Sodium Influence Upon the Transport Kinetics of *p*-Aminohippurate in Rabbit Kidney Slices[1] (37608)

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In the last 15 years, numerous studies involving intestinal systems have shown a dependence of the active transport of organic solutes on the presence of Na (1). For example, one school of thought has it that the Na ion interacts with the same carrier system that the organic solute does. Sodium, allosterically, increases the affinity of the carrier for the organic solute, but does not necessarily affect the maximal velocity of transport for the organic solute (2). Reabsorption of organic compounds (e.g., glucose) by the kidney tubule is thought to occur by a similar process (3). However, to date, very little information has been collected in regard to the possible effects of Na on the secretion of organic solutes in the kidney. Chung *et al.* (4) have provided clear evidence showing the dominant role of Na in determining the transport characteristics of phenolsulfonophthalein and para-aminohippurate (PAH) in rabbit kidney slices. Vogel *et al.* (5, 6) have demonstrated in frog kidney a proportionality existing between the reabsorption of Na and secretion of PAH. The paucity of information concerning the role of Na in the active uptake of organic compounds in a kidney system prompted us to perform comparable studies using the weak organic acid, PAH, as a model organic solute in order to elucidate the specific function of Na.

Methods. This series of experiments was performed using Dutch-Cross F_1 rabbits (approximately 2.5–3.0 kg). The rabbits were sacrificed and the kidneys were immediately removed and placed in an ice-cold low Na medium (composition in mmoles: Na acetate, 10; KCl, 40; $CaCl_2$, 1.5; choline Cl, 60; Tris-HCl, 40). Renal cortex slices approximately 0.4–0.5 mm thick and weighing approximately 250–350 mg were cut with a Stadies-Riggs tissue slicer or a Brinkmann-Mickle Original McIllwain Tissue Chopper and placed in an identical chilled, low Na medium for 25 min. Slices were incubated in a medium whose final volume was 10 ml; the gas phase was 100% oxygen. The high Na medium used had the following composition (in mmoles): Na acetate, 10; NaCl, 90; KCl, 40; $CaCl_2$, 1.5; and Tris-HCl, 10. The composition of the low Na medium was identical to that of the preincubation medium (vide supra). All incubations were done in a Dubnoff metabolic shaker at 25° and slices were blotted and weighed at the end of the incubations; the calculations were based upon this wet weight. The slices were then homogenized in water and the concentrations of PAH in the medium and in the homogenate were determined by the methods described by Smith *et al.* (7).

There were no significant changes in PAH concentration following incubation at the volume used in low Na media. However, significant changes in the concentration of PAH following incubation were observed in high Na media. In the kinetic studies, medium PAH concentration is defined as that concentration of PAH in the medium following incubation.

The oxygen consumption of the slices was measured using a Warburg manometric respirometer as described by Cross and Taggart (8).

Results. The initial step was to measure PAH accumulation as a function of time.

[1] This work was carried out under a Grant-in-Aid from the American Heart Association and supported in part by the Hawaii Heart Association.

These experiments included paired observations from the same animal; comparison was made between the effects of a control medium and one containing iodoacetate and nitrogen had upon PAH accumulation at a constant Na concentration (10 or 100 mM) and the fixed PAH concentration (70 μM). Subtracting the accumulation of PAH obtained in the poisoned slice from that obtained in the control slice for each corresponding time period gives the value for active accumulation during that period of time for the respective concentration of Na employed. Normalizing the values of active PAH uptake relative to that of the 30-min time period in the 100 mM ($n = 6$) and 10 mM ($n = 4$) Na media gives the results as expressed in Fig. 1. As is seen, PAH uptake in the high Na appears to be linear up to the 60-min time period after which it levels off. The curve for a low Na concentration is also linear for the time period of 0–60 min. Therefore, any time period selected between 0–60 min would include a maximal rate of active PAH uptake from either a medium containing 10 or 100 mM Na. The 45-min period was chosen for incubation during the kinetic investigation; this time would confidently satisfy requirements needed for a maximal rate of accumulation or initial velocity (V_i) in either of the two Na-containing media.

The next group of experiments was designed to show the effect of Na on the accumulation of PAH. The results are as follows: with the bathing medium containing 100 mM Na and 70 μM PAH, the mean slice to medium ratio (S/M) is 9.43 ± 0.47 ($n = 20$). When the medium contained 10 mM Na the mean S/M is 1.60 ± 0.10 ($n = 20$), a value being significantly lower than the corresponding value at the high Na concentration ($p < 0.001$ for paired t test).

