^+, ALANINE AND OUABAIN ON KINETIC PARAMETERS

In one experiment, six kidneys were examined at the following concentrations of *p*-aminohippurate, 0.05, 0.1, 0.2 and 0.4 mM in the media except for in the 10 mM Na^+ medium, in which *p*-aminohippurate concentrations were 0.1, 0.2, 0.4 and 0.8 mM, respectively. Each V and K_t was obtained as described previously [12]. Each value represents mean of two experiments.

$[Na^+]_0$ (mM)	Medium	V (mmol/kg cell water per 30 min)	K_t (mM)
10	+2.4 mM K^+	2.26	1.05
	+92.4 mM K^+	2.80	3.08
50	+2.4 mM K^+	2.36	0.37
	+52.4 mM K^+	2.22	0.74
80	+40 mM mannitol	2.53	0.29
	+40 mM alanine	2.34	0.48
100	ouabain-free	2.33	0.28
	+1 mM ouabain	2.21	1.40

Effects of K^+, alanine and ouabain on kinetic parameters

In order to gain insight into the inhibitory effects of high K^+, alanine and ouabain on *p*-aminohippurate uptake, we examined the kinetic parameters, apparent Michaelis constant, K_t, and the maximal rate of uptake, V, for *p*-aminohippurate. Table IV summarizes the data obtained under various conditions. High medium K^+ concentration increased K_t without changing V. Similar effects of K^+ on the kinetic parameters were observed by Crane et al. [20] and Eddy and Hogg [21]. 40 mM alanine and 1 mM ouabain also affect K_t without influencing on V. It was found that K_t increased when the $\Delta\bar{\mu}_{Na^+}$ was decreased.

Discussion

We previously postulated a model for the *p*-aminohippurate-Na^+ interaction at the peritubular membrane of newt kidney proximal tubule [12], in which we assumed that *p*-aminohippurate is transported across the membrane only in

an electrically neutral form of carrier-Na^+-*p*-aminohippurate. The data from the present study together with those of the previous study [12] indicate that the decrease in the $\Delta\bar{\mu}_{Na^+}$ was linearly related to the decrease in *p*-aminohippurate uptake (*p*-aminohippurate uptake = $-0.039 + 0.122\ \Delta\bar{\mu}_{Na^+}$, $\gamma = 0.961$) (Fig. 5). These results suggest that the $\Delta\bar{\mu}_{Na^+}$ is required for *p*-aminohippurate uptake in newt kidney. However, the electrochemical potential gradient of *p*-aminohippurate ($\Delta\bar{\mu}_{PAH}$) toward cell exterior was $10.1 \cdot 10^3$ J/mol at 30 min incubation period in the basic medium, so that the $\Delta\bar{\mu}_{Na^+}$ ($8.83 \cdot 10^3$ J/mol) toward cell interior could not sufficiently account for accumulation of *p*-aminohippurate. The present assumptions of equal activity coefficients and uniform distribution may be oversimplificated and estimates of the concentrations of Na^+ and organic solute at cytoplasmic side of membrane are subject to considerable uncertainty [3,22]. For example, (1) an unstirred layer may be present in the microenvironment at the cell membrane [23,24]; (2) a fraction of *p*-aminohippurate binds to the membrane and cytoplasmic proteins [25—27]; (3) volume-average intracellular Na^+ concentration determined using standard analytic techniques does not represent the effective intracellular Na^+ activity [7,22], and (4) nuclear sequestration of Na^+ is present [22,28]. Considering these facts, the true $\Delta\bar{\mu}_{PAH}$ may be lower and $\Delta\bar{\mu}_{Na^+}$ higher than the estimated values in the present study. However, it is difficult to think that the $\Delta\bar{\mu}_{Na^+}$ is the direct driving force for *p*-aminohippurate transport because *p*-aminohippurate uptake increased up to 60 min and the transport complex is electrically neutral [12].

