pH Gradient-stimulated Transport of Urate and *p*-Aminohippurate in Dog Renal Microvillus Membrane Vesicles

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ABSTRACT The transport mechanism of urate and *p*-aminohippurate (PAH) was evaluated in microvillus membrane vesicles isolated from the renal cortex of the mongrel dog. Imposition of a transmembrane pH gradient ($pH_o < pH_i$) markedly accelerated the uptake of [^{14}C]urate and [^{3}H]PAH and caused the transient accumulation ("overshoot") of each anion above its final level of uptake. The transport of urate and PAH under both stimulated ($pH_o < pH_i$) and basal ($pH_o = pH_i$) conditions was insensitive to valinomycin-induced K^+ diffusion potentials. The pH gradient-stimulated uptake of 25 μM [^{14}C]urate and 1.0 μM [^{3}H]PAH was significantly inhibited by 1.2 mM PAH, urate, furosemide, salicylate, or probenecid. The effect of each inhibitor on [^{14}C]urate transport was identical to the effect of the same inhibitor on [^{3}H]PAH flux. We conclude that the transport of urate and PAH in dog renal microvillus membrane vesicles occurs via a pH gradient-stimulated electroneutral carrier-mediated process such as 1:1 H^+-anion cotransport or OH^--anion exchange. Such a transport mechanism may possibly play a role in effecting uphill urate reabsorption in the proximal tubule. Moreover, this study demonstrates that secondary active solute transport in epithelial membranes may be coupled to the electrochemical gradient of an ion other than Na^+.

INTRODUCTION

Because of the complexities involved in studying transtubular transport processes that are bidirectional, it has been difficult to characterize the specific membrane events underlying organic anion transport in the intact proximal tubule (1). An important new approach for elucidating basic mechanisms of transtubular solute transport is to investigate the transport properties of plasma membrane vesicles isolated from tubular cells (2). For example, studies of isolated renal microvillus membrane vesicles have provided the best evidence in support of the concept that uphill glucose flux from the proximal tubular lumen into the cell occurs via carrier-mediated cotransport with Na^+ energized by the electrochemical Na^+ gradient at the luminal membrane (3). Carrier-mediated transport pathways for uric acid (4) and *p*-aminohippurate (PAH)[1] (5) have been identified in renal brush border membrane vesicles, but coupling between organic anion flux and electrochemical ion gradients has not been previously demonstrated. In the present communication, we report the existence of a carrier-mediated transport system in dog renal cortical microvillus membrane vesicles that, in response to transmembrane pH gradients, can effect the uphill accumulation of urate and PAH.

METHODS

Membrane isolation. Renal microvillus membrane vesicles were isolated from the mongrel dog renal cortex by the Mg-aggregation method previously described for isolation from the rabbit kidney (6), with the following modifications: (*a*) the homogenizing solution consisted of 200 mM mannitol, 50 mM Tris, 80 mM Hepes, pH 7.5; (*b*) the three low-speed centrifugations were performed at 2,930, 4,380, and 6,120*g*; (*c*) the three high-speed collections were performed at 32,600 *g*; (*d*) the purified membranes were suspended in 200 mM mannitol, 50 mM Tris, 80 mM Hepes, 10 mM $MgSO_4$, pH 7.5, at a concentration of 10–20 mg protein/ml. Purity of the membranes was similar to that reported (6); the enrichment in specific activity (final pellet per homogenate) of the luminal marker, γ-glutamyl transpeptidase, was ten to seventeen times, whereas that of the basolateral marker, Na,K-ATPase, was < 1.0.

Solute uptake studies. Uptake of [^{14}C]urate and [^{3}H]PAH was assayed at 20°C by the rapid filtration technique previously employed in this laboratory (7). Each experiment was performed in triplicate with membranes prepared on the same day or stored on ice for 24–48 h. No significant difference in organic anion transport properties between fresh

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[1] *Abbreviation used in this paper*: PAH, *p*-aminohippurate.

membranes and those stored on ice was noted. Each experiment was repeated at least twice on different membrane preparations. Although absolute solute uptake expressed per milligram membrane protein varied from preparation to preparation, relative effects of experimental maneuvers were extremely reproducible and, therefore, unless otherwise stated, the results of representative experiments are illustrated. In general, uptake of [^{14}C]urate (20–30 μM) and [^{3}H]PAH (1–2 μM) was assayed simultaneously. At these concentrations there is no inhibitory interaction between urate and PAH. All experimental solutions contained 10 mM $MgSO_4$ in addition to the constituents noted in the figure legends. In general, pH was varied by employing potassium phosphate buffers. The precise composition of each experimental solution is given in the figure legends. The pH of every experimental solution was checked before each experiment.

