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THE KINETIC PARAMETERS OF RENAL TRANSPORT OF *p*-AMINOHIPPURATE *IN VITRO*

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SUMMARY

1. Influx and efflux of *p*-amino[^{3}H]hippurate in separated renal tubules, prepared from cortex of rabbit kidney, have been measured under a variety of conditions after preequilibration with unlabeled *p*-aminohippurate.

2. *p*-Aminohippurate transport is characterized by a rapid and a slow component which are described in influx experiments by $(A_1 - A_1 \exp(-s_1 t))$ and $(A_2 - A_2 \exp(-s_2 t))$, respectively, where A_1 and A_2 are the equilibria accumulations, s_1 and s_2 are constants, and t is time. At 0.075 mM medium *p*-aminohippurate, $A_1 = 3.2$, $A_2 = 7.8$, $s_1 = 1.88$ min^{-1}, $s_2 = 0.056$ min^{-1}. The efflux of *p*-aminohippurate is also described by similar equations and constants.

3. Preincubation with probenecid or 2,4-dinitrophenol resulted in a proportionate decrease of A_1 and A_2, whereas s_1 and s_2 were unaffected.

4. At high medium concentrations of *p*-aminohippurate, A_1 and A_2 were also reduced to the same extent, while s_1 and s_2 remained unaltered. Influx of *p*-aminohippurate approached a saturation limit under these conditions.

5. It is concluded that the rapid component represents active transport of *p*-aminohippurate, presumably occurring at the peritubular membrane. An analysis of the data shows that the decrease of A_2 observed under various conditions may be explained as a secondary effect of the accompanying decrease of A_1. It is proposed that the slow component is due to intracellular compartmentation of *p*-aminohippurate.

INTRODUCTION

The kinetics of the transport of *p*-aminohippurate in kidney slices have been studied by various investigators[1-4]. It has been found that influx of *p*-aminohippurate occurs very rapidly in the first 2 min and is followed by a steady but slow increase in the uptake of *p*-aminohippurate which eventually leads to a high accumulation of this substance inside the renal cells. It has been proposed that the rapid transport of *p*-aminohippurate may be due to a facilitated transport across the basal cell membrane[1] or distribution of this substance within the extracellular space of the slices[3],

Abbreviation: T/M_{PAH}, the ratio between the tubular and medium concentration of *p*-aminohippurate.

whereas the slow transport has been considered to constitute active transport of *p*-aminohippurate[1–3]. However, conclusive evidence of any of these views has not been forthcoming.

The present article documents the results of a kinetic analysis of *p*-aminohippurate transport with a preparation of separated renal tubules[5] in order to eliminate some of the complicating features of the slice technique (*e.g.* the existence of an extracellular space[3], and the possibility that the slow component of *p*-aminohippurate transport might be due to a diffusion barrier between the exterior and interior part of the slices). It is found that a rapid and a slow component for transport of *p*-aminohippurate persist under these conditions. However, our experimental observations indicate that, in contrast to earlier views, active transport of *p*-aminohippurate must be ascribed to the rapid component, whereas the slow component may be due to intracellular compartmentation.

METHODS

Preparation of separated renal tubules

Young male and female rabbits, weighing about 2–3 kg, were killed by a blow on the neck and exsanguinated. Separated renal tubules were prepared by treatment with collagenase as described by BURG AND ORLOFF[5] but with the following modifications: In order to remove erythrocytes from the tissue preparation, the excised kidneys were first perfused with 15 ml of an electrolyte solution containing 110 mM NaCl, 20 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10 mM sodium phosphate buffer (pH 7.4), and gassed with 100% O_2; the resultant mixture is referred to hereafter as the preparation medium. 5 ml of a freshly prepared solution of 0.3% collagenase (Nutritional Biochemical Corp., Cleveland) dissolved in preparation medium were used for the injection into the renal artery after clamping the renal vein with a hemostat. The incubation of the minced cortex tissue was carried out under continuous O_2 supply for 50 min at 25° with 30 ml preparation medium containing 0.1% collagenase. Then the suspension was filtered through a single layer of surgical gauze and centrifuged for 1 min at 100 × *g*. The renal tubules were washed with serum according to BURG AND ORLOFF[5], and the centrifuged preparation was suspended in approx. 25 ml of experimental medium (125 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM sodium phosphate buffer (pH 7.4), 10 mM sodium acetate, and 5.5 mM D-glucose, gassed with 100% O_2, and containing various amounts of unlabeled *p*-aminohippurate according to the design of the experiment). The tubules were centrifuged off at 50 × *g* for 1 min, weighed, and resuspended in the experimental medium described above to give a 1% (wet wt./vol.) suspension of tubules.

Influx experiments

Samples (15–20 ml) of the tubule suspension mentioned above were preincubated for 45 min at 25° under continuous O_2 supply in the special flasks described by BURG AND ORLOFF[5] in order to obtain a constant concentration of unlabeled *p*-aminohippurate in the tubules. Then a tracer amount of *p*-amino[2-^{3}H]hippuric acid (Radiochemical Centre, Amersham) was added immediately to the flasks and the solution vigorously stirred. At different time intervals 0.6 ml suspension was pipetted off with a broad-tipped plastic constriction pipette, and the tubules were

separated from the medium by a special filtration cell (Fig. 1) in which medium was sucked off through a millipore filter (Sartorius-Membran-Filter Type SM 11302, GmbH, Göttingen, West Germany). The millipore filter was immediately flushed twice with 1 ml ice-cold saline in order to remove adhering medium. With the apparatus and filters used in the present study, filtration took place very rapidly, making it possible to complete the sampling and the washing procedure within 5 sec. Control experiments with labeled inulin ([*carboxy*-^{14}C]inulin, New England Nuclear Corporation) established that only a negligible amount of medium was left on the filter after washing with ice-cold saline. The influx was followed closely after addition of labeled *p*-aminohippurate by taking out samples at 15-sec intervals during the first 2 min. Later on, the time interval was lengthened according to the duration of the kinetic study, the total number of samples in each experiment being 20–30.

