Paradoxical Effects of Pyrazinoate and Nicotinate on Urate Transport in Dog Renal Microvillus Membranes

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Abstract

The effects of pyrazinoate and nicotinate on urate transport in microvillus membrane vesicles isolated from canine renal cortex were evaluated. An outwardly directed gradient of pyrazinoate stimulated uphill urate accumulation, suggesting urate-pyrazinoate exchange. An inside-alkaline pH gradient stimulated uphill pyrazinoate accumulation, which suggested pyrazinoate- OH^- exchange. Pyrazinoate- OH^- exchange and urate- OH^- exchange were similarly sensitive to inhibitors, implying that both processes occur via the same transport system. In addition, an inward Na^+ gradient stimulated uphill pyrazinoate accumulation, suggesting Na^+ -pyrazinoate cotransport. Inhibitor studies demonstrated that Na^+ -pyrazinoate cotransport takes place via the same pathway that mediates Na^+ -lactate cotransport in these membrane vesicles. Previously we found that urate does not share this Na^+ -dependent cotransport pathway. Nicotinate inhibited transport of pyrazinoate by the anion exchange pathway and the Na^+ cotransport pathway, suggesting that it is a substrate for both transport systems. Finally, in the presence of an inward Na^+ gradient, low doses of pyrazinoate or nicotinate stimulated urate uptake, and higher doses of pyrazinoate or nicotinate inhibited urate accumulation, thereby mimicking in vitro the paradoxical effects of drugs on renal urate excretion that have been observed in vivo. These findings indicate that the paradoxical effect of uricosuric drugs at low doses to cause urate retention may result at least in part from stimulation of urate reabsorption across the luminal membrane of the proximal tubular cell.

Introduction

Pyrazinoate, a metabolite of the antituberculous drug pyrazinamide, suppresses the renal excretion of urate (1, 2). This effect has been attributed to inhibition of the secretory component of bidirectional urate transport in the proximal tubule and forms the basis for the pyrazinamide suppression test in man (3). When given in higher doses, pyrazinoate actually enhances urate excretion (2, 4). Several other substances, such as salicylate, also have the paradoxical property of inhibiting urate excretion when administered in low doses and of stimulating urate excretion when given in high doses (5).

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Previous work has identified a probenecid-sensitive anion exchanger that can account for uphill urate reabsorption across the luminal (microvillus, brush border) membrane of the proximal tubular cell of dog and rat (6-9). This transport system mediates the exchange of urate for any of several anions for which a cell-to-lumen electrochemical gradient is present across the luminal membrane. The known substrates for exchange with urate via this pathway are OH^- , Cl^- , *p*-aminohippurate (PAH),¹ several monocarboxylic acid anions (i.e., valerate, lactate, beta-hydroxybutyrate, pyruvate, and acetoacetate), the monovalent forms of certain dicarboxylic acid anions (i.e., maleate, succinate, alpha-ketoglutarate, and oxaloacetate), and the tricarboxylic acid anion *cis*-aconitate (6-8).

We now report that pyrazinoate and nicotinate, another substance that causes urate retention (10), are substrates for the dog renal microvillus membrane anion exchange pathway shared by urate. Moreover, pyrazinoate and nicotinate are also substrates for an organic anion- Na^+ cotransport pathway not shared by urate. We demonstrate an interaction between these two luminal membrane transport systems in vitro that can explain the paradoxical effects of pyrazinoate and other compounds on urate excretion in vivo.

Methods

Membrane preparation. Microvillus membrane vesicles were isolated from mongrel dog renal cortex by the Mg^{++} aggregation method previously described (6). In general, the membranes were prepared and suspended (25-30 mg membrane protein/ml) in a medium of 200 mM mannitol, 10 mM MgSO_4 , and 80 mM Hepes titrated to pH 7.5 with 41 mM KOH (8). Membrane vesicles were frozen and stored at -70°C until used (7, 8).

Solute uptake studies. Uptake of radiolabeled solutes was assayed at 20°C by the rapid filtration technique previously described (11), using a stopping and washing solution of 170 mM Na gluconate, 2 mM K probenecid, 2.5 mM Tris, and 4 mM Hepes, pH 7.5 (8). Each experiment was performed in triplicate on at least three separate occasions, each time using a different membrane preparation. For experiments with pH and voltage clamps (see below), it was necessary to maintain the intravesicular and extravesicular K^+ concentrations approximately equal. Thus, the combination of KH_2PO_4 and K_2HPO_4 that would give the desired pH with the desired total K^+ concentration was empirically determined for each experimental solution. Additional details of the experimental methods are given in the figure legends. Unless stated otherwise, the figures illustrate absolute uptake values from single representative experiments.

