Biochimica et Biophysica Acta, 601 (1980) 490—499

BBA 78948

KINETICS OF *p*-AMINOHIPPURATE TRANSPORT IN RABBIT KIDNEY SLICES

ROLE OF Na^+

JAN MAXILD, JESPER V. MØLLER and M. IQBAL SHEIKH

Institute of Medical Biochemistry, University of Århus, 8000 Århus C (Denmark)

(Received February 13th, 1980)

Key words: p-Aminohippurate transport; Na^+ dependence; Oxidative metabolism; Ouabain; (Kidney cortex)

Summary

Active accumulation of *p*-aminohippurate by cortex slices of rabbit kidney in vitro requires 2.5—3 h at 25°C to attain a steady-state value. The uptake process can be divided into a rapid and a slow component. The slow component makes the largest contribution to the final accumulation of *p*-aminohippurate. Rapid uptake is dependent on oxidative metabolism, but is not affected by Na^+ or ouabain. By way of contrast, Na^+ is required for the slow uptake process to occur. Efflux of *p*-aminohippurate accumulated in the presence of Na^+ is stimulated by transfer to media with a low concentration of Na^+. On the other hand, there is only evidence of a stimulatory effect of Na^+ on *p*-aminohippurate influx at medium concentrations above 30 mM. The results indicate the existence of both Na^+-dependent and Na^+-independent *p*-aminohippurate transport, respectively.

Introduction

Accumulation of *p*-aminohippurate by kidney slices is widely used as a measure of tubular transport of *p*-aminohippurate in vitro. Kinetic studies of *p*-aminohippurate uptake by renal cortical slices have shown that transport occurs rapidly during the first minutes [1—3] and is followed by a slow phase, which takes more than 1 h to go to completion [4—5]. The slow equilibration of *p*-aminohippurate in kidney slices contrasts with the ready transtubular transport of the compound in vivo [6—7]. Various factors such as predominant uptake of *p*-aminohippurate by tubules in the exterior part of the slices during the initial period, and intracellular events [1], such as slow diffusion across

internal membranes [8], have been proposed to account for the slow cellular uptake. In agreement with the latter view a rapid and a slow phase have been demonstrated in separated tubules from rabbit kidney [8], where medium *p*-aminohippurate is in direct contact with the transporting membranes. The biphasic nature of *p*-aminohippurate uptake has assumed renewed importance in connection with the studies on the Na^+-dependency of the *p*-aminohippurate uptake. Many studies have shown that Na^+ is required for accumulation of *p*-aminohippurate by the rabbit kidney in vitro [9—12], but in a recent investigation we found that, in the absence of Na^+ in the medium, uptake of *p*-aminohippurate proceeded quickly during the initial phase in Na^+-depleted slices [13]. Results obtained with metabolic inhibitors also indicated the probable existence of a Na^+-independent component in the uptake process [13]. We wish here to report experiments which throw further light on the effect of Na^+ on the kinetics of active transport of *p*-aminohippurate in vitro. Rabbit-kidney slices were used for these experiments rather than separated tubules [14], since the integrity of the latter preparation could not be maintained at low Na^+ concentrations, in agreement with observations by Podevin et al. [12].

Methods

The general experimental procedures were essentially the same as those recently described by us [13]. Kidneys were excised from anesthetized and exsanguinated rabbits and cortical slices were prepared with a razor blade. The uptake of *p*-aminohippurate was studied both in freshly prepared and Na^+-depleted renal cortical slices under aerobic and anaerobic conditions.

Na^+-depletion. Kidney slices were depleted of Na^+ by preincubation in 100% N_2 at 0°C for 1 h, in a medium containing 145 mM choline chloride/5 mM potassium acetate/0.5 mM $CaCl_2$/0.7 mM $MgSO_4$/15 mM Tris buffer (pH 7.4). The Na^+ and ATP concentrations of the slices were lowered to 10 and 0.2 mmol/kg tissue, respectively, under these conditions. In order to restore the ATP concentration to the normal level (approx. 2 mmol/kg tissue) the slices were transferred to an oxygenated medium of the same composition, where they were stored for 1 h at 25°C before measurement of *p*-aminohippurate uptake.