Efflux experiments

In these experiments the tubule suspension was preincubated for 45 min at 25° in experimental medium to which labeled *p*-aminohippurate had been added. After the incubation, the tubules were centrifuged for 1 min at 120 × *g* and 0°. Medium adhering to the tubules was removed by suspending and centrifuging the tubules twice in 10 ml ice-cold physiological saline. An appropriate amount of experimental medium was then added to the centrifuged preparation and mixed quickly. Samples (0.6 ml) of the tubule suspension were taken out at different time intervals, filtered, and washed in the same manner as described in influx experiments.

Radioactive measurements

The millipore filters, containing the washed tubules, were dried under an infrared lamp, transferred to counting vials, and dissolved* by vigorous agitation in 10 ml of the scintillation medium described by BRAY[6]. The counting vials were shaken overnight to extract the radioactivity from the tubules into the scintillation medium. Samples for determination of labeled *p*-aminohippurate in the medium were prepared by pipetting 100 μl of the medium onto millipore filters. They were dried and treated in the same manner as the tubule-containing filters. Radioactivity was measured with a Packard liquid scintillation counter.

Determination of p-aminohippurate accumulation in tubules

3 ml of the tubule suspension were centrifuged for 2 min at 480 × *g* and 0° at the end of influx experiments and at zero time in efflux experiments for the chemical determination of *p*-aminohippurate in the tubules. *p*-Aminohippurate was extracted from the sediment with 8 ml 5% trichloroacetic acid and determined by the method of SMITH *et al.*[7]. The supernatant was also analyzed for *p*-aminohippurate after deproteinization with trichloroacetic acid. The intertubular space was estimated by adding labeled inulin to a control sample of the tubule suspension without labeled *p*-aminohippurate. The sample was subsequently centrifuged, extracted with 5% trichloroacetic acid, and the radioactive contents were determined by liquid scintillation counting. The supernatant was also assayed for radioactivity after addition of trichloroacetic acid. The dry weight of the tubules was determined after drying

* Sartorius membrane millipore filters are readily soluble in the scintillation medium described by BRAY[6].

the sediment in an oven at 105° overnight and was approx. 10%. The amount of *p*-aminohippurate in the tubules was calculated as the difference between the *p*-aminohippurate content of the packed tubules and that of the inulin space. This figure was then divided by the tubule water content (wet weight of tubules − dry weight − inulin space) to obtain the intratubular concentration of *p*-aminohippurate from which the accumulation of *p*-aminohippurate (T/M_{PAH}, *i.e.* the ratio between the tubular and medium concentration of *p*-aminohippurate) was calculated. It should be noted that we have used a thin suspension of tubules (1% wet wt./vol., containing 0.35% tubule water) in order to avoid large changes in the concentration of radioactivity in the medium during the influx and the efflux experiments.

RESULTS

A series of experiments was carried out in which influx and efflux of labeled *p*-aminohippurate in renal tubules prepared from the kidneys of the same rabbit were

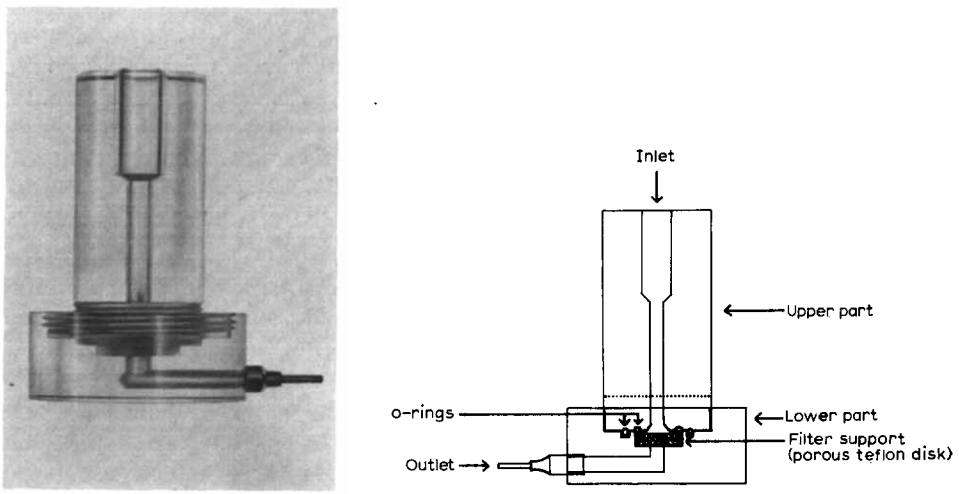

Fig. 1. Suction-filtration cell for rapid separation of isolated renal tubules from the incubation medium. The cell is made of plexiglass and consists of an upper and lower part, which can be screwed together. The millipore filter is placed on the filter support which is made of a porous teflon disk and pressed with rubber O-rings by screwing the upper part. The separation of the tubules from the medium occurs within a sec.

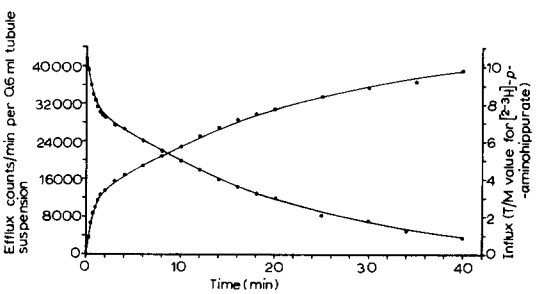

Fig. 2. Influx and efflux of labeled *p*-aminohippurate in separated renal tubules. The experiments were carried out as described in METHODS. The medium concentration of unlabeled *p*-aminohippurate was 0.075 mM.

compared. A representative experiment is shown in Fig. 2. It can be seen that the influx curve shows a very rapid uptake during the first 2 min, resulting in an accumulation of approx. 3.5. Then a pronounced decline in the rate of uptake occurs, and the accumulation of labeled *p*-aminohippurate rises slowly but steadily as time proceeds, approaching in an exponential manner the accumulation of unlabeled *p*-aminohippurate at the end of the experiment. The efflux curve of Fig. 2 shows the converse changes of those found for influx, *i.e.* a rapid efflux during the first 2 min, followed by a slower run-out of labeled *p*-aminohippurate into the medium.