Voltage and pH clamps. In one of the experiments to be described, the effect on urate uptake of imposing an outward gradient of pyrazinoate was evaluated to test for direct coupling of pyrazinoate efflux to urate influx. This strategy necessarily required that the direct effect of the pyrazinoate gradient be distinguished from indirect effects of the pyrazinoate gradient to generate transmembrane diffusion potentials or pH gradients. In dog renal microvillus membrane vesicles, at least

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

a small component of urate uptake may be sensitive to alterations in the transmembrane electrical potential difference (7), and urate uptake is markedly affected by variations in the transmembrane pH gradient (6, 8). Accordingly, the effect of the pyrazinoate gradient was evaluated by using vesicles with equal internal and external K^+ concentrations in the presence of the K^+ ionophore valinomycin (3 mg/ml) to short-circuit anion diffusion potentials (voltage clamp) and in the presence of the K^+ - H^+ exchanger nigericin (150 μ g/ml) to collapse pH gradients (pH clamp). In other experiments, the effects of outward OH^- gradients on organic anion uptake were evaluated. These experiments were performed in the presence of the K^+ -valinomycin voltage clamp to short-circuit H^+ / OH^- diffusion potentials. The efficacy of the valinomycin voltage clamp and the nigericin pH clamp in these membranes has been demonstrated previously (8).

Materials. [2- ^{14}C]Urate (61 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, IL; [^{14}C]L-lactate (165 mCi/mmol) and [3H]-D-glucose (30 Ci/mmol) from New England Nuclear, Boston, MA; probenecid and valinomycin from Sigma Chemical Co., St. Louis, MO; and nigericin from Eli Lilly & Co., Indianapolis, IN. [^{14}C]Pyrazinoate (62 mCi/mmol) and unlabeled pyrazinoate were the generous gifts of Dr. I. M. Weiner, Department of Pharmacology, SUNY Upstate Medical Center, Syracuse, New York.

Results

Our initial method to test the hypothesis that pyrazinoate shares the urate exchange pathway was to determine whether pyrazinoate would exchange with urate. As shown in Fig. 1, the uptake of 7 μ M [^{14}C]urate was assayed in the absence or presence of an outward gradient (10 mM inside, 0.2 mM outside) of pyrazinoate. The experiment was conducted by using media of pH 6.5 in the presence of both a valinomycin voltage clamp and a nigericin pH clamp. The outward gradient of pyrazinoate accelerated the rate of urate uptake and caused the transient accumulation of urate (overshoot) above equilibrium. In two other similar experiments not illustrated, the 2-min accumulation of urate was 3.1- and 4.0-fold higher than the equilibrium level of urate uptake. These findings indicate that the outward movement of pyrazinoate must be directly

Figure 1. Stimulation of urate uptake by an outward pyrazinoate gradient. Membrane vesicles were preincubated for 120 min at 20°C in the presence of 150 μ g/ml nigericin, 3 mg/ml valinomycin, and either 10 mM pyrazinoate, 87 mM mannitol, 160 mM K^+ , 2.5 mM $MgSO_4$, 110 mM phosphate, 20 mM Hepes, pH 6.5 (●, ○), or 10 mM gluconate, 62 mM mannitol, 168 mM K^+ , 3 mM $MgSO_4$, 117 mM phosphate, 24 mM Hepes, pH 6.5 (■). Uptake of 7 μ M [^{14}C]urate was then measured in the presence of 120 mM mannitol, 165 mM K^+ , 0.06 mM $MgSO_4$, 118 mM phosphate, 0.5 mM Hepes, pH 6.5, and either 0.2 mM gluconate (■), 0.2 mM pyrazinoate (●), or 0.2 mM pyrazinoate and 2 mM K-probenecid (○).

coupled to urate entry. The rate of urate uptake in the presence of the outward pyrazinoate gradient was inhibited >90% by 2 mM probenecid, which is consistent with the concept that urate-pyrazinoate exchange occurs via the previously described, probenecid-sensitive urate exchange system.

