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ROLES OF SODIUM AND POTASSIUM IONS ON *P*-AMINOHIPPURATE TRANSPORT IN RABBIT KIDNEY SLICES

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SUMMARY

This investigation was principally undertaken to test the ionic gradient hypothesis as applied to active *p*-aminohippurate uptake in the rabbit kidney cortical slice preparation. Efflux of *p*-aminohippurate from the slice was shown to be independent of external Na^+ concentration. Transferring slices from a low sodium preincubation to a high sodium incubation medium containing *p*-aminohippurate increased intracellular concentrations of both Na^+ and K^+, and *p*-aminohippurate accumulation occurred. Transferring slices from a low sodium preincubation to a high sodium incubation medium containing ouabain and *p*-aminohippurate resulted in a net increase in intracellular Na^+ concentration but no *p*-aminohippurate accumulation occurred. Different combinations of preincubation and incubation media gave a high to low array of intracellular Na^+ concentrations and these directly reflected their respective *p*-aminohippurate uptake. These results suggest that the Na^+-gradient hypothesis does not adequately explain the transport of organic acids in rabbit kidney. These results also suggest that Na^+ possibly has an intracellular role through its stimulation of $(Na^+ + K^+)$-ATPase channeled to energizing the *p*-aminohippurate accumulative mechanism.

INTRODUCTION

Cation dependence of active organic acid transport in various kidney preparations has been firmly established. For example, in isolated flounder tubules [1], K^+ is needed for active uptake of phenolsulfonphtalein from the bathing medium into the cell (Step I), while active transport of phenolsulfonphthalein across the luminal membrane (Step II) is Ca^{2+} dependent. Taggart et al. [2] have shown in rabbit kidney slices that *p*-aminohippurate is maximal only when tissue Na^+ and K^+ concentrations are close to normal. Ross et al. [3] demonstrated a 50–70 % reduction in *p*-aminohippurate uptake in dog kidney slices if K^+ and Ca^{2+} were omitted from the bathing medium. Foulkes and Miller [4] went a step further and suggested that the K^+ stimulatory effect on *p*-aminohippurate accumulation occurred at a peritubular membrane transport site.

In the last 15 years the importance of Na^+ to active intestinal transport of organic solutes has been pointed out by many investigators. One school of thought [5] favors the Na^+-gradient hypothesis, in which active solute accumulation is not directly supported by the expenditure of metabolic fuels but is effected through a simultaneous down-hill translocation of Na^+ from the extracellular to intracellular space. In fact, Khuri et al. [6] also suggested that D-glucose reabsorption in the renal proximal tubule occurs via a carrier linkage to an extracellular to intracellular Na^+ gradient. Another school of thought [7] holds that intracellular, not extracellular, Na^+ is the stimulus for maximal solute accumulation in epithelial systems. However, relative to renal secretory phenomena, the possible stimulating or modifier effects of cations, especially Na^+, has not been adequately explored. Vogel and Kroger [8] showed in a doubly perfused frog kidney that transport of *p*-aminohippurate is much more dependent on the Na^+ concentration in the arterial (glomerular) perfusion fluid than it is on that in the peritubular fluid, a fact which suggests that *p*-aminohippurate secretion depends upon Na^+ reabsorption.

Earlier experiments in our laboratory [9] demonstrated a dominant role for Na^+ in *p*-aminohippurate uptake in the rabbit kidney slice. Following this, we showed in a kinetic study [10] that the K_m for *p*-aminohippurate uptake is independent of the Na^+ concentration in the medium, while V for *p*-aminohippurate uptake is directly proportional to the Na^+ concentration in the medium. These results are not consistent with the Na^+-gradient hypothesis, as formulated by Crane [11].

In the present experiments, we further examined the cation dependence of active organic acid secretion by testing the various hypotheses mentioned above with a special focus on the Na^+-gradient hypothesis. A preliminary account of parts of this work has already been given [12].

MATERIALS AND METHODS

Preparation of tissue and media. Experiments were performed using adult Dutch-Cross F_1 rabbits (approx. 2.0–3.0 kg). The rabbits were sacrificed by injecting air through an auricular vein, and the kidneys were promptly removed and chilled in an ice-cold low Na^+ Cross-Taggart medium. The techniques employed for preparation of kidney cortex slices and the aerobic incubation in the media used have been described previously [10]. Media with varying Na^+ concentrations were prepared by replacing choline in isomolar quantities with Na^+. Further modifications to the media, whether at 0 or 25 °C, will be mentioned in Results.