In order to characterize further the transport of *p*-aminohippurate in the tubule preparation, the effect of probenecid and 2,4-dinitrophenol which act as inhibitors of *p*-aminohippurate accumulation in renal cortical slices[8,9] was studied. In these experiments the tubule suspension was preincubated for 45 min with various concentrations of the inhibitor in question together with unlabeled *p*-aminohippurate before the addition of labeled *p*-aminohippurate. The influx of labeled *p*-aminohippurate was measured for 40 min as usual, although only the initial uptake pattern from a typical experiment is shown in Fig. 3. Here, Curve A functions as a control (no addition of inhibitor). The uptake proceeds in the same way as in the previous influx experiment, but it should be noted that the time scale of Fig. 3 has been broadened in comparison with that of Fig. 2, which makes it more easy to observe the exponential course of the rapid uptake of labeled *p*-aminohippurate. Curve B shows the effect of incubating the tubules with a low concentration of 2,4-dinitrophenol (50 μM). It is seen that a rapid and a slow component of *p*-aminohippurate transport persist under these conditions. However, the amount of radioactivity taken up in the first 2 min is reduced by approx. 35% in comparison with that of the control, and a similar decrease of the slow component is observed. Curves C and D represent the *p*-aminohippurate uptake in tubules incubated with probenecid at a concentration of 5 and 500 μM, respectively. The changes are similar to those observed with 50 μM 2,4-dinitrophenol except that the reduction of the rapid and slow components is more pronounced in both cases. Finally, the effect of a high concentration of 2,4-dinitrophenol (5 mM) is illustrated by Curve E. In this case the rapid transport is almost completely abolished and cannot be clearly distinguished from the slow transport of *p*-aminohippurate.

The effect of probenecid and 2,4-dinitrophenol on the run-out of labeled *p*-aminohippurate was also studied (Fig. 4). In these experiments tubules were allowed to take up unlabeled *p*-aminohippurate and tracer amounts of labeled *p*-aminohippurate for 45 min in the presence of inhibitor, and the run-out of labeled *p*-aminohippurate from the tubules to a medium containing unlabeled *p*-aminohippurate and inhibitor at the same concentration as in the incubation medium was measured. The efflux was followed for 40 min but only the first 10-min run-out pattern is shown in Fig. 4. Note that the concentrations of inhibitor used to obtain Curves b–e of Fig. 4 were the same as those of Curves B–E of Fig. 3. As might be expected, the concentration of labeled *p*-aminohippurate at zero time is decreased in the presence of the inhibitor compared with that of the control (Curve a). A comparison with Fig. 2 indicates that a reduction of the rapid and the slow components, analogous to that observed in the influx experiments, was induced by the inhibitors.

The results of the influx and efflux experiments have been evaluated quantitatively by resolving the curves into double exponential equations. In the case of

influx: $T/M_{PAH} = -A_1 \exp(-s_1 t) - A_2 \exp(-s_2 t) + A_3$ in which A_1, A_2, s_1, s_2, and A_3 are constants, and $A_3 = A_1 + A_2$, the equilibrium value for T/M_{PAH}. For efflux $T/M_{PAH} = A_1 \exp(-s_1 t) + A_2 \exp(-s_2 t) + C$, where C denotes the radioactivity remaining in the tubules when equilibrium has been attained. A_3 was taken as the

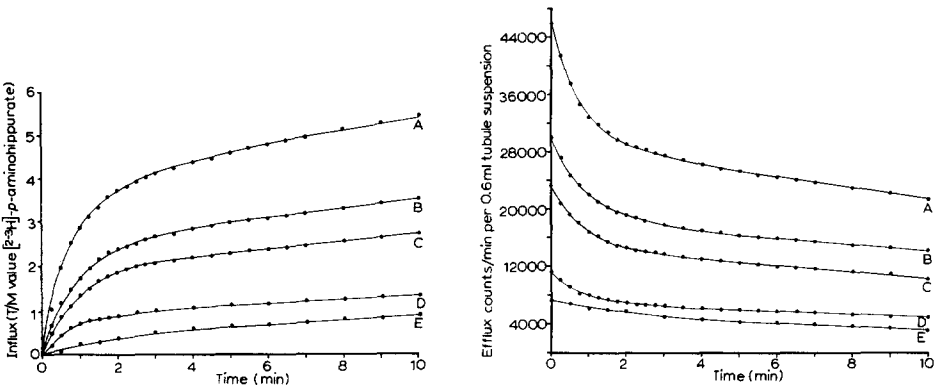

Fig. 3. Effect of inhibitors of *p*-aminohippurate accumulation on influx of labeled *p*-aminohippurate in separated renal tubules. Tubules were preincubated for 45 min with 0.075 mM *p*-aminohippurate in presence or absence of inhibitor. A, control (no inhibitor); B, 50 μM 2,4-dinitrophenol; C, probenecid 5 μM; D, 500 μM probenecid; E, 5 mM 2,4-dinitrophenol.

Fig. 4. Effect of inhibitors of *p*-aminohippurate accumulation on efflux of labeled *p*-aminohippurate in separated renal tubules. Tubules were preincubated for 45 min with 0.075 mM *p*-aminohippurate and a tracer amount of labeled *p*-aminohippurate in the presence or absence of inhibitor. A, control (no inhibitor); B, 50 μM 2,4-dinitrophenol; C, 5 μM probenecid; D, 500 μM probenecid; E, 5 mM 2,4-dinitrophenol.

value obtained for T/M_{PAH} following 45-min equilibration. The results for all influx and efflux experiments of the types presented above have been summarized in Table I. The table reveals the remarkable fact that the exponential coefficients, s_1 and s_2, are invariant in the presence or absence of inhibitor. Besides, the values calculated for s_1 and s_2 are not significantly different in influx and efflux experiments. On the other hand, A_3 is decreased to various degrees according to the concentration of inhibitor, whereas A_1/A_3 and A_2/A_3 remain constant.