Uptake experiments. Slices were evenly distributed among 12 Warburg vessels, containing 3 ml of the electrolyte medium with the following composition: 0—145 mM NaCl, 145—0 mM choline chloride (to make the sum of Na^+ and choline, 145 mM), 5 mM potassium acetate, 0.5 mM $CaCl_2$, 0.7 mM $MgSO_4$, 15 mM Tris buffer (pH 7.4), *p*-amino[^{3}H]hippurate (Radiochemical Centre, Amersham) and unlabelled *p*-aminohippurate (0.075 mM). In a few experiments the effect of CN^- (10 mM) or F^- (10 mM) on the aerobic uptake of *p*-aminohippurate in Na^+-depleted slices was also studied (Fig. 1).

The uptake of *p*-aminohippurate was measured in a Warburg respirometer under the following conditions: Shaker speed 100 cycles/min, gas phase 100% O_2 or 100% N_2, temperature 25°C, incubation period 1—60 min. The centre well contained KOH for CO_2 absorption. After incubation, the slices were blotted on a piece of filter paper and quickly transferred to conical flasks containing 2.5 ml 5% trichloroacetic acid. The tissue samples were grounded with a spatula and extracted for 30 min with continuous shaking. The extracts were

then centrifuged for 10 min at 2000 rev./min. A sample of medium (1 ml) was deproteinized by adding 6 ml of 5% trichloroacetic acid. Supernatants obtained by centrifugation of the extracted tissue and media samples were used for subsequent analysis.

Efflux experiments. In a series of experiments the release of *p*-amino[^{3}H]-hippurate already accumulated by kidney slices was examined after transfer into electrolyte media containing only unlabelled *p*-aminohippurate. The slices were preincubated for 2 h at 25°C in an oxygenated electrolyte medium with the following composition: 145 mM NaCl/5 mM potassium acetate/0.5 mM $CaCl_2$/0.7 mM $MgSO_4$/15 mM Tris buffer (pH 7.4)/0.075 mM *p*-amino[^{3}H]-hippurate. After the incubation the slices were washed twice in 10 ml ice-cold electrolyte solution, in order to remove labelled *p*-aminohippurate adhering to the renal tissue. They were then transferred to other Warburg vessels containing 3 ml electrolyte medium, having various concentrations of Na^+ (0—145 mM) and unlabelled *p*-aminohippurate (0.075 mM). The release of labelled *p*-aminohippurate was stopped after various time intervals by separating medium from slices.

Analytical methods. The trichloroacetic acid supernatants were analyzed for *p*-aminohippurate by a diazotation procedure of Smith et al. [15]. *p*-Amino-[^{3}H]hippurate was measured by addition of 0.2 ml supernatant to 10 ml of the scintillation fluid described by Bray [16] and radioactivity was measured in a Tri-carb Scintillation counter (Packard). The cpm were converted to dpm by the addition of [^{3}H]toluene to the solutions. The concentration of ATP in the tubular cells was determined by enzymatic spectrophotometry as described elsewhere [17]. Na^+ and K^+ concentrations of renal tissue and media were estimated by atomic absorption spectrometry.

Estimation of extracellular space and dry weight of kidney slices. The inulin space of the kidney slices was determined by the addition of [*carboxy*-^{14}C]inulin (Radiochemical Centre, Amersham) to the medium in a separate series of experiments and was found to be 26.9 ± 1.8 and 16.1 ± 0.8% under aerobic and anaerobic conditions, respectively. The decrease of the extracellular space under anaerobic conditions is presumably brought about by distension of renal cells as a consequence of imbibition of water under these conditions. The dry weight of the slices (20.2 ± 1.6%) was also determined in control experiments after drying the renal tissue in an oven at 105°C overnight.

