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Uric acid transport in rat renal basolateral membrane vesicles

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Pathways for urate transport across the basolateral membrane of rat proximal tubule cells were investigated using membrane vesicles isolated from rat renal cortex. The presence of an anion exchange mechanism(s) operative in the mode of α-ketoglutarate/urate; Cl^-/urate and $OH^-(HCO_3^-)$/urate as well as a mediated conductive mechanism was assessed from tracer flux measurements. In the presence of an inwardly directed Na^+ gradient an α-ketoglutarate dependent concentrative accumulation of PAH but not urate was observed suggesting an absence of the mediated exchange of α-ketoglutarate for urate. The imposition of an outwardly directed Cl^- gradient stimulated urate uptake in the absence but not the presence of conditions designed to minimize membrane potential development suggesting an indirect electrostatic coupling of urate uptake to a Cl^- gradient-induced diffusion potential. Conditions favoring the development of an inside-positive K^+ diffusion potential was observed to induce an inhibitor-sensitive, concentrative accumulation of urate in the absence of Cl^-. The stimulation of urate uptake measured in the presence of an inside-alkaline pH gradient was not of sufficient magnitude to suggest the apparent conductive urate uptake was secondary to a membrane voltage induced, inside alkaline pH gradient and the operation of an $OH^-(HCO_3^-)$/urate exchanger. The evidence obtained from the present investigation suggests rat basolateral membrane urate transport occurs by a mediated, conductive mechanism and is not coupled to Cl^-, α-ketoglutarate or HCO_3^-. This finding, when considered together with existing evidence supporting the presence of transport pathways mediating the active uptake of urate across the brush-border membrane, suggests the transtubular reabsorptive flux of urate in rat proximal tubules occurs by active uptake at the apical membrane and passive efflux at the basolateral membrane.

Introduction

The renal handling of uric acid, a major metabolic end product of purine metabolism, is confined largely to the proximal tubule which mediates bidirectional transepithelial transfer with species dependent net reabsorption or net secretion [1–3]. Among mammals, net reabsorption occurs in human, monkey, rat and mongrel dog whereas net secretion of urate occurs in rabbit and pig. Recent evidence suggests the species-specific net reabsorption or secretion of uric acid may reflect possible differences in the presence or absence of membrane transport pathways for urate in the brush-border and basolateral membrane. For example, brush-border membrane vesicle studies of urate transport in rat and dog, net reabsorbers of urate, indicate the presence of an anion exchange mechanism with affinity for both urate and PAH [4–7]. In contrast, when investigated in brush-border membranes isolated from pig and rabbit, net secretors of urate, no evidence for the presence of a urate/anion exchange mechanism was obtained, rather, evidence supporting the presence of a mechanism mediating conductive urate translocation was described [8,9].

Presently, the membrane transport pathways for urate across the basolateral membrane remain to be completely defined with regard to possible coupling to other ions via co- or counter-transport or with regard to the presence or absence of transport mechanisms correlating to species demonstrating net urate secretion or reabsorption. In the rat, evidence for a basolateral membrane urate/Cl^- exchange mechanism has been described from the stimulatory effect of an imposed Cl^- gradient on urate uptake by membrane vesicles [10]. Although also yet to be confirmed, a

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Abbreviations: PAH, *p*-aminohippuric acid; TMA, tetramethylammonium; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid; Mes, *N*-morpholinoethanesulfonic acid; DIDS, 4,4′-diisothiocyanostilbene2,2 disulfonic acid; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid.

possible role for uricase in rat basolateral membrane urate transport has been suggested from the observed dependence of mediated urate transport on the presence of copper [11,12]. More recently, the mechanism of urate transport across the basolateral membrane of a species demonstrating net secretion of urate has been investigated using membrane vesicles isolated from pig kidney [13]. The indirect effect of an imposed Na^+ gradient to stimulate urate uptake in the presence but not the absence of α-ketoglutarate suggested the presence of a α-ketoglutarate/urate exchange mechanism as a pathway for urate transport across the pig basolateral membrane. Given the small number of studies devoted to characterizing pathways for basolateral membrane urate transport we have assessed the possible presence of four urate transport mechanisms (or modes of the same mechanism) in rat renal basolateral membrane vesicles: α-ketoglutarate/urate exchange, Cl^-/urate exchange, HCO_3^-/urate exchange and conductive urate transport. Of the basolateral membrane transport pathways examined, only evidence supporting the presence of a conductive mechanism was obtained and the possible significance of this observation is discussed in the context of species-dependent net secretion or reabsorption of urate.