The results of this analysis show that the effect of probenecid and of 2,4-dinitrophenol in both influx and efflux experiments is characterized by a proportionate decrease of rapid and slow transport of *p*-aminohippurate which strongly suggests a causal relationship between the two phenomena. One possible explanation for this finding would be that the rapid component represents cellular uptake of *p*-aminohippurate across the peritubular membrane and the slow component a subsequent transport across the luminal membrane of the renal cells. This possibility is critically examined in the discussion section where it has been shown that the extent to which *p*-aminohippurate is taken up by slow transport (approx. 72% of the total uptake) cannot be accounted for on the basis of intraluminal *p*-aminohippurate. We have therefore examined in greater detail the possibility that the slow component in fact reflects intracellular compartmentation of *p*-aminohippurate. A schematic illustration of such a model has been given in Fig. 5. According to this model, *p*-aminohippurate is first transported by an active mechanism across the peritubular membrane to a readily accessible part of the cytoplasm (designated as Compartment I),

TABLE I

EXPONENTIAL ANALYSIS OF INFLUX AND EFFLUX EXPERIMENTS IN THE PRESENCE OR ABSENCE OF INHIBITORS

The table gives a summary of all experiments in which the tubules were equilibrated with 0.075 mM unlabeled *p*-aminohippurate. s_1 and s_2 are the respective exponential terms, and A_1 and A_2, the respective equilibria accumulations of the rapid and slow component as explained in the text $A_3 = A_1 + A_2$. The values given are means ± S.D.

Type of experiment	*Inhibitor*	*Concn. (μM)*	*Number of experiments*	s_1 (min^{-1})	s_2 (min^{-1})	A_3	A_1/A_3	A_2/A_3
Influx	None		20	1.88 ± 0.01	0.056 ± 0.002	11.0 ± 2.0	0.28 ± 0.01	0.72 ± 0.01
	2,4-Dinitrophenol	50	6	1.84 ± 0.03	0.053 ± 0.003	7.8 ± 1.0	0.28 ± 0.01	0.72 ± 0.01
	Probenecid	5	6	1.84 ± 0.02	0.056 ± 0.001	6.0 ± 0.7	0.29 ± 0.01	0.71 ± 0.01
		500	6	1.86 ± 0.02	0.051 ± 0.003	2.9 ± 0.5	0.27 ± 0.02	0.73 ± 0.02
Efflux	None		18	1.84 ± 0.01	0.052 ± 0.003	10.7 ± 1.5	0.27 ± 0.02	0.73 ± 0.02
	2,4-Dinitrophenol	50	6	1.85 ± 0.02	0.050 ± 0.002	7.4 ± 0.8	0.26 ± 0.02	0.74 ± 0.02
	Probenecid	5	6	1.83 ± 0.03	0.053 ± 0.003	5.8 ± 0.6	0.28 ± 0.01	0.72 ± 0.01
		500	6	1.83 ± 0.01	0.048 ± 0.001	2.6 ± 0.4	0.26 ± 0.01	0.74 ± 0.01

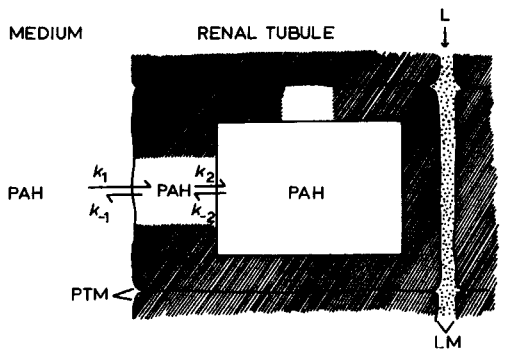

Fig. 5. Schematic illustration of model for *p*-aminohippurate (PAH) transport, based upon intracellular compartmentation of *p*-aminohippurate. k_1 and k_{-1} are the rate constants for influx and efflux of *p*-aminohippurate from medium to Compartment I (hatched area) of cytoplasm. Inside the cell *p*-aminohippurate diffuses from Compartment I to Compartment II (clear area). The rate constants for transfer between Compartments I and II are k_2 and k_{-2} ($k_2 = k_{-2}$). PTM, peritubular membrane; LM, luminal membrane; L, lumen.

from which it diffuses slowly to a subcellular region (Compartment II). The rate constants for influx and efflux of *p*-aminohippurate across the peritubular membrane are k_1 and k_{-1}, and those for transfer between Compartments I and II are denoted as k_2 and k_{-2} (we consider here that $k_2 = k_{-2}$). The mathematical relationship between the rate constants and the experimentally determined values for s_1, s_2 and A_3 has been derived and is discussed in the APPENDIX. The rate constants computed on the basis of the exponential analysis given in Table I are shown in Table II, from which it appears that k_1 is depressed in the presence of inhibitor, whereas k_{-1} and k_2 are unaffected. These findings are in accordance with the view that probenecid and 2,4-dinitrophenol inhibit an active transport process across the peritubular membrane. Besides, the invariability of k_2 is in agreement with the model, since the rate constant for a diffusion process would not be expected to be changed by probenecid or 2,4-dinitrophenol. Finally, as is shown in the APPENDIX, A_1/A_2 is a measure of the ratio between the distribution volumes of Compartments I and II (V_1/V_2). Thus, in accordance with expectations, A_1/A_3 and A_2/A_3 remain unaltered in the presence of inhibi-

TABLE II

RATE CONSTANTS FOR *p*-AMINOHIPPURATE TRANSPORT IN THE PRESENCE OR ABSENCE OF INHIBITORS

The values for s_1, s_2, and A_3 given in Table I were used to derive rate constants on the basis of the model for *p*-aminohippurate transport given in Fig. 5.