Calculation of p-aminohippurate accumulation. The amount of *p*-aminohippurate in the intracellular part of slices was calculated as the difference between the *p*-aminohippurate content of renal tissue and that of the inulin space. This figure was then divided by the tubular water content (wet weight of tissue minus inulin space minus dry weight) in order to obtain the tubular concentration of *p*-aminohippurate (T_{PAH}) from which the accumulation of *p*-aminohippurate (T/M_{PAH}) was calculated.

Results

Fig. 1 shows uptake of *p*-aminohippurate by Na^+-depleted slices in the absence of Na^+ in the incubation medium. It is seen that in an oxygen atmosphere uptake occurs rapidly, leading to a final value of T/M_{PAH} of approx. 1.5

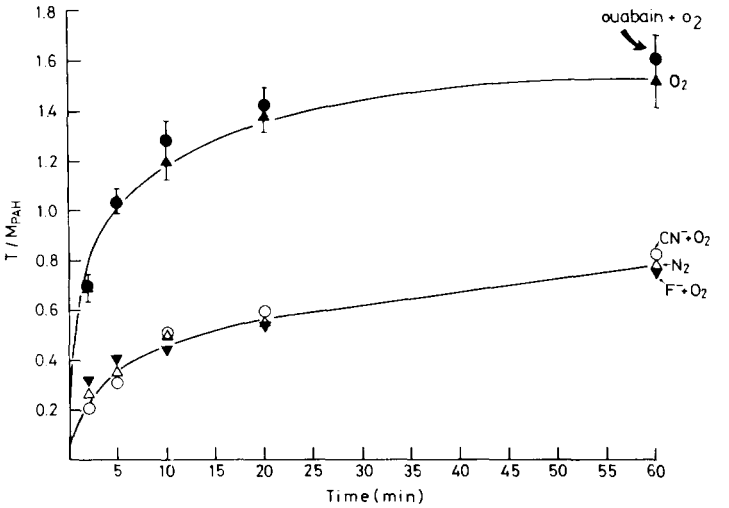

Fig. 1. Uptake of *p*-aminohippurate in Na^+-depleted slices under aerobic and anaerobic conditions. Na^+ concentration of slices was reduced to approx. 10 mM by the depletion procedure described in the Methods section. The uptake of *p*-aminohippurate was measured at a medium concentration of 0.075 mM *p*-amino[^{3}H]hippurate in a Na^+-free medium. ▲, 100% O_2; △, 100% N_2 atmosphere; ○, 10 mM CN^-; ▼, 10 mM F^-; ●, addition of 2 mM ouabain to the Na^+-depletion and *p*-aminohippurate uptake medium. Ordinate: T/M_{PAH}, ratio between the concentration of *p*-aminohippurate in the tubule water and medium. Abscissa: time of incubation.