Materials and Methods

Membrane preparations

Basolateral membrane vesicles were prepared from male, Sprague-Dawley rats (225–250 g) by differential and Percoll density gradient centrifugation as previously described [14]. The basolateral membrane fraction was enriched 9–12-fold compared to the homogenate in specific activity of the basolateral membrane marker Na^+/K^+-ATPase and constituted 9–14% of the total homogenate Na^+/K^+-ATPase activity [15]. The specific activity of maltase, a luminal membrane marker enzyme, was enriched 1–2-fold and constituted 1–2% of the homogenate maltase activity [16]. Both freshly prepared and frozen basolateral membrane preparations were used in this study as preliminary experiments indicated freezing (−70°C) had no effect on urate transport activity.

Upon removing the basolateral membrane fraction from the Percoll density gradient brush-border membrane vesicles were further isolated from the remaining membranes by divalent cation aggregation as previously described [17]. The brush-border membrane preparation was enriched approx. 14-fold compared to the homogenate in maltase specific activity and represented approximately 7% of homogenate activity. The Na^+/K^+-ATPase activity measured in the brush-border preparation was 1–2-fold greater than in the homogenate and represented 1–2% of the total homogenate activity. Brush-border membrane vesicles were resuspended in 100 mM TMA gluconate, 80 mM Hepes-TMA (pH 7.5) and centrifuged $35\,000 \times g$ for 30 min. The brush-border membrane pellet was again resuspended and recentrifuged and the vesicles were stored frozen (−70°C) until used. Membrane protein was determined by a sodium dodecyl sulfate-Lowry assay with bovine serum albumin as the standard [18].

Isotopic flux measurements

Thawed (20–25°C) or freshly prepared basolateral membrane vesicles were washed twice into buffers designated for each experiment by resuspension and centrifugation at $35\,000 \times g$ for 30 min. The second pellet was resuspended to 10–30 mg of protein/ml in the same media and isosmotic solutions of appropriate ionic composition were added to obtain the desired intravesicular solution described for each experiment in the figure and table legends. The membrane suspension was incubated for 120 min at room temperature to facilitate complete transmembrane equilibration of the added media. For experiments testing the effect of HCO_3^- on brush-border and basolateral membrane urate transport the membranes were gassed continuously with humidified 100% N_2 or 90% N_2/10% CO_2 during the pre-equilibration period. The extravesicular media were prepared similarly and the final composition for each experiment is given in the figure and table legends. Intravesicular [^{3}H]PAH and [^{14}C]urate content was assayed in triplicate at 37°C by a rapid filtration technique previously described [19]. The uptake reaction was quenched by rapid dilution with isosmotic K gluconate, 2 mM TMA probenecid, 10 mM Hepes-TMA (pH 7.4) kept at 4°C. The diluted membrane suspension was passed through a 0.65 μm Millipore filter (DAWP) and washed with an additional 9 ml of quench buffer. The filters were dissolved in 3 mL of Ready-Solv (HP) Beckman and counted by scintillation spectroscopy. The sequence of quenching, filtration and washing occurred within a 15-s period. The timed uptake values obtained were corrected for the nonspecific retention of isotope by the filters. While absolute PAH and urate uptake value varied from preparation to preparation, relative changes resulting from experimental manipulations were highly reproducible.

Materials

Valinomycin, probenecid, DIDS, SITS, oxonate, nicotinate and α-ketoglutarate were purchased from Sigma (St. Louis, MO). Pyrazinoate was purchased from Aldrich (Milwaukee, WI). [^{3}H]PAH and [^{14}C]-urate were obtained from New England Nuclear (Boston, MA) and American Radiolabelled Chemical (St. Louis, MO). Except where indicated, all solutions were prepared from distilled-deionized water and passed through a 0.22 μm Millipore filter.