Type of experiment	*Inhibitor*	*Concn.* (μM)	k_{-1} (min^{-1})	k_1 (min^{-1})	k_2 (min^{-1})
Influx	None		1.75 ± 0.02	19.0 ± 2.5	0.057 ± 0.002
	2,4-Dinitrophenol	50	1.71 ± 0.02	13.3 ± 1.5	0.055 ± 0.003
	Probenecid	5	1.70 ± 0.01	10.2 ± 1.0	0.058 ± 0.001
		500	1.73 ± 0.03	5.0 ± 1.4	0.053 ± 0.002
Efflux	None		1.74 ± 0.02	18.6 ± 2.0	0.056 ± 0.003
	2,4-Dinitrophenol	50	1.75 ± 0.02	13.0 ± 1.3	0.054 ± 0.002
	Probenecid	5	1.73 ± 0.03	10.0 ± 0.9	0.057 ± 0.002
		500	1.75 ± 0.02	4.6 ± 1.2	0.052 ± 0.001

TABLE III

RATE CONSTANTS AND EQUILIBRIA ACCUMULATIONS AT VARIOUS CONCENTRATIONS OF *p*-AMINOHIPPURATE IN THE MEDIUM

The values given are the means of seven experiments.

Final concn. of p-aminohippurate in medium (mM)	k_{-1} (min^{-1})	k_1 (min^{-1})	k_2 (min^{-1})	A_3	A_1/A_3	A_2/A_3
0.07	1.70 ± 0.01	17.2 ± 0.8	0.056 ± 0.003	10.1 ± 1.0	0.28 ± 0.01	0.72 ± 0.01
0.12	1.69 ± 0.01	15.9 ± 0.9	0.058 ± 0.001	9.4 ± 0.9	0.27 ± 0.02	0.73 ± 0.02
0.20	1.73 ± 0.03	14.5 ± 0.6	0.052 ± 0.002	8.4 ± 0.5	0.28 ± 0.02	0.72 ± 0.02
0.42	1.71 ± 0.02	11.1 ± 0.7	0.055 ± 0.004	6.5 ± 0.3	0.29 ± 0.01	0.71 ± 0.01
0.70	1.75 ± 0.03	8.9 ± 0.5	0.057 ± 0.002	5.1 ± 0.4	0.26 ± 0.03	0.74 ± 0.03
0.90	1.73 ± 0.04	7.6 ± 0.3	0.053 ± 0.003	4.4 ± 0.2	0.27 ± 0.02	0.73 ± 0.02
1.70	1.75 ± 0.02	4.4 ± 0.5	0.054 ± 0.001	2.5 ± 0.2	0.29 ± 0.01	0.71 ± 0.01

tor (Table I). It can be concluded that the model proposed is consistent with the experimental data presented above.

We have tried to test further the theory that the slow component of *p*-aminohippurate transport is due to intracellular compartmentation by measuring the rate of labeled *p*-aminohippurate transport at different concentrations of unlabeled *p*-aminohippurate in the medium. Influx experiments were performed in the usual manner except that the samples of the tubule suspension were preincubated for 45 min with seven different concentrations of unlabeled *p*-aminohippurate in the medium, before the addition of labeled *p*-aminohippurate (Table III). It is seen from the table that k_1 and A_3 are depressed gradually as the concentration of unlabeled *p*-aminohippurate rises in the medium. In contrast, k_{-1}, k_2, A_1/A_3 and A_2/A_3 are unaltered.

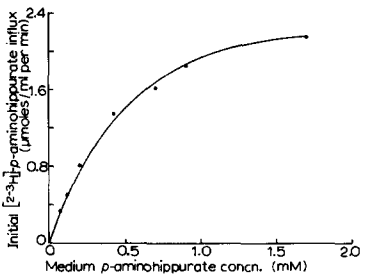

Fig. 6. Uptake of labeled *p*-aminohippurate in tubules as a function of the medium concentration of *p*-aminohippurate. The tubules were preincubated with different concentrations of unlabeled *p*-aminohippurate (Table III). Initial rate of labeled *p*-aminohippurate was calculated as μmoles/ml per min.

The dependence of rapid component influx on medium concentration of *p*-aminohippurate is shown in Fig. 6. It is apparent that the influx approaches a maximum at the highest medium concentration (1.7 mM), in agreement with the view that it is a transport process. Furthermore, the invariability of k_2 substantiates the view that it is due to diffusion of *p*-aminohippurate. Finally, it should be noted that the requirement that V_1/V_2 should be constant is fulfilled by the constant values obtained for A_1/A_3 and A_2/A_3.

DISCUSSION

The use of isolated renal tubules has made it possible to study directly the transfer of *p*-aminohippurate from medium to renal cells, and the suction-filtration technique has permitted us to investigate the course of the rapid component of *p*-aminohippurate transport, which occurs within the first 2 min. The existence of a rapid and a slow component for renal transport of *p*-aminohippurate under these conditions confirms and extends previous results obtained with kidney slices. However, our results show that the rapid component cannot be due to diffusion into extracellular space as suggested by ROSS AND FARAH[3], since it was established in control experiments by addition of labeled inulin to the tubule suspension that virtually all the medium was washed off the tubules during the sampling procedure. Furthermore, the accumulation of labeled *p*-aminohippurate (approx. 3.5) obtained in the first

