Na⁺-gradient-dependent stimulation of renal transport of *p*-aminohippurate

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Transport of *p*-aminohippurate was studied by the use of a preparation of rabbit kidney basolateral-membrane vesicles and in rat kidney-cortex slices under anaerobic conditions. With both preparations clear evidence of Na⁺-gradient stimulation of *p*-aminohippurate transport ('overshoot') was obtained. These results thus indicate that a significant aspect of active *p*-aminohippurate transport is by co-transport with Na⁺, and they appear to resolve previous disagreements concerning the role of Na⁺. Vesicle studies with a potential-sensitive dye suggested that *p*-aminohippurate may be transported electroneutrally, i.e. in a 1:1 complex with Na⁺.

Many studies have shown the presence of Na⁺ co-transport processes in luminal-membrane vesicles prepared from kidney cortex. This is presumably the basis for the active renal re-absorption of a wide variety of organic solutes (Murer & Kinne, 1980; Kragh-Hansen *et al.*, 1982*a,b*). On the other hand the energy source for active transport of organic anions that are secreted by the kidney is less well-defined (Møller & Sheikh, 1982). Studies on the uptake of *p*-aminohippurate, a prototype organic anion, by kidney-cortex slices have indicated that Na⁺ + K⁺-stimulated ATPase is involved in active accumulation (Spencer *et al.*, 1979; Maxild *et al.*, 1981; Sheikh *et al.*, 1981). However, the exact mechanism by which Na⁺ energizes *p*-aminohippurate transport has not been settled, since previous investigations failed to demonstrate an 'overshoot' of *p*-aminohippurate uptake by basolateral-membrane vesicles in the presence of an extravesicular-to-intravesicular Na⁺ gradient (Berner & Kinne, 1976; Kinsella *et al.*, 1979; Eveloff *et al.*, 1979), the usual criterion for demonstration of an Na⁺ co-transport mechanism. As an alternative it has been proposed that *p*-aminohippurate transport is directly linked to Na⁺ + K⁺-stimulated ATPase, rather than being influenced indirectly by the Na⁺ and K⁺ gradients established by this enzyme *in vivo* (Gerencser & Hong, 1975). The purpose of the present paper is to provide new data that are relevant for an understanding of the role of Na⁺ for the active transport of *p*-aminohippurate.

Materials and methods

Studies with membrane vesicles

Basolateral-membrane and luminal-membrane

vesicles were prepared from a homogenate of rabbit kidney cortex by a procedure including CaCl₂/MgCl₂ precipitation and differential and density-gradient centrifugation on a self-orienting Percoll gradient (Sheikh *et al.*, 1982). The membrane fractions (containing 8–10 mg of protein/ml) were stored in 300 mM-mannitol in 25 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/Tris buffer, pH 7.0, before use.

For measurements of *p*-aminohippurate uptake 50 µl of membrane-vesicle suspension was added to 100 µl of incubation medium as described in the legend to Fig. 1, containing *p*-amino[Gly-1-¹⁴C]hippurate (The Radiochemical Centre, Amersham, Bucks., U.K.) at a specific radioactivity of 374 mCi/mmol. Uptake of *p*-aminohippurate was stopped after various time intervals by the addition of 850 µl of the same ice-cold incubation medium but without *p*-aminohippurate and containing 1 mM-probenecid. The mixture was rapidly filtered through a Sartorius (Göttingen, W. Germany) membrane filter (0.45 µm, type SN 11106), which was washed twice with 2.5 ml of the same buffer. After drying, radioactivity on the filters was counted in a liquid-scintillation counter.

Changes in membrane potential were recorded spectrophotometrically by use of the dye 3,3'-diethyloxadycarbocyanine iodide (Eastman Kodak Co., Rochester, NY, U.S.A.) as previously described (Kragh-Hansen *et al.*, 1982*a*).

Experiments with kidney slices

Uptake of *p*-aminohippurate by rat kidney slices was studied under anaerobic conditions. The slices were depleted of Na⁺ and K⁺ by incubation for 3 h at 2°C under 100% N₂ in a medium containing

150 mM-choline chloride, 0.5 mM- CaCl_2 , 0.7 mM- MgSO_4 and 2 mM-ouabain in 25 mM-Hepes/Tris buffer, pH 7.0. Subsequent transport studies were performed in a Warburg respirometer under the following conditions: shaker speed, 100 cycles/min; gas phase, 100% N_2 ; temperature, 25°C; incubation period, 0–120 min.