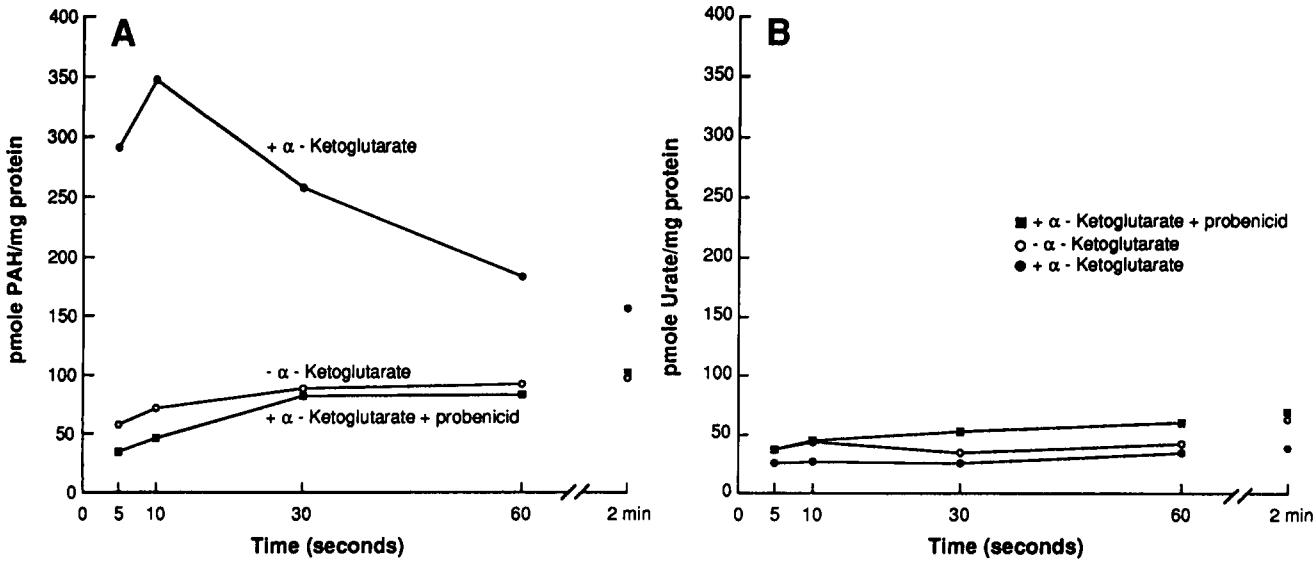

Fig. 1. Effect of α-ketoglutarate on PAH and urate influx. Basolateral membrane vesicles were pre-equilibrated with 125 μM KCl, 10 mM TMA-Hepes (pH 7.4). Uptake of PAH (50 μM) (A) and urate (50 μM) (B) occurred from extravesicular solutions containing 100 mM NaCl, 25 mM KCl, 10 mM TMA-Hepes (pH 7.4) and: ●, 10 μM α-ketoglutarate; ○, 10 μM Cl^-; ■, 10 μM αketoglutarate, 1 μM probenicid. A representative experiment of three independent observations is illustrated.

Results

Membrane transport pathways for urate at the basolateral side of rat renal proximal tubule cells was first investigated by testing for the possible presence of α-ketoglutarate/urate exchange. Utilizing the presence of a basolateral membrane Na^+-dicarboxylate cotransport mechanism [20–22] an outwardly directed α-ketoglutarate concentration gradient was induced by imposing an inwardly directed Na^+ gradient in the presence of 10 μM α-ketoglutarate. The ability of an inwardly directed Na^+ gradient to generate an outwardly directed α-ketoglutarate gradient is demonstrated indirectly by the effect of α-ketoglutarate on PAH uptake as shown in Fig. 1A. Previously, the presence of a rat basolateral membrane α-ketoglutarate/PAH exchange mechanism was proven in part by the demonstration of an α-ketoglutarate dependent, active accumulation of intravesicular PAH which could only have resulted from the generation of an outwardly directed gradient of α-ketoglutarate [23,24]. The ability of an outwardly directed α-ketoglutarate gradient to serve as a driving force for the active uptake of PAH is shown in Fig. 1A by the concentrative accumulation of PAH measured in the presence but not the absence of α-ketoglutarate. In contrast to the stimulation of PAH uptake measured in the presence of α-ketoglutarate, Fig. 1B shows no effect of α-ketoglutarate on basolateral membrane vesicle urate uptake when assayed in the same vesicle preparation under identical conditions. This observation suggests an absence of α-ketoglutarate/urate exchange in rat renal basolateral membrane. The possible presence of a basolateral membrane α-ketoglutarate/urate exchange mechanism was further investigated by examining the effect of increasing concentrating of α-ketoglutarate on PAH and urate uptake. Table I describes the parabolic relationship of the effect of increasing extravesicular α-ketoglutarate concentrations on basolateral membrane PAH uptake consistent with a cis-inhibitory effect occurring at higher α-ketoglutarate concentrations. In contrast, the magnitude of basolateral membrane urate uptake measured

TABLE I

Effect of increasing α-ketoglurate concentration on PAH and urate uptake

Basolateral membrane vesicles were pre-equilibrated with 125 mM KCl, 10 mM TMA-Hepes (pH 7.4). The 10-s uptake of PAH (50 μM) and urate (50 μM) occurred from extravesicular solutions containing 100 mM NaCl, 25 mM KCl, 10 mM TMA-Hepes (pH 7.4) and α-ketoglutarate at the concentrations shown below. PAH and urate uptake are expressed as a percentage of uptake measured in the absence of α-ketoglutarate. The mean ± S.E. ($n = 4$) PAH and urate uptake in the absence of α-ketoglutarate was 74.5 ± 17 pmol/mg and 40 ± 7 pmol/mg, respectively.