2 min shows that, in contrast to the view put forward by FOULKES AND MILLER[1], an active process is involved in the transport of *p*-aminohippurate related to the rapid component. Other similar indications are the inhibition of the accumulation related to the rapid component by probenecid and 2,4-dinitrophenol and the saturation of influx at high concentrations of *p*-aminohippurate. These findings raise the question of the relationship between rapid transport of *p*-aminohippurate and tubular secretion of this substance *in vivo*. It is here of interest that the half-maximal value for influx of *p*-aminohippurate calculated on the basis of Fig. 6 is approx. 0.4 mM, which agrees reasonably well with the plasma concentration of *p*-aminohippurate that is needed to obtain half-maximal values for *p*-aminohippurate secretion in the functioning kidney (0.5–0.6 mM)[13]. It can be calculated from Fig. 6 that the maximal rate of rapid influx is 2.7 μmoles/min per ml tubule water *in vitro*. Assuming that the water content of kidney cortex from a 3-kg rabbit is 8 ml (10 g cortex, 20% dry weight), the value for rapid influx in cortex tissue from two kidneys would be $8 \times 2.7 =$ approx. 22 μmoles/min per ml which is about half of that found for tubular secretion of *p*-aminohippurate *in vivo*[13]. However, it should be taken into account that our studies *in vitro* were performed at 25°, and the rate of uptake would presumably be higher at 37°. Although it is recognized that some uncertainties are involved in comparing studies performed *in vitro* and *in vivo*, the data nevertheless strongly suggest that the rapid uptake of *p*-aminohippurate observed in separated renal tubules constitutes a rate-limiting step in the tubular secretion of this substance *in vivo*. Furthermore, the observation that *p*-aminohippurate is accumulated intracellularly[10–12] is most readily explained by the existence of a rapid transport process at the peritubular membrane.

The slow component of *p*-aminohippurate transport was considered, within the framework of the model outlined in Fig. 5, to be due to intracellular compartmentation, resulting from the existence of intracellular diffusion barrier(s) for *p*-aminohippurate, and this hypothesis was shown to be in good agreement with the experimental data. However, in assessing the validity of a kinetic theory it is equally important to consider if the results could be accounted for by other models. Let us first examine the possibility that Compartment II represents intraluminal space rather than an intracellular compartment. When discussing this possibility it should be taken into account that renal tubules collapse in the absence of glomerular filtration[14–16], and we must therefore expect a relatively low value for the luminal volume in our preparations, *i.e.* a small value for $V_2/V_1 + V_2$. This would seem to contradict the observation that the major part of the total accumulation of *p*-aminohippurate is derived from the slow component. However, it should be recalled here that the rate constants given in Tables II and III were derived under the assumption that $k_2/k_{-2} = 1$, but it is possible to compute rate constants and distribution volumes for $k_2/k_{-2} \neq 1$. This subject has been treated extensively in the APPENDIX where it has been shown that definable values for rate constants can be computed for k_2/k_{-2} varying between 0 and 9 (Eqn. 13). It suffices to mention here that the need for making assumptions about the magnitude of k_2/k_{-2} stems from the fact that we are unable to measure directly the concentration of *p*-aminohippurate in Compartments I and II. The following relationship between the distribution volumes of Compartments I and II and k_2/k_{-2} has been derived: $V_1/V_2 = k_2/k_{-2} \cdot A_1/A_2$ (Eqn. 10 of the APPENDIX). A graphical representation of this relationship has been shown in Fig. 7 from

which it appears that for $k_2/k_{-2} = 1$, $V_2/V_1 + V_2 = 0.72$, whereas for the extreme value of $k_2/k_{-2} = 9$, $V_2/V_1 + V_2 = 0.23$ which might be a more realistic value for the luminal space. However, $k_2/k_{-2} = 9$ would require the existence of a concentrative mechanism for *p*-aminohippurate between cell and lumen, but previous studies[10–12] have shown that the concentration of *p*-aminohippurate in the tubular lumen does not exceed the intracellular concentration of *p*-aminohippurate in proximal tubules which secrete *p*-aminohippurate. Furthermore, in contrast to expectations, the model would imply that k_2/k_{-2} is not influenced by probenecid and 2,4-dinitrophenol. This can be seen directly from the relationship that $V_1/V_2 = k_2/k_{-2} \cdot A_1/A_2$. On the basis of this analysis it appears improbable that Compartment II is localized to the luminal space.

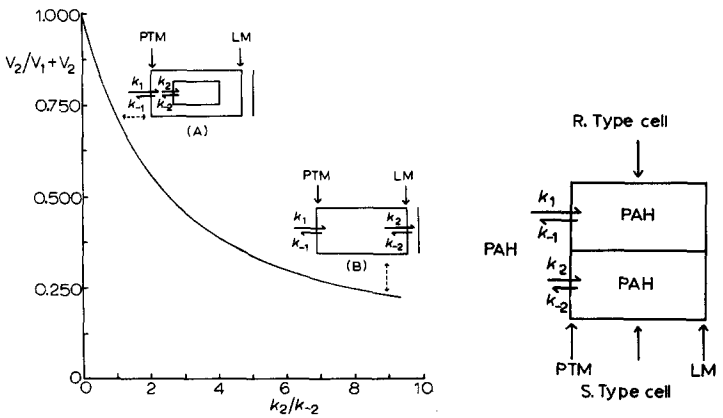

Fig. 7. Models for *p*-aminohippurate transport in which the slow component is considered to be due to processes occurring after the uptake of *p*-aminohippurate *via* rapid transport. The unbroken line shows $V_2/V_1 + V_2$ as a function of all possible values of k_2/k_{-2}. Model A is identical with that proposed in Fig. 5 and presupposes that k_2 is of the same order of magnitude as k_{-2}, whereas Model B is only consistent with the kinetic data at maximal values for k_2/k_{-2}. PTM, peritubular membrane; LM, luminal membrane.

Fig. 8. Schematic illustration of model for *p*-aminohippurate (PAH) transport in which the rapid and slow components are supposed to be due to transport into two different types of cells. R, rapidly transporting cell; S, slowly transporting cell; PTM, peritubular membrane; LM, luminal membrane.