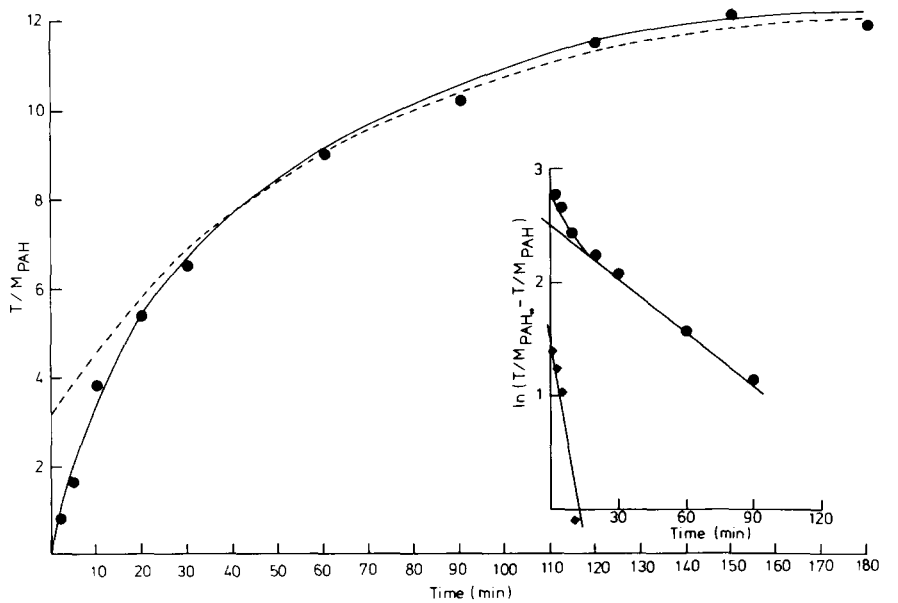

Fig. 2. Uptake of *p*-aminohippurate by freshly prepared kidney slices as a function of time. Uptake was measured in a medium containing 145 mM NaCl/5 mM potassium acetate/0.5 mM $CaCl_2$/0.7 mM $MgSO_4$/15 mM Tris buffer/0.075 mM *p*-amino[^{3}H]hippurate. The inset shows an exponential analysis of *p*-aminohippurate accumulation vs. time, indicating biphasic uptake. The broken line shows uptake corresponding to the slow component in the inset $T/M_{PAH} = 3.15 + 12.7\ (1 - e^{-0.0157t})$.

after 60 min. The uptake is much slower under anaerobic conditions or in the presence of CN^- and F^- to inhibit cellular metabolism. The difference between the two curves emphasizes that the quick uptake, although not resulting in an appreciable accumulation of *p*-aminohippurate, is energized by cellular metabolism. The low degree of accumulation observed under aerobic conditions may either be attributed to residual Na^+ remaining in the slices (about 10 mM Na^+), or it could represent active transport of *p*-aminohippurate by a Na^+-independent mechanism. Evidence in favor of the latter view is provided by the finding that pre-incubation with ouabain for 1 h at a medium concentration of 2 mM does not result in any changes of the uptake rate under aerobic conditions. It has previously been demonstrated that ouabain has a profoundly inhibitory effect on Na^+-dependent *p*-aminohippurate accumulation [11].

Fig. 2 shows uptake of *p*-aminohippurate by kidney slices under aerobic conditions in the presence of 145 mM Na^+ in the medium. Uptake occurs rapidly in the initial period, resulting in a T/M value around 3.5 after 10 min. Thereafter, *p*-aminohippurate uptake gradually levels off, reaching a steady-state value of around 13 after 2.5—3 h. As shown by the broken line the latter part of the uptake curve (after 20—30 min) can be approximated by an exponential uptake curve, which accounts for about 70% of the total uptake. We shall refer to this part as the slow component of uptake, while the extra uptake of *p*-aminohippurate after smaller time periods is referred to as the rapid component of uptake. It should be noted that the words 'slow' and 'rapid' in this context refer solely to the relative magnitude of efflux rate constants of the slow and rapid component, and not to the rates of concentrative uptake by the slices. *

Fig. 3 compares the properties of slices, depleted of Na^+ and K^+ by preincubation in media free of alkali cations, with those of slices used immediately after preparation. It is seen that the depleted slices rapidly gain Na^+ and K^+ so that the level is the same as in the untreated slices after a 10 min incubation period. There is no difference in the uptake of *p*-aminohippurate, ATP concentration and Q_{O_2} in the two sets of experiments. Fig. 3 thus demonstrates that there is no deleterious effect of removal of Na^+ on tubule function with respect to *p*-aminohippurate and various metabolic parameters.

Fig. 4 shows *p*-aminohippurate uptake in Na^+-depleted slices in the presence of various medium concentrations of Na^+ (0—145 mM). It is seen that over this range of Na^+ concentrations in the medium there is no difference in the transport rate of *p*-aminohippurate in the initial time period. Hence, the rapid component of *p*-aminohippurate transport is apparently not affected by Na^+. However, uptake of *p*-aminohippurate after the initial incubation period is strongly influenced by Na^+. Over a 60 min incubation period appreciable accumulation is obtained at 30 and 145 mM Na^+. It should be noted that at lower Na^+ concentrations *p*-aminohippurate uptake approaches steady state after 1 h, i.e., the feature of a slow component in the uptake curve is gradually lost. This suggests an enhanced efflux of Na^+-dependent *p*-aminohippurate transport, which could

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* Uptake of a compound from a medium with a constant concentration into a cellular compartment, governed by influx and efflux rate constants of k_1 and k_{-1}, respectively, is given by:

$T/M = k_1/k_{-1}\ (1 - e^{-k_{-1}t})$

where t is time after the start of the uptake experiment.