	α-Ketoglutarate (μM)						
	0	5	10	25	50	100	150
PAH	100	273 ± 5	311 ± 21	332 ± 19	316 ± 17	267 ± 8	248 ± 9
Urate	100	101 ± 5	100 ± 5	105 ± 3	106 ± 4	108 ± 5	98 ± 6

at increasing extravesicular α-ketoglutarate concentrations remained at the same level observed in the absence of α-ketoglutarate. This observation suggests our inability to detect the presence of basolateral membrane α-ketoglutarate/urate exchange was not the result of measuring urate uptake at suboptimal α-ketoglutarate concentrations and further supports the absence of α-ketoglutarate/urate exchange in rat basolateral membrane.

Recently, evidence for the presence of a rat renal basolateral membrane Cl^-/urate exchange mechanism was obtained from membrane vesicle studies of urate uptake [10]. In an attempt to verify the presence of a Cl^-/urate exchange mechanism in rat basolateral membrane we examined the effect of imposing an outwardly directed Cl^- concentration gradient on urate uptake in the presence and absence of conditions expected to blunt the possible development of an inside-positive, Cl^- gradient-induced diffusion potential. As shown in Table II, compared to level of urate uptake measured in the presence of equal intra- and extravesicular Cl^-, urate uptake was markedly stimulated in the presence of an outwardly directed Cl^- gradient suggesting a coupling consistent with the presence of a Cl^-/urate exchange mechanism. The nature of coupling of urate uptake to the imposed Cl^- gradient was assessed by determining the effect of maneuvers designed to minimize the development of membrane potential ($K_o^+ = K_i^+$ + valinomycin) on Cl^- gradient-induced urate uptake. As shown to the right in Table II, when measured in valinomycin pretreated membranes the level of urate uptake in the presence of an outwardly directed Cl^- gradient was essentially indistinguishable from the level measured where intra- and extravesicular Cl^- was equal. This observation suggests the Cl^- gradient-induced stimulation of urate uptake measured in the absence of valinomycin occurred via conductive urate uptake driven by an inside-positive voltage difference.

TABLE II

Effect of Cl^- concentration gradient on urate influx

Basolateral membrane vesicles were pre-equilibrated with 125 mM KCl, 10 mM TMA-Hepes (pH 7.4). The 10-s uptake of urate (30 μM) occurred from extravesicular solutions containing: $Cl_o^- = Cl_i^-$, 125 mM KCl, 10 mM TMA-Hepes (pH 7.4); $Cl_o^- < Cl_i^-$, 112.5 mM K gluconate, 12.5 mM KCl, 10 mM TMA-Hepes (pH 7.4). Where indicated membranes were preincubated with valinomycin (0.25 mg/ml) or an equivalent volume of ethanol (1%) for a minimum of 30 min. The data shown (means ± S.E. in pmol urate/mg protein) was compiled from four experiments each performed using a different membrane preparation.

$Cl_o^- = Cl_i^-$ +valinomycin	$Cl_o^- < Cl_i^-$ −valinomycin	$Cl_o^- < Cl_i^-$ +valinomycin
29.5 ± 0.8	44 ± 2.5	27.3 ± 0.9

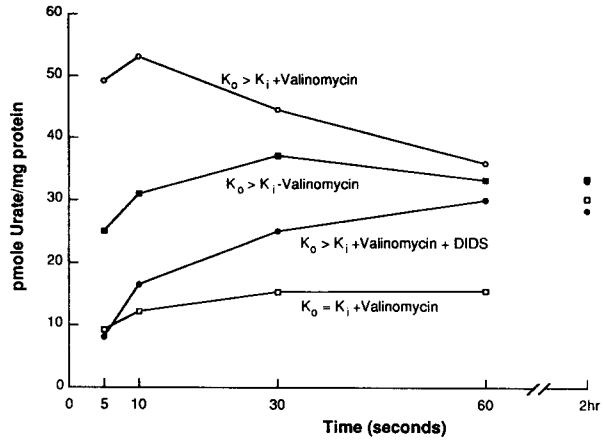

Fig. 2. Effect of valinomycin-induced K^+ diffusion potential on urate influx. Basolateral membrane vesicles were pre-equilibrated with: ●, ■, ○, 125 mM TMA gluconate, 10 mM TMA-Hepes (pH 7.4); □, 62.5 mM K gluconate, 62.5 mM TMA gluconate, 10 mM TMA-Hepes (pH 7.4). Uptake of urate (30 μM) occurred from extravesicular solutions containing: ○, 100 mM K gluconate, 25 mM TMA gluconate, 10 mM TMA-Hepes (pH 7.4) and where indicated 1 mM DIDS; □, 62.5 mM K gluconate, 62.5 mM TMA gluconate, 10 mM TMA-Hepes (pH 7.4). Where indicated membranes were incubated with valinomycin (0.5 mg/ml) or an equivalent volume of ethanol (1%) for a minimum of 30 min. A representative experiment of three independent observations each performed with a different membrane preparation is shown.