If urate-pyrazinoate exchange does occur via the same transport pathway that mediates urate- OH^- exchange, then pyrazinoate- OH^- exchange should also take place. To test this prediction, we measured the uptake of 29 μ M [^{14}C]pyrazinoate in the presence (pH_o 6.5; pH_i 7.5) or absence (pH_o 7.5; pH_i 7.5) of an outward OH^- gradient (Fig. 2). This experiment was conducted in the presence of a valinomycin voltage clamp. As indicated in Fig. 2, imposing an outward OH^- gradient stimulated the rate of pyrazinoate uptake and induced a transient overshoot above equilibrium, consistent with transport of pyrazinoate via exchange for OH^- . Although not illustrated, the rate of pyrazinoate uptake in the presence of an outward OH^- gradient was inhibited >90% by 2 mM probenecid, as expected if pyrazinoate- OH^- exchange occurs via the urate exchange pathway.

If pyrazinoate- OH^- exchange is mediated by the same transport system that catalyzes urate- OH^- exchange, then OH^- gradient-stimulated pyrazinoate influx should be sensitive to inhibition by the same agents that inhibit OH^- gradient-stimulated urate influx. This prediction was tested in the experiments illustrated in Fig. 3. The rate of uptake of 24 μ M [^{14}C]pyrazinoate (Fig. 3 A) or 60 μ M [^{14}C]urate (Fig. 3 B) was determined in the presence of an outward OH^- gradient and varying concentrations of unlabeled pyrazinoate, urate, or nicotinate. For each of the three agents, the dose-response curve for inhibition of [^{14}C]pyrazinoate uptake was similar to that for inhibition of [^{14}C]urate uptake. For example, the concentration of urate causing 50% inhibition was just above 0.3 mM for both the transport of [^{14}C]pyrazinoate and the transport of [^{14}C]urate. The concentration of nicotinate causing 50% inhibition was 0.1 mM in both cases. Pyrazinoate was a slightly less potent inhibitor than nicotinate. These data provide further support for the concept that urate and pyrazinoate share the same transport system.

Figure 2. Stimulation of pyrazinoate uptake by an inside-alkaline pH gradient. Membrane vesicles were preincubated for 120 min in the presence of 3 mg/ml valinomycin, 100 mM mannitol, 165 mM K^+ , 5 mM $MgSO_4$, 78 mM phosphate, 40 mM Hepes, pH 7.5. Uptake of 29 μ M [^{14}C]pyrazinoate was then measured in the presence of either 89 mM mannitol, 165 mM K^+ , 1 mM $MgSO_4$, 126 mM phosphate, 8 mM Hepes, pH 6.5 (●), or 159 mM mannitol, 165 K^+ , 1 mM $MgSO_4$, 88 mM phosphate, 8 mM Hepes, pH 7.5 (○).

Figure 3. Effect of inhibitors on pH gradient-stimulated uptake of [^{14}C]pyrazinoate and [^{14}C]urate. The 2-s uptake of $24\ \mu\text{M}$ [^{14}C]pyrazinoate (A) and the 3-s uptake of $60\ \mu\text{M}$ [^{14}C]urate (B) were assayed at external pH 6.5 using vesicles preincubated at pH 7.5 according to the protocol employed in Fig. 2. Inhibitors were added as K salts. Each datum represents the mean $\pm$ SE for three separate experiments performed on different membrane preparations.

In rabbit renal microvillus membrane vesicles, Na^+ -nicotinate cotransport takes place and is inhibited by pyrazinoate but not by PAH (12). We therefore tested for the presence of Na^+ -pyrazinoate cotransport in dog microvillus membranes, as shown in Fig. 4. Imposing an inward Na^+ gradient accelerated the rate of uptake of $34\ \mu\text{M}$ [^{14}C]pyrazinoate, and caused a transient overshoot above equilibrium that was consistent with Na^+ -pyrazinoate cotransport.