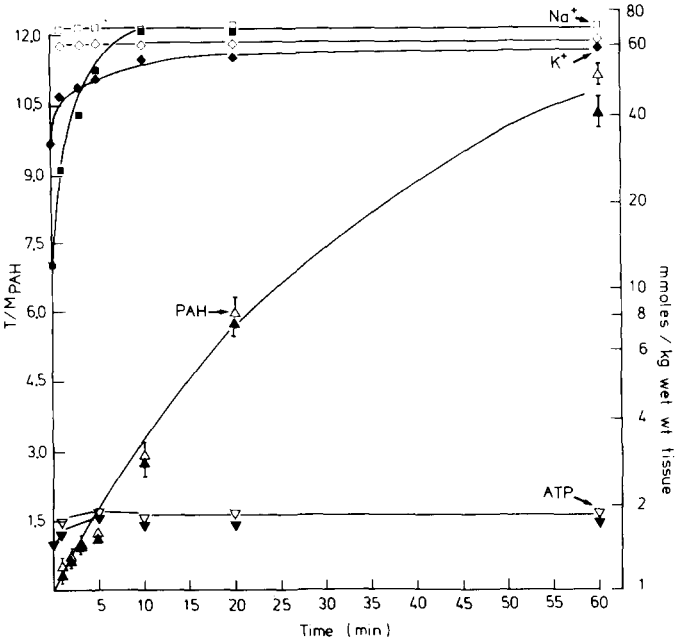

Fig. 3. Comparison of *p*-aminohippurate uptake by Na^+-depleted and freshly prepared slices. Closed and open symbols indicate results obtained on Na^+-depleted and freshly prepared slices, respectively. The uptake medium contained 145 mM NaCl/5 mM potassium acetate/0.5 mM $CaCl_2$/0.7 mM $MgSO_4$/15 mM Tris buffer/0.075 mM *p*-amino[^{3}H]hippurate.

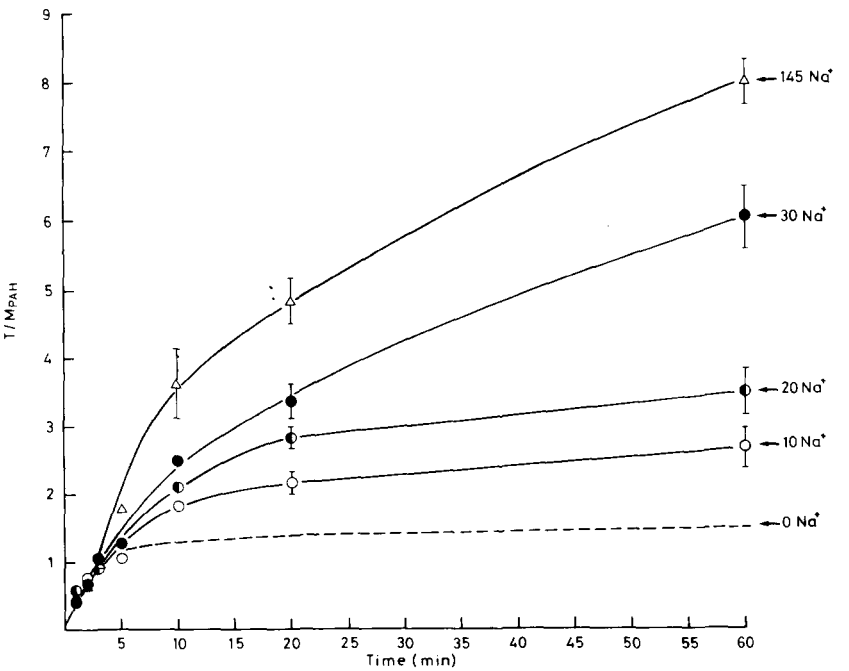

Fig. 4. Uptake of *p*-aminohippurate by Na^+-depleted slices as a function of various medium concentrations of Na^+. The broken line is taken from the data of aerobic uptake of *p*-aminohippurate in Fig. 1.