Experimental Procedure. Three different types of experiments were performed. The first was a runout experiment as described by Ross et al. [3] Groups of slices weighing 150-300 mg were incubated aerobically (gaseous phase O_2) at 25 °C for 120 min with $1 \cdot 10^{-3}$ M *p*-aminohippurate in the 100 mM Na^+ medium. The slices were blotted on filter paper and transferred every 1.0 min through a series of 10 beakers, each containing 1.5 ml of oxygenated 10 or 100 mM Na^+ medium. The amount of *p*-aminohippurate released into the beakers, as well as that remaining in the slices at the end of incubation, was determined. From these values, the initial content of *p*-aminohippurate in the slices was calculated. The rate of decrease in concentration of *p*-aminohippurate in the slice as a function of time was plotted semi-logarithmically. The slope of this line represents the rate of runout.

In the second series of experiments the effect of *p*-aminohippurate uptake on cellular concentrations of Na^+ and K^+ was examined. Groups of slices were preincubated at 0 °C in either a 10 or 100 mM Na^+, 40 mM K^+, 0.2 % inulin Cross-Taggert medium. The preincubation times of slices in the 10 and 100 mM Na^+ media were 25 min, and 3 h, respectively. The above preincubation times were sufficient for intracellular concentrations of both Na^+ and K^+ to approach their concentrations in their respective bathing medium. For the determinations of intracellular concentrations of Na^+ and K^+, the inulin space of each group of slices was determined as a measure of the extracellular space and the appropriate corrections were made for the extracellular ions. The mean inulin space after preincubation and incubation was 13.0 ± 1.1 % ($n = 20$) and 21.2 ± 2.3 % ($n = 20$), respectively. After preincubation, the slices were transferred to the appropriate incubation medium containing *p*-aminohippurate and 0.2 % inulin at 25 °C. Samples of slices were taken at various times of the incubation period for the measurement of *p*-aminohippurate, Na^+ and K^+.

In a third series of experiments we attempted to see if there was a relationship between *p*-aminohippurate influx and Na^+ influx or efflux. In the Na^+ influx experiments cortical slices were preincubated in a 10 mM Na^+ Cross-Taggart medium at 0 °C for 25 min. After preincubation the slices were divided into four approximately equal groups (by mass) and then each groups of slices was placed into either of four different incubation media for influx measurements. The incubation media used were: two 10 mM Na^+ media containing trace $^{22}Na^+$, one of which also contained 70 μM *p*-aminohippurate plus trace *p*-[^{14}C]aminohippurate; and two 100 mM Na^+ media containing trace $^{22}Na^+$, one of which also contained 70 μM *p*-aminohippurate plus trace *p*-amino[^{14}C]hippurate. The two time periods used for taking influx samples by removing slices from the incubation medium were a 0–2.5-min and a 2.5–5-min period. A total 5-min influx measurement seemed valid since a pilot experiments in monitoring efflux under similar metabolic conditions, net efflux was negligible. Specific activity samples for both *p*-amino[^{14}C]hippurate and $^{22}Na^+$ were taken with each group of slices taken as a sample. After taking a sample, the tissue was blotted to remove adhering surface radioactivity. The β emissions of both ^{14}C and ^{22}Na were counted after solubilization of the tissue and the counts were corrected against standard curves for both quenching and ^{22}Na masking of ^{14}C counts. The extracellular space was determined separately under similar conditions after a 5-min incubation and was found to be 15.4 ± 1.6 % ($n = 20$).

In the Na^+ efflux experiments cortical slices were preincubated in a 100 mM Na^+ Cross-Taggart medium containing $^{22}Na^+$ at 0 °C for 3 h. After preincubation the slices were blotted, divided into four groups and then each group of slices was placed into either of four different incubation media for efflux measurements. The incubation media used were: two 10 mM Na^+ media, one of which contained 70 μM *p*-aminohippurate plus trace *p*-amino[^{14}C]hippurate; and two 100 mM Na^+ media, one of which contained 70 μM *p*-aminohippurate. Only a 0–5-min time period was used for taking efflux measurements because the specific activity of ^{22}Na in the tissue did not change appreciably during this time. The tissue sample was treated as in the influx experiments (vide supra) and counting corrections were similar to those in the above-mentioned influx experiments. For preliminary experiments, the extracellular spaces after preincubation and incubation were 13 and 17 %, respectively

($n = 10$), the tissue water content was 80 % ($n = 60$), and the intracellular Na^+ concentration after preincubation was 100 mM/l intracellular water ($n = 25$).