Numerous studies have shown that the replacement of Na^+ with K^+ has a greater inhibitory effect on Na^+-coupled transport processes than that with other cations [3]. The present study also shows that elevation of medium K^+

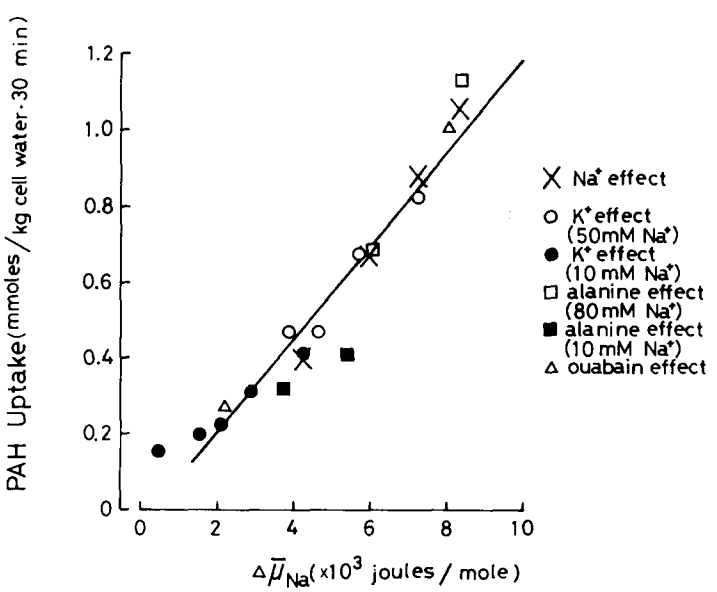

Fig. 5. Relationship between *p*-aminohippurate (PAH) uptake and the $\Delta\bar{\mu}_{Na^+}$. Each point represents the mean values of the data from Fig. 1 (○,●), Tables II (□,■) and III (△) and Ref. 12 (X).

concentration decreased *p*-aminohippurate uptake (Fig. 1). Four possibilities of the inhibitory effects of high K^+ concentration may be thought as follows: (1) competition with Na^+ for a common binding site in the Na^+-dependent processes [1,2,20,21]; (2) marked swelling of the tissues, then alterations in membrane and/or cellular functions [3,29]; (3) depolarization of the cells [4], and (4) change in permeability of the membrane for the organic acid [30]. From the present results of K^+ effects on *p*-aminohippurate uptake and the $\Delta\bar{\mu}_{Na^+}$ (Fig. 1) and that addition of alanine or ouabain showed similar effects on the kinetic parameters as those of high K^+ concentration (Table IV), the inhibitory effect of high K^+ concentration on the uptake seems to be mainly due to the depolarization of the cells. This idea appears to be consistent with that of Gibb and Eddy [6], who showed that valinomycin increased amino acid transport in ascites tumor cells and suggested that a K^+ gradient influenced amino acid transport only indirectly, i.e. by modifying the electrical potential difference and thus the $\Delta\bar{\mu}_{Na^+}$. In addition, the results that the decrease in intracellular negativity decreased *p*-aminohippurate uptake in this study are consistent with those of Podevin et al. [31], who have shown that conditions which created an interior-positive membrane potential inhibited the Na^+-dependent transport of *p*-aminohippurate in rabbit cortical slices.

The $\Delta\bar{\mu}_{Na^+}$ did not alter V in the present study (Table IV), suggesting that the rate of translocation of the transport complex across the membrane is not affected. This suggestion appears to be consistent with that the transport complex is electrically neutral and thus the rate of translocation is not affected by the electrical fields. However, the nature of the effect of the $\Delta\bar{\mu}_{Na^+}$ on K_t (table IV) is not fully clear at present. An asymmetry in the substrate-specific Na^+-dependent process at both sides of the membrane has been suggested by many authors [1,2,9,21,24]. Recently, Toggenburger et al. [11] have indicated 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 optimal phlorizin binding to the brush border membrane from rabbit small intestine. Also, they showed that when the electrical gradient was reduced, the extent of reduction in phlorizin binding was approximately the same as that in the apparent affinity of phlorizin. Our present study is consistent with the findings described above. Therefore, in conclusion, the role of the $\Delta\bar{\mu}_{Na^+}$ in active *p*-aminohippurate transport is confirmed to be the maintenance of the asymmetry in the *p*-aminohippurate-Na^+-coupled process at both surface of the peritubular membrane of newt kidney proximal tubule. Consequently, the concentration gradient of the ternary complex is formed across the membrane which results in an active *p*-aminohippurate transport.

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