Na^+ -lactate cotransport has been documented in microvillus membrane vesicles from rat (13), dog (8), and rabbit (14), and occurs via a pathway shared by many other monocarboxylic acid anions (14). This Na^+ -cotransport system is not shared by urate, inasmuch as Na^+ -lactate cotransport is not inhibited by urate (8), and the initial rate of urate uptake is not stimulated by Na^+ (7). If Na^+ -pyrazinoate cotransport takes place via the same pathway that mediates Na^+ -lactate cotransport, then Na^+ -stimulated pyrazinoate uptake should be sensitive to inhibition by the same substances that inhibit Na^+ -stimulated lactate uptake. This prediction was tested in the experiments illustrated in Fig. 5. The rate of influx of $32\ \mu\text{M}$

Figure 4. Stimulation of pyrazinoate uptake by an inward Na^+ gradient. Membrane vesicles were prepared and suspended in 200 mM mannitol, 41 mM K^+ , 10 mM MgSO_4 , 80 mM Hepes, pH 7.5. Uptake of $34\ \mu\text{M}$ [^{14}C]pyrazinoate was then measured in the presence of 40 mM mannitol, 8 mM K^+ , 2 mM MgSO_4 , 16 mM Hepes, pH 7.5, and either 140 mM Na-gluconate (●) or 140 mM K-gluconate (○).

Figure 5. Effect of inhibitors on Na^+ gradient-stimulated uptake of [^{14}C]pyrazinoate and [^{14}C]lactate. The 2-s uptake of $32\ \mu\text{M}$ [^{14}C]pyrazinoate (A) and the 5-s uptake of $15\ \mu\text{M}$ [^{14}C]lactate (B) were assayed in the presence of 140 mM Na^+ according to the protocol employed in Fig. 4. Inhibitors were added as Na salts. Each datum represents the mean $\pm$ SE for three separate experiments performed on different membrane preparations.

[^{14}C]pyrazinoate (Fig. 5 A) or $15\ \mu\text{M}$ [^{14}C]lactate (Fig. 5 B) was measured in the presence of an inward Na^+ gradient and varying concentrations of unlabeled pyrazinoate, lactate, or nicotinate. For each of the three agents, the dose-response curve for inhibition of [^{14}C]pyrazinoate uptake was similar to that for inhibition of [^{14}C]lactate uptake. For example, the concentration of nicotinate causing 50% inhibition was slightly under 1.0 mM for both the transport of [^{14}C]pyrazinoate and the transport of [^{14}C]lactate. The concentration of lactate or pyrazinoate causing 50% inhibition was $\sim 2.5\ \text{mM}$ in both cases. At concentrations of 2.5 mM and below, none of the three substances inhibited the Na^+ -stimulated uptake of $0.3\ \mu\text{M}$ [^3H]glucose measured at 2 s under identical conditions (not shown), which argues against significant alterations in the electrochemical Na^+ gradient. Even at a concentration of 5 mM, nicotinate, pyrazinoate, and lactate inhibited glucose uptake by only 1, 15, and 17%, respectively. Thus, these data suggest that pyrazinoate and lactate share the same Na^+ cotransport system in dog renal microvillus membranes.

We then attempted to demonstrate paradoxical effects of pyrazinoate and nicotinate on urate uptake in our membrane vesicle preparation. In Fig. 1, we showed that an outward pyrazinoate gradient could stimulate urate accumulation. In Fig. 4, we showed that an inward Na^+ gradient could induce the uphill accumulation of pyrazinoate. That is, an inward Na^+ gradient at least transiently could generate an outward pyrazinoate gradient. It therefore seemed possible that adding pyrazinoate to the external medium in the presence of an inward Na^+ gradient might stimulate urate accumulation. On the other hand, adding higher concentrations of pyrazinoate might reduce urate uptake as *cis* inhibition at the external face of the membrane became the predominant effect, as observed in Fig. 3. In the experiment illustrated in Fig. 6, the uptake of $48\ \mu\text{M}$ [^{14}C]urate was assayed in the presence of an inward Na^+ gradient with 0, 0.1, or 5 mM pyrazinoate added to the external medium at the initiation of the uptake measurement. The lower dose of pyrazinoate stimulated the accumulation of urate and induced a transient overshoot. In contrast, the higher dose of pyrazinoate inhibited the rate of urate uptake.