Analytical Methods. Tissue water was determined gravimetrically from the difference between wet and dried tissue (95 °C overnight), Na^+ and K^+ by flame photometry after the dried tissue was digested in 0.1 M HNO_3, *p*-aminohippurate and inulin by the method of Smith et al. [13] and Schreiner [14], respectively. Using appropriate descrimination procedures, ^{14}C and ^{22}Na were counted simultaneously in Aquasol (New England Nuclear) in a Packard Tri-Carb series 3380 liquid scintillation spectrometer.

Materials. ^{22}Na was obtained from New England Nuclear as NaCl and *p*-amino-[^{14}C]-hippurate from International Chemical and Nuclear Ouabain or G-strophanthin was obtained from Sigma Chemical Co. All other reagents were of commercial grade.

RESULTS

Effect of varying external Na^+ concentration on the rate of runout of preaccumulated p-aminohippurate

We attempted to isolate the runout component by minimizing or eliminating the re-uptake phenomenon (i.e. acetate omission and metabolic inhibitor addition to incubation media). Paired observations for 10-min *p*-aminohippurate runouts into acetate-free, iodoacetate-containing 10 and 100 mM Na^+ media resulted in no significant difference between the mean runouts ($n = 6$) in the two incubation media as shown in Fig. 1. As is seen in the mean 10-min runouts of *p*-aminohippurate into both high and low Na^+ media approximated 20 %.

This series of experiments strongly suggest that extracellular Na^+ plays no role in runout of *p*-aminohippurate from the renal proximal tubule cell.

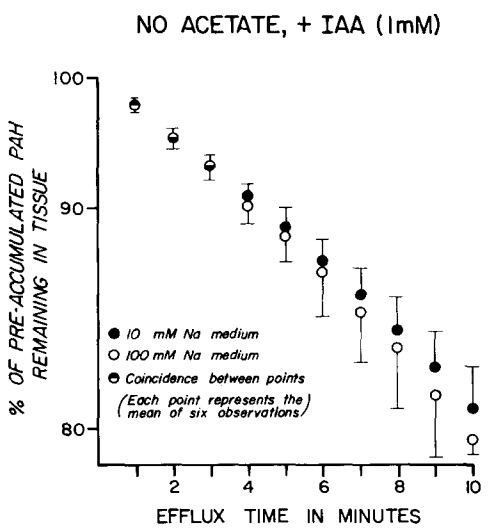

Fig. 1. Runout of *p*-aminohippurate (PAH) into paired 10 and 100 mM Na^+ media. IAA, iodoacetate.

Relationship between p-aminohippurate accumulation and intracellular cation composition

(A) *Changes in intracellular cation concentration during incubation.* The first group of experiments was designed to examine Na^+ and K^+ intracellular concentrations relative to *p*-aminohippurate uptake before and after incubation in either a 10 or a 100 mM Na^+ medium. As shown in Fig. 2, after transferring the slices from a preincubation medium containing 10 mM Na^+ and 40 mM K^+ to an incubation medium containing 100 mM Na^+, 40 mM K^+ and 70 μM *p*-aminohippurate, both Na^+ and K^+ intracellular concentrations increased; however, only the intracellular K^+ concentration increased after transferring preincubated slices to a 10 mM Na, 40 mM K^+ medium containing 70 μM *p*-aminohippurate. The final intracellular K^+ concentrations were not significantly different in either the 10 or 100 mM Na^+ incubation media; however, net uptake of *p*-aminohippurate occurred only in the 100 mM Na^+ medium.

The results of this group of experiments suggest that the Na^+-gradient hypothesis may indeed be operating in conjunction with active *p*-aminohippurate uptake, although this does not negate a coupling role for K in *p*-aminohippurate accumulation as suggested by Burg and Orloff [15]. This observation holds, at least, for K^+ concentrations equal to or greater than 40 mM in the bathing medium.