In the foregoing discussion we have considered models in which the slow component is considered to reflect events taking place after the rapid uptake of *p*-aminohippurate. We should now consider the alternative possibility that the slow transport in analogy with rapid transport is due to active transport of *p*-aminohippurate across the peritubular membrane. It should be noted that this presupposes the existence of two different populations of cells, one of which is endowed with the rapid transport system (Cells R in Fig. 8) and the other with a slow transport mechanism for *p*-aminohippurate (Cells S in Fig. 8). In this case a decrease of A_2 in the presence of probenecid and 2,4-dinitrophenol must represent a direct inhibition of slow component. Besides, the reduction of A_2 at high concentrations of unlabeled *p*-aminohippurate would indicate saturation of the slow transport system. It should be realized that although there is nothing self-contradictory in these assumptions, the remarkable constancy of A_1/A_2 under various experimental conditions would

imply that the two different transport systems are inhibited exactly to the same degree by the inhibitors; furthermore, that both transport systems are saturated at the same concentration of unlabeled *p*-aminohippurate in the medium. In contradistinction, the model proposed in Fig. 5 actually predicts that A_1/A_2 should remain constant.

On the basis of this analysis and the arguments advanced above, it appears most probable that the slow component is a consequence of intracellular compartmentation of *p*-aminohippurate, although a final conclusion cannot rest upon a kinetic analysis alone but must involve the application of other experimental approaches as well. Besides, it can be calculated that the rate of influx due to rapid transport is approx. 12 times higher than that of slow transport. Therefore, it is concluded that, irrespective of the nature of the slow component, rapid transport of *p*-aminohippurate is the major factor of active transport of *p*-aminohippurate in isolated renal tubules.

APPENDIX

We describe here the mathematical formulation of the kinetics of a two-compartment model for *p*-aminohippurate transport in renal tubules. The first step is envisaged to be the transport of *p*-aminohippurate across the peritubular cell membrane to the readily accessible part of the cytoplasm which constitutes Compartment I. In order to account for the slow component of *p*-aminohippurate transport the existence of a second compartment is indicated. *A priori* this compartment may either be localized within the tubular cells or the tubular lumina, and in order to derive general expressions we place no restrictions on the rate constants for transfer between the two compartments. Therefore, in contrast to the model outlined in Fig. 5, k_2 is not considered to be necessarily of the same magnitude as k_{-2}. It is assumed that the two compartments are placed in series (*i.e.* *p*-aminohippurate in Compartment II does not exchange directly with the medium) and that labeled *p*-aminohippurate is instantly miscible with the unlabeled compound in each compartment.

Influx experiments

In these experiments a small amount of labeled *p*-aminohippurate is added to a tubule suspension in which the tubules have already been equilibrated with unlabeled *p*-aminohippurate. The concentration of labeled *p*-aminohippurate is described by the following equations

$$\mathrm{d}({}^1c)/\mathrm{d}t = k_1({}^0c_t) + k_{-2}({}^2c_t) - (k_{-1} + k_2)\,({}^1c_t) \tag{1}$$

$$\mathrm{d}({}^2c)/\mathrm{d}t = k_2({}^1c_t) - k_{-2}({}^2c_t) \tag{2}$$

in which ${}^0c_t$, ${}^1c_t$, and ${}^2c_t$ refer to the concentration of labeled *p*-aminohippurate in medium, Compartment I, and Compartment II, respectively, at time t. The designation of the rate constants (k_1, k_{-1}, k_2, k_{-2}) corresponds to that shown in Fig. 5. As the uptake of labeled *p*-aminohippurate proceeds, a decrease in the concentration of radioactivity in the medium from ${}^0c_0$ to ${}^0c_t$ occurs which is given by

$${}^0c_0 - {}^0c_t = f_1({}^1c_t) + f_2({}^2c_t) \tag{3}$$

where $f_1 = V_1/M$ and $f_2 = V_2/M$, V_1 and V_2 representing the volumes of Compartments I and II, and M the volume of the medium.

Eqns. 1 and 3 are combined to give

$$d({}^1c)/dt = k_1({}^0c_0) - (k_{-1} + k_2 + k_1f_1)({}^1c_t) + (k_{-2} - k_1f_2)({}^2c_t) \quad (4)$$

Eqns. 2 and 4 can be transformed into a single linear differential equation with constant coefficients[17], which can be solved for ${}^1c$ and ${}^2c$ in the usual way[18].

The solutions are of the form

$$\begin{aligned} {}^1c_t &= a_1 \exp(-s_1t) + a_1' \exp(-s_2t) + a_1'' \\ {}^2c_t &= a_2 \exp(-s_1t) + a_2' \exp(-s_2t) + a_2'' \end{aligned} \quad (5)$$

in which

$$\begin{aligned} s_1 + s_2 &= k_{-1} + k_2 + k_{-2} + k_1f_1 \\ s_1s_2 &= k_{-1}k_{-2} + k_1k_{-2}f_1 + k_1k_2f_2 \end{aligned} \quad (6)$$

a_1'' and a_2'' represent the equilibrium values for the concentration of labeled *p*-aminohippurate in the tubules (${}^1c_\infty$ and ${}^2c_\infty$) which are given by

$$\begin{aligned} a_1'' &= {}^1c_\infty = k_1/k_{-1}({}^0c_\infty) \\ a_2'' &= {}^2c_\infty = k_2/k_{-2}({}^1c_\infty) = k_1k_2/k_{-1}k_{-2}({}^0c_\infty) \end{aligned} \quad (7)$$

a_1, a_1', a_2, a_2' may be evaluated from the boundary condition that at $t = 0$, ${}^1c = {}^2c = 0$, but the expressions are not recorded here. They show a very complicated dependence on the rate constants and are of little interest in the present case, because we are unable to measure directly the concentration of *p*-aminohippurate in Compartments I and II.