Results and discussion

Uptake of *p*-aminohippurate by basolateral membranes of rabbit kidney cortex is shown in Fig. 1. In the presence of an extravesicular-to-intravesicular Na^+ gradient a marked 'overshoot' of *p*-aminohippurate is observed, which reaches a maximum after 1 min. The peak value is about 4-fold higher than the equilibrium concentration of *p*-aminohippurate, which is approached after 5–10 min. By contrast, *p*-aminohippurate uptake is not energized by a KCl gradient, in which case the uptake rate is only a little higher than that observed in the presence of probenecid at a concentration (1 mM) that is expected to block carrier-mediated transport of *p*-aminohippurate. Fig. 1 also demonstrates that Na^+ energization of *p*-aminohippurate transport is a particular feature of the basolateral membrane, since no 'overshoot' is observed with a luminal-membrane preparation.

Fig. 1. Uptake of *p*-aminohippurate by preparations of rabbit kidney basolateral-membrane and luminal-membrane vesicles

The incubation medium contained 150 mM-NaCl and 25 mM-Hepes/Tris buffer (▲) or 150 mM-KCl and 25 mM-Hepes/Tris buffer (△) or 150 mM-NaCl, 1 mM-probenecid and 25 mM-Hepes/Tris buffer (●) in the experiments with basolateral-membrane vesicles. ■, Uptake of *p*-aminohippurate by luminal-membrane vesicles in the presence of 150 mM-NaCl in 25 mM-Hepes/Tris buffer. All experiments were performed at pH 7.0 and at 25°C.

The present observations correspond to the finding by Podevin *et al.* (1978) of a concentrative uptake of *p*-aminohippurate by slices of rabbit kidney under anaerobic conditions. Normally oxidative metabolism is required for accumulation of *p*-aminohippurate by this system (for a review see Weiner, 1973). However, Podevin *et al.* (1978) reported accumulation of the organic anion under anaerobic conditions in the presence of an extracellular-to-intracellular Na^+ gradient. Previous studies on *p*-aminohippurate uptake by basolateral-membrane vesicles were made in rat (Berner & Kinne, 1976), dog (Kinsella *et al.*, 1979) and flounder (Eveloff *et al.*, 1979). In order to ascertain whether species difference might have been responsible for the failure to observe Na^+ -dependent 'overshoot' of *p*-aminohippurate uptake in these latter studies, we have examined the uptake of the organic anion in rat kidney slices under similar conditions to those used by Podevin *et al.* (1978) with rabbit kidney slices. Fig. 2 shows that in the presence of an Na^+ gradient there is a transient accumulation of *p*-aminohippurate. The intratubular accumulation of the organic anion reaches a maximal value of 2-fold in 40 min and declines to unity after 60 min, at which time the Na^+ gradient is abolished. The uptake of *p*-aminohippurate is slower in the presence of a K^+ gradient. Accumulation is not observed under these conditions, and is only a little higher than that observed in the presence of a transport-blocking concentration of probenecid.

The above results suggest that co-transport with Na^+ is a significant aspect of active *p*-aminohippurate transport by the basolateral membranes of proximal-tubule cells in mammalian species. In previous studies with vesicles some increase in permeability was observed in the presence of an NaCl gradient, but with no clear evidence for transient accumulation inside the vesicle lumen (Berner & Kinne, 1976; Kinsella *et al.*, 1979; Eveloff *et al.*, 1979). We suspect that previous failures to observe Na^+ -gradient-dependent accumulation of *p*-aminohippurate may have been due to technical problems. In contrast with luminal-membrane vesicles, reasonably pure and functionally competent preparations of basolateral membranes have been difficult to obtain. The method for preparation of basolateral-membrane vesicles included a $\text{Ca}^{2+}/\text{Mg}^{2+}$ precipitation step that was important in order to obtain vesicles with satisfactory transport properties (Sheikh *et al.*, 1982).

We have attempted to evaluate, by spectrophotometric recording of absorbance changes of the potential-sensitive dye 3,3'-diethyloxadicyanone iodide, whether Na^+ and *p*-aminohippurate are co-transported in a 1:1 ratio, or whether more than 1 Na^+ ion is required for each co-transport cycle. In the former case the transport

Fig. 2. Effect of Na^+ and K^+ gradients on the anaerobic uptake of *p*-aminohippurate by Na^+ + K^+ -depleted rat kidney slices

After depletion of the slices as described in the Materials and methods section, they were transferred to an anaerobic medium containing 0.075 mM-*p*-amino[^{14}C]hippurate and 150 mM- NaCl (▲) or 150 mM- KCl (△) or 150 mM- NaCl and 1 mM-probenecid (●), dissolved in 25 mM-Hepes/Tris buffer, pH 7.0. ♦, Intracellular uptake of Na^+ after transfer to the 150 mM- NaCl medium. T/M values represent the ratio between concentration in intracellular water and incubation medium, calculated as previously described (Sheikh *et al.*, 1981).