Identity of retained ^{14}C counts per minute. Urate conversion to allantoin has been reported in rat renal microvillus membranes (8). After 90-min incubations of dog renal microvillus membranes with 0.37 mM [^{14}C]urate at 20°C, >90% of the retained ^{14}C counts per minute could be eluted from the vesicles and identified as urate by the column chromatography method of Abramson et al. (9).

Binding vs. transport. The 90-min uptake of 26 μM urate and 1.2 μM PAH was assayed in media of varying mannitol concentrations. For both urate and PAH, a plot of uptake vs. 1/osmol was linear ($r = 0.99$ and 0.98, respectively). An estimate of binding was made from extrapolation of the regression line to infinite osmolarity (1/osmol = 0) (10). This analysis indicated that under standard incubation conditions (350 mosmol), only 4% of the urate uptake and 25% of the PAH uptake could be attributed to binding. No correction has been made for the component of solute uptake representing binding because the effects of experimental maneuvers on transport were always greater than could be attributed to alterations in binding.

Materials. [2-^{14}C]urate (57 mCi/mmol) was obtained from Amersham, Corp. (Arlington Heights, Ill.), [^{3}H]PAH (6.1 Ci/mmol) from New England Nuclear (Boston, Mass.), uricase, probenecid, and valinomycin from Sigma Chemical Co. (St. Louis, Mo.), and furosemide from Hoechst Pharmaceuticals, Inc. (Cincinnati, Ohio). As valinomycin was stored, 5 mg/cm^3 in 95% ethanol, equivalent volumes of ethanol were added to control incubations.

RESULTS

The effect of imposed pH gradients on urate (22 μM) and PAH (1.2 μM) uptake is illustrated in Fig. 1. With membrane vesicles preincubated in pH 7.5 buffer, the uptakes of both urate and PAH were markedly accelerated when incubations were performed at pH 6.0 (6.0/7.5) as compared with pH 7.5 (7.5/7.5). Indeed, a transient accumulation of each organic anion (overshoot) was noted to a level greater than four times the eventual equilibrium value. The eventual level of anion uptake was identical in the presence and absence of an initial pH gradient, indicating that equilibrium had indeed been established. These results suggest that the imposition of a large transmembrane pH gradient ($pH_o < pH_i$) stimulates the transient accumulation of urate and PAH against their concentration gradients, analogous with the Na^+ gradient-

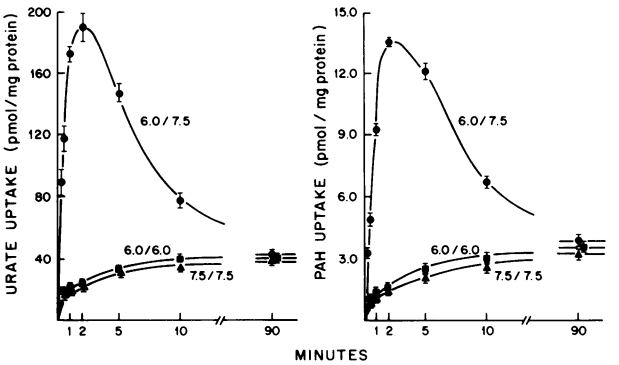

FIGURE 1 Effect of pH on anion uptake. ▲, 7.5/7.5: Membrane vesicles were preincubated for 120 min at 20°C in 170 mM mannitol, 42 mM Tris, 67 mM Hepes, 36 mM K^+, 18 mM sulfate, pH 7.5, and then uptake of 22 μM urate and 1.2 μM PAH was assayed in the presence of 155 mM mannitol, 8 mM Tris, 13 mM Hepes, 104 mM K^+, 4 mM sulfate, 52 mM phosphate, pH 7.5. ●, 6.0/7.5. Membranes were similarly preincubated at pH 7.5, but anion uptake was assayed in the presence of 200 mM mannitol, 8 mM Tris, 13 mM Hepes, 60 mM K^+, 4 mM sulfate, 52 mM phosphate, pH 6.0. ■, 6.0/6.0. Membranes were preincubated in 200 mM mannitol, 42 mM Tris, 67 mM Hepes, 18 mM sulfate, pH 6.0, and then anion uptake was assayed in the presence of 180 mM mannitol, 8 mM Tris, 13 mM Hepes, 60 mM K^+, 4 mM sulfate, 52 mM phosphate, pH 6.0. Each point represents the mean±SE for three determinations.