Similar effects were noted with nicotinate (Fig. 7). Adding 0.04 mM nicotinate to the external medium in the presence

Figure 6. Paradoxical effects of pyrazinoate on urate uptake. Membrane vesicles were preincubated for 120 min in the presence of 105 mM mannitol, 126 mM K^+ , 5 mM $MgSO_4$, 86 mM phosphate, 42 mM Hepes, pH 6.5. Uptake of 48 μM [^{14}C]urate was then measured in the presence of 57 mM mannitol, 100 mM Na^+ , 53 mM K^+ , 1 mM $MgSO_4$, 39 mM phosphate, 84 mM Hepes, pH 6.5, and 0 ($\blacksquare$), 0.1 ($\blacktriangle$), or 5 mM ($\bullet$) pyrazinoate.

of an inward Na^+ gradient stimulated the accumulation of 65 μM [^{14}C]urate. However, adding 0.8 mM nicotinate to the medium inhibited urate uptake. Neither 0.1 mM pyrazinoate nor 0.04 mM nicotinate affected urate uptake measured in the absence of an inward Na^+ gradient (not illustrated). Thus, paradoxical effects of drugs on urate absorption across the luminal membrane of dog proximal tubular cells were demonstrable in vitro.

Discussion

Our studies suggest that the paradoxical effects exerted on renal urate excretion by such drugs as pyrazinoate may arise from stimulation and inhibition of urate absorption across the luminal membrane of the proximal tubular cell, as schematically illustrated in Fig. 8. In the absence of pyrazinoate, urate absorption across the luminal membrane occurs in exchange for intracellular OH^- (6) and several organic anions (8) for which a cell-to-lumen electrochemical gradient normally exists (process 1). If the concentrations of OH^- and exchangeable organic anions within the cell are not saturating for the urate exchange process, then urate absorption across the luminal membrane will be stimulated when the concentration of exchangeable anions in the cell rises. Pyrazinoate, which can be accumulated within the cell by Na^+ -cotransport across the luminal membrane (process 2), can serve as an exchange partner for urate and thereby stimulate urate absorption (process 3). However, the higher the intratubular concentration of pyrazinoate, the more it will displace urate from the external

Figure 7. Paradoxical effects of nicotinate on urate uptake. Uptake of 65 μM [^{14}C]urate was assayed according to the protocol employed in Fig. 6 except that 0 ($\blacksquare$), 0.04 ($\blacktriangle$), or 0.8 mM ($\bullet$) nicotinate rather than pyrazinoate was added to the experimental medium.

Figure 8. Schematic model to explain the paradoxical effects of pyrazinoate on urate transport across the luminal membrane of the proximal tubular cell. See text for details.

transport site of the anion exchanger. Ultimately, this *cis* inhibitory effect of pyrazinoate will predominate, and the rate of urate absorption will fall below the base-line rate measured in the absence of pyrazinoate.

Certainly, our studies do not rule out the possibility that drugs such as pyrazinoate may reduce urate excretion by inhibiting urate secretion across the basolateral membrane of the proximal tubular cell. However, the feasibility of the scheme we have proposed is underscored by the fact that the same pyrazinoate concentrations found to stimulate or inhibit absorption of urate across the luminal membrane in Fig. 6 actually cause urate retention or uricosuria, respectively, in the intact dog (4). In addition, species differences in the effects of pyrazinoate support the concept that the drug reduces urate excretion by stimulating urate absorption across the luminal membrane via anion exchange rather than by inhibiting urate secretion across the basolateral membrane. For example, pyrazinoate reduces urate excretion in the dog (2, 4) and rat (15), two species in which there is active, mediated urate reabsorption in the proximal tubule (16, 17) and in which urate transport via anion exchange has been demonstrated in renal microvillus membrane vesicles (6–9). In contrast, pyrazinoate does not reduce urate excretion in the rabbit (18). Rabbit proximal tubules secrete urate actively but have no mediated urate reabsorption (19, 20). Urate transport via anion exchange can not be demonstrated in rabbit renal microvillus membrane vesicles (8). In fact, pyrazinoate has not been found to reduce urate excretion in any animal—such as the pig (21), chicken (22), snake (23), or guinea pig (4)—in which the tubular transport of urate is in the direction of net secretion. A possible explanation for the inability of pyrazinoate to reduce urate excretion in animals that are net secretors of urate is that the luminal membranes of their proximal tubular cells may contain few or none of the anion exchangers whose principal function is to mediate active urate reabsorption.

In summary, we have demonstrated paradoxical effects of pyrazinoate and nicotinate on urate absorption into luminal membrane vesicles isolated from dog proximal tubular cells. These findings suggest that the paradoxical effect of uricosuric drugs at low doses to cause urate retention in vivo may result at least in part from stimulation of urate reabsorption and may not result simply from inhibition of urate secretion as commonly believed.

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