Fig. 3. Absorbance changes of 3,3'-diethyloxadadicarbo-cyanine iodide during uptake of L-malate and *p*-aminohippurate by basolateral-membrane vesicles

Curve 1 shows the absorbance changes caused by addition of 5 mM-L-malate to dye/vesicle suspension in the presence of a 150 mM- NaCl gradient taken from a previous publication (Sheikh *et al.*, 1982). Curves 2 and 3 depict the rate of uptake of *p*-aminohippurate (1 mM) in the presence of a 150 mM- KCl gradient and a 150 mM- NaCl gradient respectively. Curve 4 shows the effect of a 150 mM- KCl gradient on the rate of uptake of 5 mM-L-malate. All the results shown were obtained with an intravesicular medium composed of 300 mM-mannitol in 25 mM-Hepes/Tris buffer, pH 7.0. Protein concentration was 0.25 mg/ml, and the dye concentration was 7 $\mu\text{g}/\text{ml}$.

extravesicular to the intravesicular space (Kragh-Hansen *et al.*, 1982a). The absorption increase is lowered towards the original value as a function of time. A small optical response is observed on the addition of L-malate in the presence of a KCl gradient (curve 4). A quite different result is obtained for *p*-aminohippurate. Here the increase observed is small in the presence of an Na^+ gradient and is unchanged in the presence of a KCl gradient (compare curve 2 and curve 3). Further studies indicated that the response is non-specific, since it persisted after heating of the vesicles at 80°C for 30 min and after treatment with a disintegrating concentration of the non-ionic detergent dodecyl octa(ethylene glycol) ether (1 mg/ml of vesicle protein (Møller *et al.*, 1980). Furthermore, the response is proportional to the *p*-aminohippurate concentration up to a concentration of 25 mM (results not shown), although the concentration of *p*-aminohippurate needed to sustain half-maximal transport of *p*-aminohippurate across the baso-

process should be electroneutral, since *p*-aminohippurate bears one negative charge at pH 7.0, whereas electrogenic transport would be demonstrable if the Na^+/p -aminohippurate transport ratio were larger than unity. In Fig. 3 the broken line (curve 1) shows previous data on L-malate, which is co-transported with Na^+ as a positively charged complex (Sheikh *et al.*, 1982). It is seen that after addition of L-malate there is a rapid change of absorption in the presence of an Na^+ gradient. This corresponds to the movement of positive charge ('depolarization') through the membrane from the

lateral membrane is only about 0.5 mm (Møller & Sheikh, 1982). We therefore consider that the absorption increase is unrelated to depolarization of the membranes. We thus have no evidence that *p*-aminohippurate transport is electrogenic, consistent with an Na⁺/*p*-aminohippurate stoichiometric ratio of 1:1. The same conclusion was reached by Kikuta & Hoshi (1979), who in electrophysiological experiments did not observe any evidence of depolarization of the basolateral membrane by high concentrations in the newt kidney.

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References

- Berner, W. & Kinne, R. (1976) *Pflügers Arch.* **361**, 269–277
- Eveloff, J., Kinne, R. & Kinter, W. B. (1979) *Am. J. Physiol.* **237**, F291–F298
- Gerencser, G. A. & Hong, S. K. (1975) *Biochim. Biophys. Acta* **406**, 108–119
- Kikuta, Y. & Hoshi, T. (1979) *Biochim. Biophys. Acta* **553**, 404–416
- Kinsella, J. L., Holohan, P. D., Pessah, N. I. & Ross, C. R. (1979) *J. Pharmacol. Exp. Ther.* **209**, 443–450
- Kragh-Hansen, U., Jørgensen, K. E. & Sheikh, M. I. (1982a) *Biochem. J.* in the press
- Kragh-Hansen, U., Jørgensen, K. E. & Sheikh, M. I. (1982b) *Biochem. J.* in the press
- Maxild, J., Møller, J. V. & Sheikh, M. I. (1981) *J. Physiol. (London)* **315**, 189–201
- Møller, J. V. & Sheikh, M. I. (1982) *Pharmacol. Rev.* in the press
- Møller, J. V., Lind, K. E. & Andersen, J. P. (1980) *J. Biol. Chem.* **255**, 1912–1920
- Murer, H. & Kinne, R. (1980) *J. Membr. Biol.* **55**, 81–95
- Podevin, R. A., Boumendil-Podevin, E. F. & Priol, C. (1978) *Am. J. Physiol.* **235**, F278–F285
- Sheikh, M. I., Maxild, J. & Møller, J. V. (1981) *Biochem. Pharmacol.* **30**, 2141–2146
- Sheikh, M. I., Kragh-Hansen, U., Jørgensen, K. E. & Røigaard-Petersen, H. (1982) *Biochem. J.* in the press
- Spencer, A. M., Sack, J. & Hong, S. K. (1979) *Am. J. Physiol.* **236**, F126–F130
- Weiner, I. M. (1973) *Handb. Physiol. Sect. 8: Renal Physiology* 521–554