dependent accumulation of glucose in renal brush border membrane vesicles (3). With dissipation of the imposed pH gradient, the driving force for uphill anion transport is removed, and the levels of intravesicular urate and PAH decline to the same equilibrium values obtained in the absence of an initial pH gradient. If the vesicles were first preequilibrated at pH 6.0, then incubation at pH 6.0 (6.0/6.0) did not induce significant stimulation of anion uptake above the control (7.5/7.5) condition, confirming that it is the imposition of a transmembrane pH gradient ($pH_o < pH_i$), and not the pH 6.0-incubation medium per se, that is responsible for the acceleration of anion uptake.

The electrical potential-dependence of urate and PAH transport was examined in the experiment described in Fig. 2. In the presence of a $K_o^+ > K_i^+$ gradient, the K^+ ionophore valinomycin enhances the relative positivity of the intravesicular space in renal microvillus membranes (11). In contrast to the striking influence of valinomycin-induced K^+ diffusion potentials on Na^+-dependent glucose transport (10), no effect of the ionophore on organic anion uptake was noted either in the presence (6.0/7.5) or absence (7.5/7.5) of an initial pH gradient ($pH_o < pH_i$). Thus, the transport of urate and PAH appears to occur via an electroneutral process, such as 1:1 H^+-anion cotransport or OH^--anion exchange.

Possibly, the cotransport of urate and PAH with

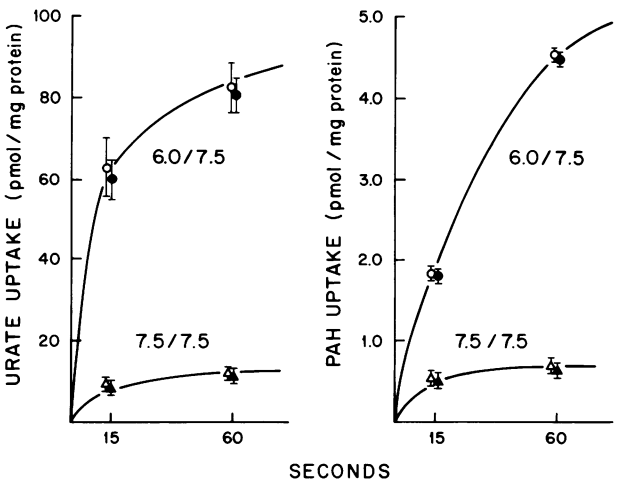

FIGURE 2 Effect of valinomycin on anion uptake. Membrane vesicles were preincubated for 100 min in 200 mM mannitol, 50 mM Tris, 80 mM Hepes, pH 7.5 with (●,▲) or without (○,△) valinomycin (0.05 mg/cm³). Uptake of 26 μM urate and 1.1 μM PAH was assayed in the presence of 200 mM mannitol, 10 mM Tris, 16 mM Hepes, 88 mM K^+, 18 mM sulfate, 52 mM phosphate, pH 6.0 (○,●) or 160 mM mannitol, 10 mM Tris, 16 mM Hepes, 132 mM K^+, 18 mM sulfate, 52 mM phosphate, pH 7.5 (△,▲). Each point represents the mean±SE for three determinations.