To determine whether the effect observed in 10 mM Na was the result of depressed tissue respiration oxygen consumption of slices was determined in both 10 mM and 100 mM Na media. The oxygen consumption of slices in the 10 mM Na, and 100 mM Na media was 0.73 ± 0.07 and 0.70 ± 0.01 μl/hr/milligram initial wet weight, respectively in 10 paired experiments.

Figure 2 shows PAH accumulation as a function of PAH concentration in either a 100 mM or 10 mM Na medium. As is seen, PAH accumulation increases curvilinearly in both media and tends to saturate at a high PAH concentration.

The Hofstee plot was used to obtain the kinetic parameters because the Lineweaver-Burke analysis tended to weight or bias the accumulation rates of PAH at the extreme medium concentrations of PAH (70 and 400 μM) (9). Hofstee analysis provided a better resolution and a more sensitive, non-

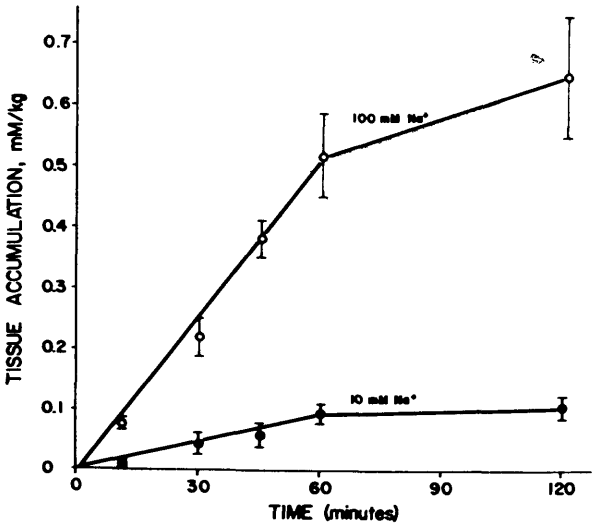

FIG. 1. Time course of active PAH accumulation in high and low Na media. (See the text for the computation of active PAH accumulation.) Vertical bars in this and Fig. 2 indicate ± SE.

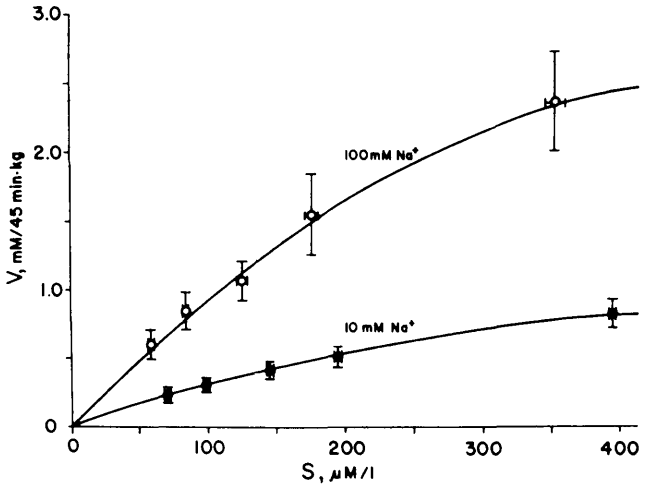

FIG. 2. Rate of accumulation (V) of PAH as a function of final medium PAH concentration (S). Each point represents the mean of 10 determinations at either the 10 mM or 100 mM Na concentration.

biased device for determining the kinetic parameters: Michaelis constant, K_m, and maximal velocity, V_{max}.

Figure 3 represents a typical experiment that was analyzed via the Hofstee plot for kinetic characteristics. The tissue concentration of PAH over a 45-min period (V) is plotted as a function of the ratio of tissue to medium PAH (V/S). The linear regression analyses were performed on the set of points pertaining to a specific Na concentration. As is seen, the V_{max} in high Na is much greater than that in the low Na, however, there is virtually no difference in the K_m of the two media.

Table I gives the mean values of V_{max} and K_m ($n = 10$) for both low and high Na media. The mean V_{max} obtained in the high Na medium is significantly greater ($p < 0.005$) than that obtained in the low Na medium. However, the mean K_m in the high Na medium is not significantly different from the mean K_m obtained in the low Na medium.