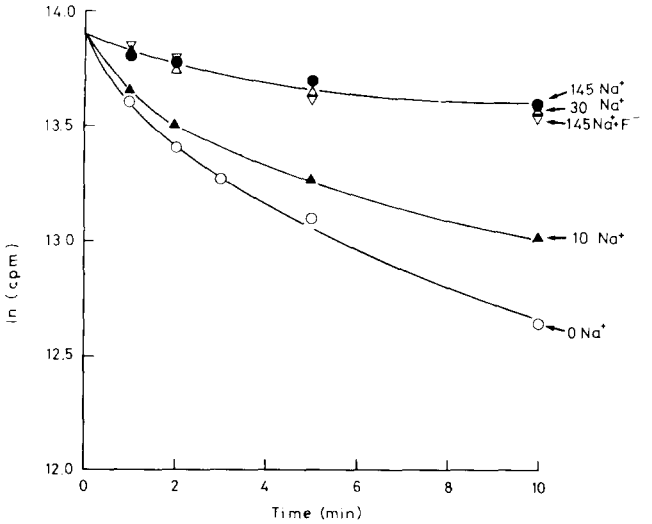

Fig. 5. Effect of various medium Na^+-concentrations on release of *p*-amino[^{3}H]hippurate from preloaded kidney slices. The kidney slices were incubated for 2 h at 25° C in an oxygenated electrolyte medium with the following composition: 145 mM NaCl/5 mM potassium acetate/0.5 mM $CaCl_2$/0.7 mM $MgSO_4$/15 mM Tris buffer/0.075 mM *p*-amino[^{3}H]hippurate. Release of radioactivity was measured after transferring slices to media containing different concentrations of Na^+ as indicated in the figure. (For details see Methods.)

be an important factor in curtailing accumulation of *p*-aminohippurate under these conditions. On the other hand, it has been concluded from autoradiographic experiments, that the long equilibration period required in experiments with high accumulation ratios may accrue from delayed access of *p*-aminohippurate to the inner part of the slices, because of extensive removal of *p*-aminohippurate from the extratubular space by accumulating tubules in the outermost portion of the slices in the initial incubation period [4]. In order to distinguish between these possibilities we have directly examined the effect of the medium concentration of Na^+ on efflux of *p*-aminohippurate (Fig. 5). In these experiments the slices were allowed to accumulate *p*-amino[^{3}H]hippurate for a period of 120 min, before transfer to media containing various concentrations of Na^+ and without labelled *p*-aminohippurate. In some experiments F^- was added as a metabolic inhibitor, but this did not affect efflux of *p*-aminohippurate. It is seen from the figure that release of *p*-amino[^{3}H]hippurate at 30 and 145 mM Na^+ in the medium is much smaller than at 0 and 10 mM Na^+. Over a 10 min period approx. 70% *p*-amino[^{3}H]hippurate is released from slices in the medium not containing Na^+. There is no difference between *p*-amino-[^{3}H]hippurate release at 30 and 145 mM Na^+, only 28% of accumulated *p*-aminohippurate being released over a period of 10 min under these conditions. Accordingly, the enhanced uptake of *p*-aminohippurate when the Na^+ concentration in the medium is raised from 30 to 145 mM Na^+ (Fig. 4) can be accounted for entirely by a stimulation of *p*-aminohippurate influx. On the other hand, an enhanced efflux at low medium concentrations of *p*-aminohippurate is an important factor, contributing to the lack of appreciable *p*-aminohippurate accumulation under these conditions.