The next series of experiments was then designed to eliminate a net K^+ movement between the extra- and intracellular compartments relative to *p*-aminohippurate accumulation. Fig. 3 shows that transferring slices from a 10 mM Na^+, 75 mM K^+ preincubation medium to an incubation medium containing either 100 or 10 mM Na^+ with 40 mM K^+ and 70 μM *p*-aminohippurate resulted in intracellular concentrations of K^+ in both media remaining approx. 78 mequiv/l intracellular

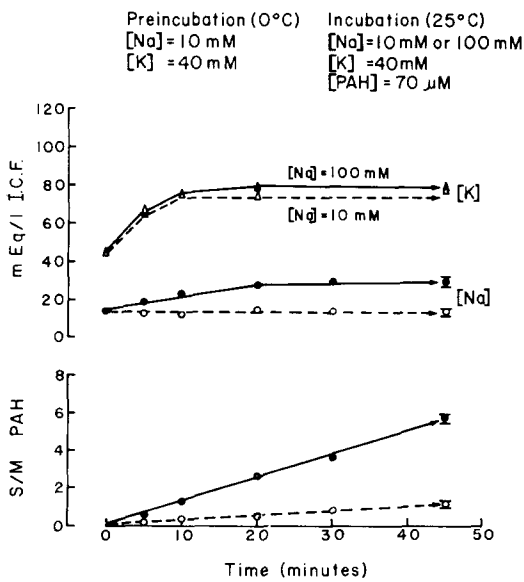

Fig. 2. Mean Na^+ and K^+ intracellular concentrations relative to mean *p*-aminohippurate (PAH) uptake ($n = 6$). Each 100 mM Na^+ observation was paired with a 10 mM Na^+.

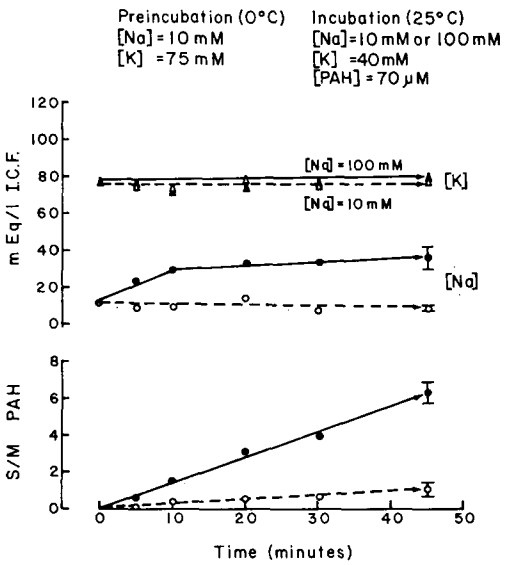

Fig. 3. Mean Na^+ and K^+ intracellular concentrations relative to mean *p*- aminohippurate (PAH) uptake ($n = 7$). Each 100 mM Na^+ observation was paired with a 10 mM Na^+.

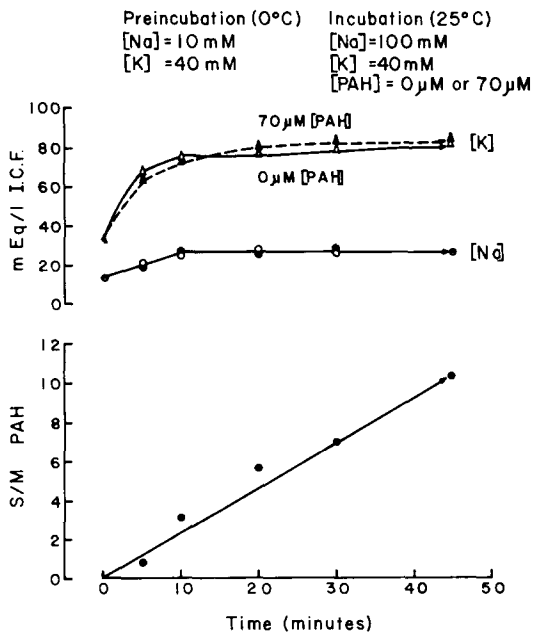

Fig. 4. Mean Na^+ and K^+ intracellular concentrations relative to presence or absence of *p*-aminohippurate (PAH) ($n = 6$). Each 70 μM *p*-aminohippurate observation was paired with one absent in *p*-aminohippurate.

fluid, while the intracellular Na^+ concentration increased only in those slices bathed in the 100 mM Na^+ medium. *p*-Aminohippurate accumulation also occurred only in slices incubated in the 100 mM Na^+ medium, suggesting that the role of K^+ flux in *p*-aminohippurate transport appears to be a minor one.