To the extent that a conductive pathway for urate uptake is present in rat renal basolateral membrane then an inside-positive, valinomycin-induced, K^+ diffusion potential should serve as a driving force for active accumulation of urate in the absence of Cl^-. As shown in Fig. 2 in the presumed absence of a transmembrane voltage difference where intra- and extravesicular K^+ was equal urate uptake by valinomycin pretreated membranes was low and slowly approached an equilibrium value measured at 2 h. The imposition of an inwardly direct K^+ gradient resulted in a marked stimulation of urate uptake by membranes pretreated with and without valinomycin but only exceeded equilibrium in membranes made sufficiently K^+ permeable by addition of valinomycin. This observation suggests an inside-positive voltage difference may serve as a driving force for the active accumulation of urate by a conductive pathway which is independent of Cl^-. The observed coupling of urate uptake to an inside-positive voltage difference may have occurred as a result of a mediated Cl^- transport process or via an illdefined 'leak' pathway possibly introduced during membrane vesicle preparation. An attempt was made to distinguish between these two possibilities by testing the effect of the anion transport inhibitor DIDS (1 mM) on conductive urate uptake. As shown in Fig. 2, K^+ gradient-induced urate uptake by valinomycin pretreated membranes was clearly reduced in the presence of DIDS which suggests conductive urate uptake occurs

by a mediated transport pathway and is not an artifact of membrane vesicle preparation.

The properties of basolateral membrane conductive urate transport were next assessed with regard to its inhibition by various substrates and inhibitors. Again, in keeping with properties expected for mediated transport, the magnitude of radiolabelled urate uptake (30 μM) was reduced 78 ± 2% ($n = 4$) by an excess (1 mM) of unlabelled urate. Interestingly, the substrates pyrazinoate, oxonate, nicotinate and PAH, which are known to interact with the urate transporter in the brush-border membrane, were observed to have no effect on the voltage sensitive pathway present in the basolateral membrane. The organic anion transport inhibitor probenecid (1 mM) was observed to have a small inhibitory effect reducing conductive urate uptake by 19 ± 4% ($n = 4$). Surprisingly, the diisothiocyanostilbene derivative DIDS (1 mM) inhibited conductive urate uptake by 87 ± 1% ($n = 4$) whereas SITS (1 mM), the singly substituted acetamido stilbene derivative, inhibited conductive urate uptake by only 11 ± 5% ($n = 4$).

In addition to the possible presence of a conductive mechanism for urate transport across the basolateral membrane the stimulation of urate uptake resulting from conditions designed to induce an inside-positive voltage difference may have been secondary to the formation of an insidealkaline pH gradient driving urate accumulation via urate/OH^-(HCO_3^-) exchange. Accordingly, the possible presence of a basolateral membrane urate/OH^-(HCO_3^-) exchanger was tested for by determining the effect of imposing an inside alkaline pH gradient (pH_o 6/pH_i 7.5) and HCO_3^- gradient on urate uptake. As shown in Table III, in the absence of CO_2 a small but significant stimulation of urate uptake was measured in the presence of an inside-alkaline pH gradient when compared to the absence of a pH gradient at pH 6 or pH 7.5. The pH gradient-induced stimulation of urate uptake was further increased when an outwardly directed HCO_3^- gradient was simultaneously imposed in the presence of CO_2. While consistent with the presence of a basolateral membrane urate/OH^-(HCO_3^-) exchange mechanism the magnitude of pH and HCO_3^- gradient-induced urate uptake suggested the origin of the anion exchange activity may have resulted from the small amount of brush-border membrane copurified in the basolateral membrane preparation.