Discussion

The present study confirms the existence of a rapid and a slow component of *p*-aminohippurate uptake by rabbit-kidney slices. Incubation with *p*-aminohippurate-containing media for about 2.5—3 h were required to approach steady-state accumulation ratios. Presumably the presence of a tissue barrier into the inner part of the slices [4] only partially accounts for the slow uptake, since the slow uptake differs from the rapid uptake process in being sensitive to Na^+. In agreement with the existence of two different modes of *p*-aminohippurate uptake there also exists a rapid and a slow phase of *p*-aminohippurate uptake in separated renal tubules of rabbit kidney [8]. We have previously suggested that the slow phase might represent intracellular compartmentation, i.e. diffusion of *p*-aminohippurate from cytoplasm into intracellular organelles by slow diffusion, following rapid equilibration at the peritubular membrane [8] *. Intracellular compartmentation suggests that perturbations of active *p*-aminohippurate transport should give rise to similar changes in the apparent rate constants of both the rapid and slow phase. This was previously found to be the case after inhibition of *p*-aminohippurate transport with probenecid and 2,4-dinitrophenol [8]. However, the present study shows a clear-cut dissociation between the Na^+ dependency of the rapid and slow component. Furthermore, the stimulatory effect of Na^+ strongly suggests an active nature of the slow uptake. We should, therefore, consider the alternative possibility, viz., that the slow component represents active transport of *p*-aminohippurate by renal cells with different efflux characteristics than those which are responsible for the rapid uptake of *p*-aminohippurate. It has been shown that the pars recta of the proximal tubules of rabbit kidney accumulates *p*-aminohippurate more intensively than the pars convoluta [19,20]. According to the study of Woodhall et al. [21] the S_2 cell type secretes *p*-aminohippurate more intensively than the S_1 and S_3 cell types. However, in all segments of the proximal tubule *p*-aminohippurate is predominantly mediated by an ouabain-sensitive process. It is, therefore, not possible to relate the existence of Na^+-independent proximal cells, suggested by the present study to any particular segment in the rabbit nephron. However, it is of interest to note that there may be species differences as to the extent to which Na^+ governs renal secretion of *p*-aminohippurate, since Ullrich et al. [22] were unable to demonstrate a reduction of *p*-aminohippurate secretion in vivo after blockade of Na^+-transport with ouabain in microperfusion experiments on the convoluted proximal tubules of the hamster kidney. It seems preferable to defer further discussion on the tubular localization of Na^+-dependent and Na^+-independent transport of *p*-aminohippurate until more detailed studies on the kinetics of the tubular transport of *p*-aminohippurate have been provided.

Concerning the effect of Na^+, Podevin et al. [12] reported transient accumulation of *p*-aminohippurate under anaerobic conditions in the presence of an extracellular to intracellular Na^+ gradient, established by transferring Na^+-

* The possibility that the slow phase represents transport from tubule cells to tubule lumen appears to be remote [8], since the tubule lumen of in vitro preparations is small, relative to that of tubule cells [18] and because there is probably no accumulation of *p*-aminohippurate in the tubule fluid, relative to that of the transporting cells [19].

depleted slices to a medium containing 145 mM Na^+. *p*-Aminohippurate accumulation under aerobic conditions is inhibited by ouabain [11], pointing to the role of (Na^+ + K^+)-ATPase in providing energy for active transport of *p*-aminohippurate. In electrophysiological studies Kikuta et al. [23] under a variety of conditions obtained evidence for an inverse correlation between the uptake of *p*-aminohippurate and the electrochemical Na^+-gradient across the peritubular membrane of the proximal tubules of the newt kidney. These findings are in agreement with the view that the Na^+-sensitive transport of *p*-aminohippurate is driven by the electrochemical Na^+-gradient across the peritubular membrane. According to the present results, the effect of Na^+ on *p*-aminohippurate transport is complex. There is only clear evidence for a stimulation of *p*-aminohippurate uptake at medium concentrations of Na^+ above 30 mM, while efflux of accumulated *p*-aminohippurate is markedly enhanced at lower concentrations of Na^+ in the medium. In this connection it should be noted, that a low medium concentration of Na^+ decreases the magnitude of the electrochemical Na^+-gradient which may limit Na^+ stimulated influx of *p*-aminohippurate. However, the enhanced efflux of *p*-aminohippurate in the absence of medium Na^+ suggests that Na^+ plays an additional and as yet unidentified role for the transport process by inhibiting efflux of *p*-aminohippurate at normal tissue levels of Na^+. It is apparent that inhibition of efflux will lead to an enhanced *p*-aminohippurate accumulation having the characteristics of a slow equilibration process. In contrast the Na^+-independent component, which makes a large contribution to the initial rate of *p*-aminohippurate uptake, is characterized by a high-efflux rate constant, resulting in a low steady-state accumulation of *p*-aminohippurate.