The next group of experiments was designed to examine possible simultaneous linked extracellular to intracellular flow of both Na^+ and its potential driver, *p*-aminohippurate. Fig. 4 shows that despite the presence or absence of 70 μM *p*-aminohippurate in the incubation media containing 100 mM Na^+ and 40 mM K^+, there was no difference between corresponding steady-state intracellular Na^+ or K^+ concentrations after incubation. The very same results were obtained in experiments using 400 μM *p*-aminohippurate as a potential driver of Na^+ in one of the 100 mM Na^+ incubation media.

The results shown in Figs. 3 and 4 also show that both intracellular Na^+ and K^+ concentrations reached a steady state after 10 min upon which active *p*-aminohippurate uptake still continued. This suggests that *p*-aminohippurate transport is independent of any net movements of either Na^+ or K^+.

(B) *Effects of ouabain on intracellular cation concentration and p-aminohippurate aptake.* An extracellular to intracellular Na^+ gradient as a possible driving force for net *p*-aminohippurate uptake was next examined. Preliminary experiments ($n = 4$) had shown that intracellular K^+ concentrations do not change while intracellular Na^+ concentrations increase when slices preincubated in a 10 mM Na^+, 40 mM K^+ medium are transferred to an incubation medium containing 100 mM Na^+, 40 mM K^+ and 1.5 mM ouabain. Steady-state Na^+ levels were reached within 20 min. Fig. 5 shows that transferring slices from a 10 mM Na^+, 40 mM K^+ preincubation medium to an incubation medium containing 100 mM Na^+, 40 mM K^+, 70 μM *p*-aminohippurate and 1.5 mM ouabain resulted in no net *p*-aminohippurate accumulation despite a net increase in intracellular Na^+ concentration.

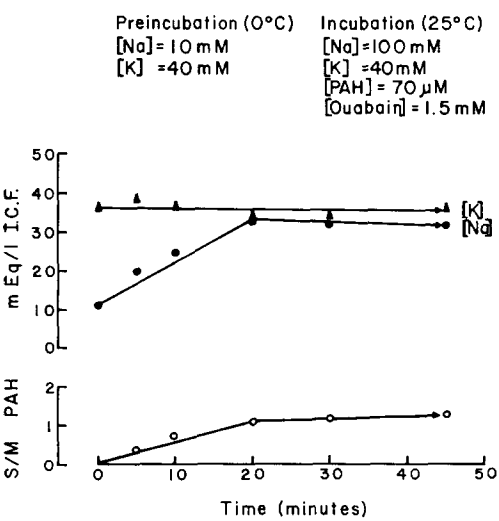

Fig. 5. Effects of ouabain on mean intracellular Na^+ and K^+ concentrations and on mean *p*-aminohippurate (PAH) transport ($n = 6$).

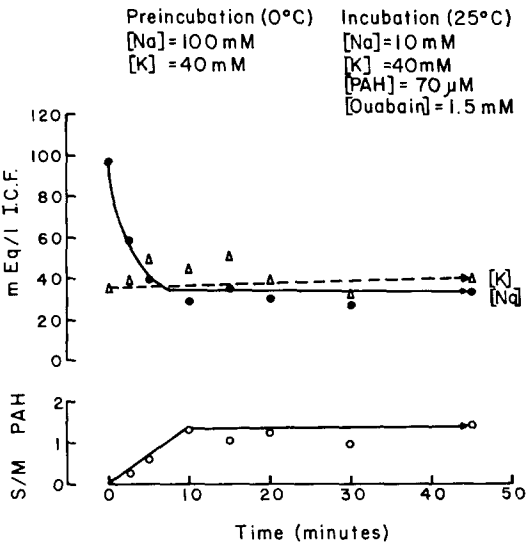

Fig. 6. Effects of ouabain on mean intracellular Na^+ and K^+ concentrations and *p*-aminohippurate (PAH) transport during net Na^+ run out ($n = 4$).

The next series of experiments was designed to examine whether a reversed intracellular to extracellular gradient of Na^+ would drive a net accumulation of *p*-aminohippurate, for Vogel and Kroger [8] had suggested a possible interaction between the oppositely directed net ionic flows of Na^+ reabsorption and *p*-aminohippurate secretion. This hypothesis was tested. Fig. 6 shows that transferring slices from a 100 mM Na^+, 40 mM K^+ preincubation medium to an incubation medium containing 10 mM Na^+, 40 mM K^+, 70 μM *p*-aminohippurate and 1.5 mM ouabain resulted in no net *p*-aminohippurate accumulation. This happened despite a net runout of Na^+. Intracellular Na^+ levels reached steady-state values within 10 min after incubation and these were far below their intracellular concentrations before incubation.