To the extent that a small amount of brush-border membrane contamination could support the anion exchange activity measured in the basolateral membrane preparation then a sizeable urate/OH^-(HCO_3^-) exchange activity should be present in brush-border membranes. The magnitude of pH and HCO_3^- gradient-driven urate uptake by brush-border membranes is shown in Fig. 3. In contrast to the basolateral membrane preparation, pH and HCO_3^- gradient-driven urate uptake was markedly stimulated compared to control when measured in brush-border membranes. This observation suggests the small pH and HCO_3^- gradient-induced stimulation of basolateral membrane urate uptake shown in Table III may originate from brush-border membrane vesicles copurified in the basolateral membrane preparation. Furthermore, among the basolateral membrane preparations used for the uptake measurements shown in Table III, an excellent correlation existed between the magnitude of brush-border membrane marker enzyme enrichment and the

TABLE III

Effect of pH and HCO_3^- on basolateral membrane urate influx

Basolateral membrane vesicles were pre-equilibrated at pH 6 under 100%N_2 or 10%CO_2/90%N_2 with 110 mM TMA gluconate, 57.3 mM K gluconate, 52 mM Mes, 45.3 mM Hepes, 25 mM TMA and at pH 7.5 substituting mannitol for Mes and $KHCO_3$ for K gluconate when under 10% CO_2/90% N_2. The 10-s uptake of urate (30 μM) under 100%N_2 or 10%CO_2/90%N_2 occurred from an extravesicular solution containing: (pH_o 6/pH_i 6)-110 mM TMA gluconate, 57.4 mM K gluconate, 52 mM Mes, 45.3 mM Hepes, 25 mM TMA; (pH_o 7.5/pH_i 7.5)-mannitol was substituted for Mes and $KHCO_3$ was substituted for K gluconate under 10%CO_2/90%N_2; (pH_o 6/pH_i 7.5)-110 mM TMA gluconate, 57.4 mM K gluconate, 47 mM Mes, 42 mM mannitol, 9 mM Hepes, 25 mM TMA and under 10%CO_2/90% 13 mM gluconate was substituted for mannitol. Membranes were preincubated with valinomycin (0.5 mg/ml) for a minimum of 30 min. The data are expressed as percent control urate uptake (15.6 ± 0.6 pmol/mg protein). The mean ± S.E. of five experiments, each performed with a different membrane preparation, is shown.

	N_2	CO_2/HCO_3^-
pH_o 6/pH_i 6	100	88 ± 7
pH_o 6/pH_i 7.5	122 ± 6	151 ± 6
pH_o 7.5/pH_i 7.5	94 ± 5	89 ± 9

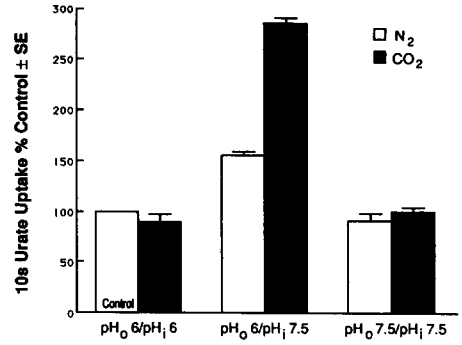

Fig. 3. Effect of pH and HCO_3^- gradients on brush-border membrane urate influx. Brush-border membrane vesicles were pre-equilibrated under 10% CO_2/90%N_2 or 100%N_2 as described in the legend to Table III. The 10-s uptake of urate (20 μM) occurred from extravesicular solutions under 10% CO_2/90% N_2 or 100% N_2 as described in the legend to Table III. Membranes were preincubated with valinomycin (0.5 mg/ml) for a minimum of 30 min. Control urate uptake was 14.8 ± 0.3 pmol/mg protein. The mean ± S.E. of three experiments, each performed with a different membrane preparation, is shown.

magnitude of urate/OH^-(HCO_3^-) exchange activity. Importantly, these results suggest the presence of, at the most, only a small basolateral membrane urate/OH^-(HCO_3^-) exchange activity which is unlikely to be sufficient to account for the magnitude of intravesicular urate accumulation resulting from an inside-positive voltage difference. Thus, basolateral membrane urate uptake in response to inside-positive voltage difference would appear to be via a conductive mechanism and not secondary to a pH gradient-driven anion exchange.

Discussion

The present investigation was conducted to gain further insight as to the membrane transport mechanism(s) mediating urate permeation across the basolateral membrane of mammalian proximal tubule cells. Specifically, the possible presence of an anion exchanger mechanism operative in the mode of α-ketoglutarate/urate exchange; Cl^-/urate exchange, HCO_3^-/urate exchange as well as a mechanism mediating conductive urate transport was assessed by tracer flux measurements using preparations of purified basolateral membrane vesicles isolated from rat kidney cortex. Of the basolateral membrane urate transport pathways examined only sufficient evidence to suggest the presence of a mediated conductive mechanism was obtained.