Acknowledgments

This work was supported by Aarhus Universitets Forskningsfond and Konsul Johs. Fogh-Nielsens og Fru Ella Fogh-Nielsens legat. Our thanks are due to Mrs. Anne Brandt Jørgensen, Mrs. Inge Lise Sheikh and Peter Vangsø Mathiasen for skillful technical assistance.

References

1 Foulkes, E.C. and Miller, B.F. (1959) Am. J. Physiol. 196, 86—92
2 Ross, C.R. and Farah, A. (1966) J. Pharm. Exp. Ther. 151, 159—167
3 Misanko, B.S., Park, Y.S. and Solomon, S. (1977) J. Endocrinol. 74, 121—128
4 Wedeen, R.P. and Weiner, B. (1973) Kidney Int. 3, 214—221
5 Podevin, R.A. and Boumendil-Podevin, E.F. (1977) Am. J. Physiol. 232, F239—F247
6 Chinard, F.P. (1956) Am. J. Physiol. 185, 413—417
7 Foulkes, E.C. (1977) Am. J. Physiol. 232, F424—F428.
8 Sheikh, M.I. and Møller, J.V. (1970) Biochim. Biophys. Acta 196, 305—319
9 Gerencser, G.A., Park, Y.S. and Hong, S.K. (1973) Proc. Soc. Exp. Biol. Med. 144, 440—444
10 Gerencser, G.A. and Hong, S.K. (1975) Biochim. Biophys. Acta 406, 108—119
11 Spencer, A.M., Sack, J. and Hong, S.K. (1979) Am. J. Physiol. 236, F126—F130
12 Povedin, R.A., Boumendil-Podevin, E.F. and Priol, C. (1978) Am. J. Physiol. 235, F278—F285
13 Maxild, J., Møller, J.V. and Sheikh, M.I. (1980) J. Physiol., in the press
14 Sheikh, M.I. and Maxild, J. (1978) Biochim. Biophys. Acta 514, 356—361
15 Smith, H.W., Finkelstein, N., Aliminosa, L., Crawford, B. and Graber, M. (1945) J. Clin. Invest. 24, 388—404
16 Bray, G.A. (1960) Anal. Biochem. 1, 279—285

17 Maxild, J. (1973) Arch. Int. Physiol. Biochim. 81, 501—521
18 Bojesen, E. and Leyssac, P.P. (1965) Acta Physiol. Scand. 65, 20—32
19 Tune, B.M., Burg, M.B. and Patlak, C.S. (1969) Am. J. Physiol. 217, 1057—1063
20 Grantham, J.J., Qualizza, P.B. and Irwin, R.L. (1974) Am. J. Physiol. 226, 191—197
21 Woodhall, P.B., Tisher, C.C., Simonton, C.A. and Robinson, R.R. (1978) J. Clin. Invest. 61, 1320—1329
22 Ullrich, K.J., Capasso, G., Rumrich, G., Papavassiliou, F. and Klöss, S. (1977) Pflüger's Arch. 368, 245—252
23 Kikuta, Y., Hayashi, H. and Saito, Y. (1979) Biochim. Biophys. Acta 556, 354—365