These two groups of experiments plus the others utilizing ouabain suggest a possible role for the $(Na^+ + K^+)$-stimulated ATPase in *p*-aminohippurate accumulation.

(C) *Role of intracellular Na^+ relative to p-aminohippurate uptake.* Utilizing experimental designs that quantitatively differentiated post-incubation intracellular Na^+ concentrations, we examined a possible correlation of intracellular Na^+ concentrations to active *p*-aminohippurate uptake. As is seen in Table I the experimental design that had 100 mM Na^+ in both its preincubation and incubation media had the highest mean intracellular Na^+ concentration, as well as the highest mean *p*-aminohippurate accumulation after a 45-min incubation period. The design that contained 10 mM Na^+ preincubation, and 100 mM incubation media had an intermediate mean *p*-aminohippurate accumulation after the 45-min incubation period. The designs that contained 100 and 10 mM Na^+ preincubation and 10 mM Na^+ incubation media had the lowest mean intracellular Na^+ concentrations after the 45-min incubation period. However, only the design that contained 100 mM Na^+ in the preincubation medium accumulated *p*-aminohippurate.

TABLE I

DIFFERENTIATION BETWEEN MEAN INTRACELLULAR Na^+ CONCENTRATIONS AND ACTIVE UPTAKE OF *p*-AMINOHIPPURATE IN DIFFERENT COMBINATIONS OF PREINCUBATION AND INCUBATION MEDIA

The concentration of K^+ in both preincubation and incubation media was 40 mM while the *p*-aminohippurate concentration in the incubation media was 70 μM.

$[Na^+]$ (mM/l)		$[Na^+]_i$ (mM/l intracellular fluid) at 45 min	Active uptake of *p*-aminohippurate at 45 min
Preincubation	Incubation		
100	100	43.4±3.1*	14.4±0.9*
10	100	27.5±2.8	8.1±0.5
100	10	11.6±1.8	3.4±0.4
10	10	12.1±1.9	1.5±0.1

* Represents mean ±S.E. of six experiments.

Relationship between p-aminohippurate influx and Na^+ influx or efflux

As seen in Table II, after preincubating slices in a 10 mM Na^+ medium and transferring them to 10 mM Na^+ and 10 mM Na^+ plus 70 μM *p*-aminohippurate incubation media, the composite mean 5-min influxes of Na^+ were not significantly different between the two media. The same results were obtained when slices were transferred from a 10 mM Na^+ preincubation medium to incubation media containing 100 mM Na^+, one of which also contained 70 μM *p*-aminohippurate. Table II also shows that there was no significant difference in mean Na^+ effluxes between media containing 70 μM *p*-aminohippurate or those lacking in *p*-aminohippurate.

These results suggest that uptake of *p*-aminohippurate has no relationship to either influx or efflux of Na^+. If the two ionic species were coupled in such a manner,

TABLE II

Na^+ INFLUX AND EFFLUX IN THE ABSENCE (CONTROL) AND PRESENCE OF *p*-AMINOHIPPURATE IN 10 AND 100 mM Na^+ MEDIA

	$[Na^+]$ in medium	
	10 mM	100 mM
(A) Na^+ influx (n = 6)		
Control	0.21±0.07	1.90±0.49
p-aminohippurate	0.19±0.03	1.98±0.60
Difference	0.02±0.04	0.08±0.12
	(n.s.)	(n.s.)
(B) Na^+ efflux (n = 3)		
Control	14.22±6.39	11.11±5.55
p-aminohippurate	11.08±1.30	10.85±0.45
Difference	3.14±4.46	0.26±213
	(n.s.)	(n.s.)

The results are expresed nM/min per g wet tissue. n.s., not significant.

there would have been a greater influx or efflux of Na^+ in the medium containing *p*-aminohippurate.