The presence of a basolateral membrane α-ketoglutarate/urate exchanger was first assessed by examining the ability of α-ketoglutarate to serve as a driving force for the accumulation of intravesicular urate. The reported presence of a basolateral membrane Na^+-dicarboxylate cotransport mechanism suggested the possibility of inducing an outwardly directed α-ketoglutarate gradient by imposing an inwardly directed Na^+ gradient in the presence of the dicarboxylate [20–22]. The formation of an outwardly directed α-ketoglutarate gradient was verified by observing the effect of α-ketoglutarate on PAH uptake in the presence of an inward Na^+ gradient. The observed concentrative accumulation of PAH measured only in the presence of α-ketoglutarate is consistent with α-ketoglutarate gradient-driven PAH uptake via a basolateral membrane α-ketoglutarate/PAH exchange mechanism previously described [23,24]. In contrast to the α-ketoglutarate dependent stimulation of PAH uptake observed in the presence of an inwardly directed Na^+ gradient, no effect of α-ketoglutarate on urate uptake was noted when assayed in the same membrane vesicle preparation under the same conditions. The absence of an apparent α-ketoglutarate/urate exchange activity suggested the possible demonstration of α-ketoglutarate dependence may require measurement of urate uptake at an appropriate α-ketoglutarate concentration. Accordingly, both PAH and urate uptake was measured in the absence and presence of increasing α-ketoglutarate concentrations to determine the optimal concentration for stimulation of urate uptake. Consistent with the cis-inhibition of PAH uptake measured in the presence of increasing α-ketoglutarate concentrations, the profile of PAH uptake was observed to increase and then decrease with the progressive increment in extravesicular α-ketoglutarate concentration. In contrast, the levels of urate uptake measured at increasing α-ketoglutarate concentrations were indistinguishable from urate uptake measured in the absence of α-ketoglutarate. The results of this experiment suggest our inability to detect urate/α-ketoglutarate exchange was not the result of assaying urate uptake at suboptimal dicarboxylate concentrations and provides further evidence for the absence of a α-ketoglutarate/urate exchange mechanism in rat basolateral membrane. The apparent absence of a basolateral membrane α-ketoglutarate/urate exchange mechanism in rat, a net reabsorber of urate contrast with the evidence to indicate its presence in the basolateral membrane of pig, a net secretor of urate [13]. However, to the extent that this anion exchange mechanism represents the pathway for active accumulation of proximal tubular urate from the blood one may expect its presence in the basolateral membrane of species demonstrating net secretion, such as pig and its absence in the basolateral membrane of species demonstrating net reabsorption, such as rat. This possibility is also consistent with our recent preliminary observations of an α-ketoglutarate-dependent stimulation of urate uptake by basolateral membrane vesicles prepared from kidneys of rabbit, a net secretor of urate.

We next examined the possible presence of a basolateral membrane Cl^-/urate exchange mechanism by attempting to confirm the observation of a Cl^- gradient-induced stimulation of urate uptake [10]. Compared to urate uptake measured in the presence of equal intra- and extravesicular Cl^- concentrations, urate uptake was significantly stimulated in the presence of an outwardly directed Cl^- gradient which suggested either a direct coupling of Cl^- efflux to urate influx consistent with the presence of a Cl^-/urate exchange mechanism or an indirect coupling of urate influx secondary to the formation of an inside-positive Cl^- gradient-induced diffusion potential. An attempt was made to identify the nature of flux coupling of urate and Cl^- by measuring Cl^- gradient-driven urate uptake in the presence of conditions expected to blunt the development of a possible Cl^- gradient-induced diffusion potential. When measured in the presence of valinomycin and equal intra- and extravesicular K^+, urate uptake in the presence of an outward Cl^- gradient was essentially indistinguishable from urate uptake measured in the absence of a Cl^- gradient. This obser-