The experimentally determined curve for the accumulation of labeled *p*-aminohippurate in the tubules as a function of time (A_t) is given by

$$A_t = -A_1 \exp(-s_1t) - A_2 \exp(-s_2t) + A_3 \quad (8)$$

where A_1, s_1 are constants relating to the rapid component, A_2, s_2 the constants of the slow component, and $A_3 = A_1 + A_2$, the equilibrium value for uptake of radioactivity in the tubules. The values computed for s_1 and s_2 in Eqn. 8 can be directly inserted into Eqn. 6. A_1 and A_2 measure the equilibrium accumulation of *p*-aminohippurate in Compartments I and II calculated on the basis of total tubule water content (T). The total amount of *p*-aminohippurate in Compartments I and II is therefore given by $A_1({}^0c_\infty)T$ and $A_2({}^0c_\infty)T$. Hence we can write that

$$A_1({}^0c_\infty)T = ({}^1c_\infty)V_1; \quad A_2({}^0c_\infty)T = ({}^2c_\infty)V_2 \quad (9)$$

Combination of Eqns. 7 and 9 gives the following expressions

$$V_1/V_2 = k_2/k_{-2} \cdot A_1/A_2 \quad (10)$$

and

$$k_1/k_{-1} = A_1/V_1 \cdot T \quad (11)$$

Eqn. 10 demonstrates the relation between k_2/k_{-2} and the distribution volumes which was shown graphically in Fig. 7. The important point here is that either k_2/k_{-2} or V_1/V_2 must be considered as an independent variable which cannot be assessed from the experimental data. This can be shown in the following way: using Eqn. 11 it is found that $k_1f_1 = (A_1T/M)k_{-1}$ which is approx. 0.01 k_{-1} with our experimental set-up. Furthermore, $k_1k_{-2}f_1 + k_1k_2f_2 = (A_3T/M)k_{-1}k_{-2}$ which is approx. 0.03 $k_{-1}k_{-2}$.

Insertion of these values into Eqn. 6 gives: $s_1 + s_2 = 1.01\, k_{-1} + k_2 + k_{-2}$ and $s_1 s_2 = 1.03\, k_{-1} k_{-2}$, from which the following expression may be derived

$$1.01\, k_{-1}^2 - (s_1 + s_2) k_{-1} + 0.97\, s_1 s_2 (1 + k_2/k_{-2}) = 0 \qquad (12)$$

It can be seen directly from Eqn. 12 that a knowledge of k_2/k_{-2} is a prerequisite of the calculation of k_{-1}. Furthermore, definable values for the rate constants (*i.e.* exclusion of negative, zero, and imaginary solutions) require that

$$0 < k_2/k_{-2} \leq \frac{(s_1 + s_2)^2}{3.92\, s_1 s_2} - 1 \simeq 9 \qquad (13)$$

The rate constants given in Tables II and III were calculated on the assumption that $k_2 = k_{-2}$. The following modified version of Eqn. 6 was used for computation of k_{-1} and k_2: $s_1 + s_2 = 1.01\, k_{-1} + 2k_2$ and $s_1 s_2 = 1.03\, k_{-1} k_2$. Calculation of k_1 represents a special problem because it requires a knowledge of the absolute value for V_1. Let Φ denote the ratio between $(V_1 + V_2)$ and total tubule water ($\Phi = (V_1 + V_2)/T$). Substitution of (ΦT) for $(V_1 + V_2)$ in Eqn. 11 and combination with Eqn. 10 gives $k_1 = A_3 k_{-1}/\Phi$ when $k_2/k_{-2} = 1$. In practice, k_1 was calculated as $A_3 k_{-1}$. Tubular secretion of *p*-aminohippurate is restricted to proximal tubules which according to BURG AND ORLOFF[16] constitute approx. 80% of the cortex of rabbit kidney. The k_1 values as given in Tables II and III may therefore underestimate the true influx constants by approx. 25%.

Efflux experiments

Run-out of labeled *p*-aminohippurate into a medium containing unlabeled *p*-aminohippurate is described by the same equations as for influx (Eqns. 1 and 2). The concentration of radioactivity in the medium at time t is related to the decreasing concentration of ^1c and ^2c by

$$^0c_t = f_1(^1c_0 - {}^1c_t) + f_2(^2c_0 - {}^2c_t) \qquad (3a)$$

The calculations proceed in a similar manner as for influx, and the following equations are obtained

$$\begin{aligned} s_1 + s_2 &= k_{-1} + k_2 + k_{-2} - k_1 f_1 \\ s_1 s_2 &= k_{-1} k_2 - k_1 k_{-2} f_1 - k_{-1} k_2 f_2 \end{aligned} \qquad (6a)$$

It can be seen that Eqn. 6a only differs from Eqn. 6 by a change in sign for the small correction factors containing f_1 and f_2.

The experimentally determined curve for accumulation of *p*-aminohippurate as a function of time is given by

$$A_3 = A_1 \exp(-s_1 t) + A_2 \exp(-s_2 t) + C \qquad (8a)$$

where c represents radioactivity remaining in the tubules under equilibrium conditions. Provided that equilibrium between Compartments I and II has been achieved before measuring efflux, Eqns. 10 and 11 are still applicable for determination of the rate constants as described in the influx experiments.

NOTE ADDED IN PROOF (Received January 14th, 1970)

After the submission of this paper for publication, a study on the transport of *p*-aminohippurate in isolated, perfused rabbit proximal tubules by TUNE, BURG AND

PATLAK[19] has appeared. These authors found that during perfusion the *p*-aminohippurate concentration was greater in tubule cells than in the luminal fluid and surrounding medium, thus confirming the view expressed here that the concentrative step of *p*-aminohippurate transport occurs at the peritubular membrane. Another notable finding was that the intracellular accumulation and tubular secretion of *p*-aminohippurate were higher in the straight part than in the convoluted part of the proximal tubule, corresponding to the model outlined in Fig. 8 of our paper. However, the big difference between the rate constants of the rapid and slow accumulation observed by us (Table I) makes it improbable that the biphasic accumulation of *p*-aminohippurate in tubules without perfusion could be attributed to the different uptake patterns of the convoluted and of the straight part of the proximal tubules.

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