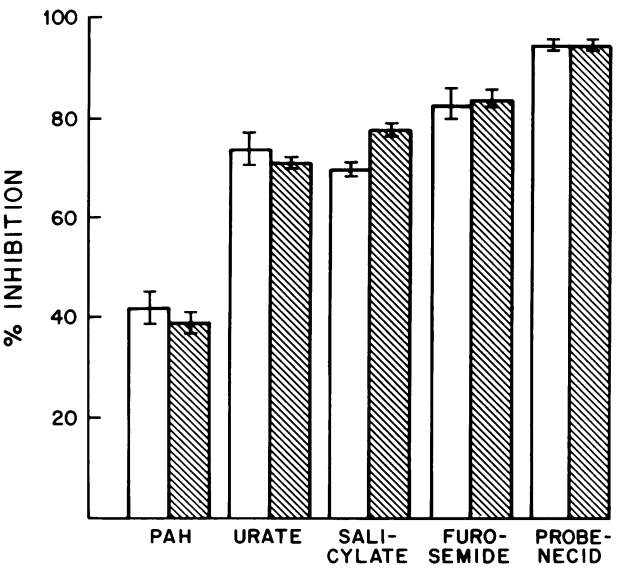

FIGURE 3 Effect of inhibitors on anion transport. Membrane vesicles were prepared and suspended in 200 mM mannitol, 50 mM Tris, 80 mM Hepes, pH 7.5, and the 15-s uptake of 25 μM urate and 1.0 μM PAH was determined in the presence of 40 mM mannitol, 25 mM Tris, 40 mM Hepes, 130 mM K^+, 130 mM phosphate, pH 6.0, without inhibitor or with 1.2 mM PAH, urate, salicylate, furosemide, or probenecid. Results shown represent the mean±SE for three separate experiments performed on different membrane preparations. □, [^{14}C]urate; ▨, [^{3}H]PAH.

K^+ could alternatively explain the electroneutral nature of anion uptake. However, such a possibility is unlikely because, in experiments not illustrated, K^+-dependence of organic anion transport could not be demonstrated. In these experiments, isosmotic replacement of potassium salts by mannitol in the pH 7.5 incubation medium had no effect on transport rate of either urate or PAH. Moreover, when Tris-Mes was used as the external pH 6.0 buffer, rather than potassium phosphate, pH gradient-stimulated uphill accumulation of urate and PAH was observed in the complete absence of K^+.

Electroneutral uphill accumulation of organic anions in response to pH gradients could, in principle, occur by simple nonionic diffusion of the undissociated acids, rather than via a specific carrier-mediated anion transport process. To clarify this issue, we tested the effect of several potential inhibitors (1.2 mM) on the pH gradient-stimulated transport of 25 μM [^{14}C]urate and 1.0 μM [^{3}H]PAH, as illustrated in Fig. 3. The inhibition caused by unlabeled PAH and urate is consistent with the fact that the organic anion transport process is saturable and, therefore, likely to be carrier mediated. Further support for the concept of carrier-mediated anion transport is provided by the observation that the uptake of urate and PAH was inhibited by furosemide and the uricosuric drugs salicylate and probenecid. All three agents inhibit carrier-mediated anion exchange in erythrocytes (12–14). Furosemide is also known to inhibit carrier-mediated cation-anion cotransport processes, such as Na^+-Cl^- cotransport in plasma membranes of the dogfish rectal gland (15). None of these agents has been reported to inhibit nonionic diffusion in any experimental system.

In Fig. 3, the effect of each inhibitor on urate transport was virtually identical to the effect of the same inhibitor on PAH flux. This finding suggests that urate and PAH may share the same pH gradient-stimulated transport system although, because urate was a more potent inhibitor than PAH, the affinity of the carrier for urate must be higher than for PAH.

DISCUSSION

We have demonstrated the existence of a carrier-mediated electroneutral transport system for urate and PAH in dog renal microvillus membrane vesicles that in response to an imposed pH gradient ($pH_o < pH_i$), can effect uphill accumulation of urate and PAH. The properties of this transport system are consistent with a process in which organic anions are 1:1 cotransported with H^+ or exchanged for OH^-. Although carrier-mediated transport pathways for uric acid (4) and PAH (5) had been previously identified in renal brush border

membrane vesicles, possible coupling between anion transport and imposed pH gradients had not been evaluated.

To the extent that a favorable electrochemical H^+ gradient exists in vivo across the luminal membrane of the proximal tubular cell as the result of the process of active acid extrusion, the organic anion transport system that we have identified can serve to mediate uphill anion uptake from the tubular lumen into the cell. Whether such a process will result in net transtubular anion reabsorption will depend on transport processes at the basolateral cell membrane and through the paracellular pathway. Urate does undergo net reabsorption in the dog proximal tubule (1). In contrast, PAH undergoes net secretion, although a significant reabsorptive flux of PAH has been identified (16). It may be that the active transport process for PAH at the basolateral membrane, poised in the direction of secretion, can overcome the luminal membrane reabsorptive mechanism so that net PAH secretion occurs.

Clearly, the precise physiological role of the luminal-membrane organic anion transport system that we have identified remains to be determined. Nevertheless, this transport system provides a possible mechanism that can account for uphill urate reabsorption. Moreover, our findings demonstrate that uphill solute transport in epithelial membranes may be coupled to the electrochemical gradient of an ion other than Na^+. Whether similar mechanisms of solute transport exist in epithelia other than the proximal tubule should be evaluated.

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