vation suggests the Cl^- gradient-induced stimulation of urate uptake was not the result of a direct coupling of urate and Cl^- via an anion exchange mechanism but, rather, the result of an indirect electrostatic coupling of urate uptake via a conductive pathway to an inside-positive, Cl^- gradient-induced diffusion potential. To the extent that a conductive pathway for urate uptake by rat basolateral membrane vesicles exists then a valinomycin-induced, inside-positive K^+ diffusion potential should serve as a driving force for the active accumulation of urate in the absence of Cl^-. Accordingly, urate uptake was measured in the presence and absence of conditions expected to result in the generation of an inside-positive voltage difference. Compared to the level of urate uptake by valinomycin pretreated membranes in the presumed absence of a transmembrane voltage difference where $K_o^+ = K_i^+$, a marked stimulation of urate uptake by valinomycin pretreated membranes was measured in the presence of an inward K^+ gradient where the development of an inside-positive voltage difference would be expected. That the increased uptake of urate occurred as a result of mediated transport rather than uptake via some form of nonmediated 'leak' pathway is suggested by the ability of the anion transport inhibitor DIDS to reduce the level of conductive urate uptake. The results of these experiments suggest both an absence of a mechanism mediating Cl^-/urate exchange and the presence of a mechanism mediating conductive urate transport across the proximal tubular basolateral membrane of rat. Previously, the presence of a Cl^-/urate exchange mechanism in rat basolateral membrane was described based largely on the observed effect of an outwardly directed Cl^- gradient to stimulate basolateral membrane vesicle urate uptake [10]. However, the possible influence of ion-gradient induced diffusion potentials on urate uptake were not tested for and the best evidence for a direct chemical coupling of Cl^- and urate, Cl^- gradient-induced concentrative accumulation of urate, was not demonstrated. The conductive pathway for basolateral membrane urate transport was further investigated by assessing its sensitivity to inhibition by substrates and anion transport inhibitors known to interact with the urate transport mechanism present in rat and dog brush-border membrane [6,7,25]. In contrast to the evidence suggesting their interaction as substrates for anion-coupled urate exchange in the brush-border membrane, the presence of excess pyrazinoate, oxonate, nicotinate and PAH had no inhibitory effect on basolateral membrane conductive urate uptake which suggests these substrates do not interact with the basolateral membrane urate transporter in rat. At a concentration of 1 mM the organic anion transport inhibitor probenecid had only a small inhibitory effect (20%) on conductive urate uptake and the 4,4′-diisothiocyanostilbene derivative (DIDS) almost completely inhibited whereas, remarkably, the 4-acetamido-4′-isothiocyanostilbene derivative (SITS) was almost without effect on conductive urate uptake.

Finally, to determine whether the apparent conductive uptake of urate was the indirect result of an inside-positive voltage difference to induce the formation of an inside-alkaline pH gradient and drive urate accumulation via OH^- or HCO_3^-/urate exchange the possible presence of basolateral membrane $OH^-(HCO_3^-)$/urate exchanger was assessed. Compared to urate uptake measured in the absence of a pH gradient at pH 6 or pH 7.5 a small but significant stimulation of urate uptake was observed in the presence of an inside-alkaline pH gradient which was still further increased when an inside-alkaline pH gradient and an outwardly directed HCO_3^- gradient was simultaneously imposed. However, while the stimulation of urate uptake suggests the possible presence of a basolateral membrane $OH^-(HCO_3^-)$/urate exchange mechanism the magnitude of stimulation observed also suggests the anion exchange activity may have resulted from the small amount of brush-border membrane present in the basolateral membrane preparation. The presence of a brush-border member anion exchanger mediating $OH^-(HCO_3^-)$/urate exchange has been previously described for both dog and rat [4,7].

In an attempt to assess the membrane origin of the anion exchange activity pH and HCO_3^- gradient-driven urate uptake was measured in preparations of purified brush-border membranes reasoning that the anion exchange activity measured must be large enough to account for the activity measured in the basolateral membrane preparation. When assayed under identical conditions in brush-border membranes $OH^-(HCO_3^-)$/urate exchange activity was observed to be more than twice the activity measured in the basolateral membrane preparation which suggests the small amount of brush-border membrane contamination as the likely origin of $OH^-(HCO_3^-)$/urate exchange activity measured in the basolateral membrane preparation. These results suggest an $OH^-(HCO_3^-)$/urate exchange mechanism is unlikely to be a pathway for urate transport across rat basolateral membrane and furthermore, that urate uptake measured in the presence of an inside-positive voltage difference was not secondary to the formation of an inside-alkaline pH gradient.

In conclusion, the results of this investigation suggest the presence of a mediated conductive mechanism for urate transport across the basolateral membrane of rat proximal tubule cells. The results further suggest an absence of a mechanism(s) coupling basolateral membrane urate transport via anion exchange with α-ketoglutarate, Cl^- or $OH^-(HCO_3^-)$. The process of urate reabsorption by the rat proximal tubule is initiated by the active uptake of filtered urate at the brush-border membrane which results from the presence of an anion

exchange mechanism coupling urate influx to the favorable concentration gradient of various intracellular anions. The results of the present investigation describing the presence of a mediated conductive mechanism for urate transport at the basolateral membrane suggests intracellular urate, being well above electrochemical equilibrium, engages this pathway for movement out of the cell down its electrochemical potential gradient. Given the rat proximal tubule reabsorbs urate within the physiological range of blood urate concentrations and the paucity of evidence to suggest the secretion observed primarily at supraphysiological blood urate concentrations occurs transcellularly, the absence of a rat basolateral membrane anion exchange mechanism poised to take up urate from the blood is not surprising. Furthermore, because urate reabsorbers such as dog and rat appear to have similar transport mechanisms for the active accumulation of urate across the brush-border membrane one may also expect the basolateral membrane of dog and other urate reabsorbing species to be devoid of anion exchangers mediating urate uptake and to possess a mediated conductive pathway for passive urate efflux as described here for rat.

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