DISCUSSION

The role of Na^+ in the transmembrane transport of organic solutes has been a controversial subject for some time. Csaky [16] observed that numerous chemically unrelated organic solutes were actively transported by intestine in the presence of Na^+ and suggested that the requirement for Na^+ was non-specific. He further noted that of a series of cations that were substituted for Na^+, those which best penetrated the cell most inhibited transport. This latter observation, coupled with his observation that carrier-linked non-active transport did not require Na^+, led Csaky to postulate that Na^+ was required intracellularly for the production of energy which could be used non-specifically. Crane [11] has proposed an alternative hypothesis which states that Na^+ is required extracellularly. The transmembrane transport of Na^+ and actively transported organic solutes is viewed as a linked process, the organic solute entering the cell along a Na^+ gradient and energy being required to extrude Na^+. By reducing extracellular Na^+ concentration, Crane [11] has demonstrated a decrease in the apparent affinity of organic solutes for their transport mechanisms.

A previous study in our laboratory [10] showed that the transport of *p*-aminohippurate into rabbit kidney cortex slices diminishes as extracellular Na^+ concentration is reduced. However, the Na^+ effect is probably not involved with the association step of *p*-aminohippurate transport; for unlike the Crane model of active organic solute transport, there was no change in the K_m for *p*-aminohippurate uptake as extracellular Na^+ concentration was changed.

The following observations are highly suggestive evidence that the Na^+-gradient hypothesis is inoperative in the transport of *p*-aminohippurate in rabbit kidney: (1) An intracellularly directed *p*-aminohippurate gradient does not drive Na^+ accumulation (Fig. 4) for if it did greater steady-state intracellular Na^+ concentration levels would be reached. (2) Net intracellularly directed flows of Na^+ in the presence of ouabain did not effect *p*-aminohippurate accumulation (Fig. 5). (3) Medium *p*-aminohippurate does not effect the extracellular to intracellular unidirectional flux of Na^+ (Table II).

It was shown in the present study that intracellular Na^+ concentrations directly reflect the extracellular Na^+ concentrations of the bathing medium. Further, as shown in Table I, there is a direct relationship between rate of *p*-aminohippurate uptake and intracellular Na^+ concentration.

Our data are most consistent with the hypothesis of Csaky [16] and the recent model proposed by Kimmich [7]. Kimmich expanded Csaky's model and proposed that intracellular Na^+ energized a $(Na^+ + K^+)$-ATPase to a phosphorylated intermediate ($E_2 \sim P$). This high energy intermediate could then be used for energizing active organic solute transport mechanisms at the mucosal membrane of epithelial cells. Unlike Csaky's model where the intracellular Na^+ concentration is placed within "critical" limits, Kimmich merely states Na^+ is required intracellularly for active organic solute transport and he implies that there is a direct relationship between the rate of active organic solute transport and Na^+ intracellular concentra-

tions. Therefore, as seen in Table I, where there are three distinctly different intracellular Na^+ concentrations, there are, presumably, three different concentrations of $E_2 \sim P$, each directly corresponding to their respective intracellular Na^+ concentration.

Based upon our data we have proposed a model for active *p*-aminohippurate transport at the peritubular membrane of the mammalian proximal renal tubule cell which invokes a chemiosmotic coupling needed to supply the energy for *p*-aminohippurate accumulation. Intracellular Na^+ plays a central role by its stimulation of phosphorylation of a $(Na^+ + K^+)$-stimulated ATPase to a high energy phosphorylated intermediate. This high energy intermediate can then energize the *p*-aminohippurate transport mechanism. Since it has been shown that there is a much greater concentration of $(Na^+ + K^+)$-ATPase located within the peritubular membrane [17] than the luminal membrane of the renal proximal cell, it seems feasible that such a coupling mechanism could occur there. This model would adequately explain Vogel's [8] results where an increase in Na^+ reabsorption was directly related to increased *p*-aminohippurate secretion and, also, Burg and Orloff's [15] observation of strophanthidin inhibiting *p*-aminohippurate accumulation in rabbit cortical kidney slices.

Finally, the kinetic data obtained from our previous study [10] fit well with our model, for, as mentioned above, Na^+ plays, at most, a minimal role in the association of *p*-aminohippurate to the transport mechanism, but intracellular Na^+ could catalyze the formation of high energy phosphate intermediate which would energize an increased mobility of the *p*-aminohippurate-carrier complex or, increase the rate of dissociation of the *p*-aminohippurate-carrier complex at the cytosol aspect of the peritubular membrane; both effects would be manifested as an increase in the V for *p*-aminohippurate accumulation.

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