Discussion. Applying Hofstee analysis to

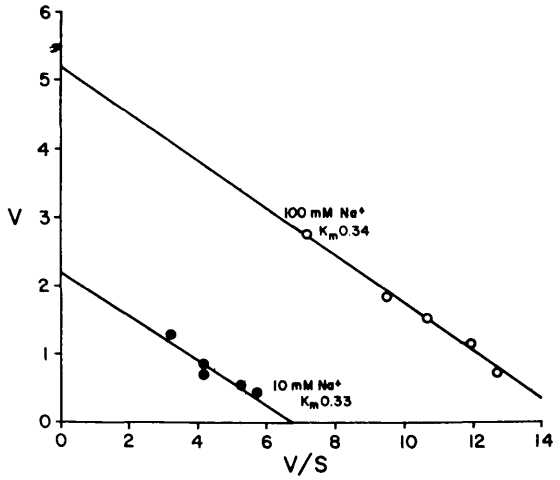

FIG. 3. A typical experiment analyzed by the Hofstee Plot. The intercept on the ordinate represents the V_{max} of accumulation for either the 10 mM or 100 mM Na concentration. The slope of the line represents the negative K_m. V_{max} and K_m are in mmole/45 min/kg wet weight and mmole/liter, respectively.

TABLE I.

Medium	V_{max} (mM/45 min • kg)	K_m (mM)
100 mM Na	4.10 ± 0.75	0.31 ± 0.03
10 mM Na	1.31 ± 0.18	0.33 ± 0.04
Δ	2.79 ± 0.67 ($p < 0.005$)	−0.02 ± 0.05 (NS)

the organic acid transport system in kidney is based upon the initial rate of accumulation (V_i) of the solute. Over a 120-min incubation period there are two slopes for PAH accumulation; the 0–60 min slope being greater than the 61–120 min slope. Using a 45-min incubation encompasses the time period for V_i.

PAH accumulations in high and low Na media compare favorably with results previously obtained under similar conditions (4). However, our results regarding the effect of Na on V_{max} and K_m are contrary to those obtained in the renal tubules of the goldfish (10). These investigators reported that the apparent K_m for phenol red transport was dependent upon external Na concentration in a reciprocal fashion, and that the V_{max} was independent of the external Na concentration. Conversely, our results indicate a direct relationship between V_{max} and external Na concentration, but no apparent relationship between K_m and the external Na concentration.

Similar results to those cited above have been reported for organic solute transport in small intestine (11). The notion, however, in intestinal systems is that there is a Na-organic solute linkage at the luminal membrane and that the solute accumulative step is at this membrane. However, accumulation of PAH in the renal epithelial cell occurs at the basal or peritubular membrane (12). Thus, it need not be presumed that the effect of Na on the mechanism of transfer necessarily be the same as in intestine since different membranes at different poles of the epithelial cells are involved. Species difference cannot be overlooked for the marked disparity of results between Hoshi and Hayashi (10) and ours.

Crucial to our argument concerning the effects of Na on the transport of PAH is also the effects played by Na on metabolism. The present study as well as that by Chung *et al.* (4) indicate that the oxygen consumption of renal slices does not appreciably change when the Na concentration of the bathing medium is likewise changed from low to normal values. Similarly, other investigations (13, 14) have shown that oxygen consumption in kidney slices remains relatively constant despite modifications in the ionic composition of the bathing medium employed. The present study suggests that changes in extracellular Na affect the transport mechanism for PAH rather than affecting the energy-producing catabolism linked to the active accumulation of PAH in the kidney slice.

Speculating upon the role of Na, one may propose that Na determines the number of carriers available for active uptake of PAH based upon the relationship between V_{max} and external Na concentration. The absence of effect of Na on K_m suggests an indirect role on PAH accumulation perhaps by affecting the metabolism linked to the carrier or by mobilizing the carrier through its binding at a site other than the active or allosteric sites.

Summary. The present investigation involving PAH uptake by rabbit kidney slices has shown (1) confirmative evidence that PAH uptake is an active process; (2) confirmative evidence that PAH uptake is dependent upon the presence of Na; (3) the K_m for PAH uptake is independent of the concentration of Na in the medium; and (4) the V_{max} for PAH uptake is directly proportional to the Na concentration in the medium.

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Received Feb. 27, 1973. P.S.E.B.M., 1973, Vol. 144.