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Pyrazinoic Acid and Urate Transport in the Rat (40274)

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The decrease in urinary excretion of urate following the administration of pyrazinamide or its active metabolite, pyrazinoic acid (PZA), has been extensively utilized as a pharmacologic aid in dissecting out the contribution of secreted urate to the urinary excretion of uric acid (1, 2). As originally proposed, the use of the "Pyrazinamide Suppression Test" was based upon the assumptions that this compound was a specific and perhaps complete inhibitor of urate secretion and was without effect on the urate reabsorptive processes (3, 4). Indirect evidence has been presented, however, that neither of these assumptions is totally valid (5–8). Published studies on the separate effects of PZA on urate reabsorption and secretion, however, have been limited and somewhat conflicting (8–11). The current studies were designed to examine the effect of PZA, in varying dosages, on net urate transport and on the urate reabsorptive and secretory mechanisms in the rat.

Methods. Male Sprague-Dawley rats with free access to food and water until the time of study were used in all experiments. Anesthesia was induced with Inactin (Promonta, Hamburg, Germany), 0.5–0.6 m*M*/kg body wt injected intraperitoneally. After a tracheostomy, the right and left jugular veins were cannulated and the urinary bladder catheterized. In the clearance experiments, the left femoral artery was cannulated for collection of blood samples. In the microinjection and precession studies, the left kidney was prepared for micropuncture as previously described (12, 13). The ureter of the left kidney was catheterized with PE-50 tubing to permit separate urine collections from each kidney. Only animals in which the urine flow rate of the left kidney was at least 85% of that from the contralateral kidney were included for study. In all animals, surgical losses of fluid were replaced with a volume of isotonic saline equal to 1% of body wt. Body temperature was maintained at 37°. Pyrazinoic acid was dissolved in a solution of sodium hydroxide (0.1 *M*); the pH was then adjusted to 7.4 with either hydrochloric acid or sodium bicarbonate. In all control periods, the diluent alone was infused to control for the effect, if any, of diluent infusion.

Clearance studies. Clearance studies were performed in diuretic rats receiving 5% mannitol in isotonic saline at a rate of 12.0 ml/hr so as to reproduce the protocol of the microinjection studies which require high urine flow rates. A priming dose of 50 μCi of [*methoxy*-^{3}H] inulin in one ml of isotonic saline was infused followed by a sustaining infusion of isotonic saline containing 25 μCi/ml of [*methoxy*-^{3}H] inulin at a rate of 1.2 ml/hr. After a 90-min equilibration period, two 20-min urine collections were obtained. 1.5 ml of arterial blood was obtained at the midpoint of each clearance period and was replaced with the same volume of blood from a donor rat.

After collection of samples in the control periods, pyrazinoic acid in a dose of either 0.40, 0.80, or 1.6 m*M*/kg body wt (50, 100, or 200 mg/kg body wt respectively) was infused intravenously as a bolus followed by the same dose infused per hour. After a 90-min equilibration period, two or three additional clearance periods were obtained. In order to control for possible changes in renal function over the time course of these experiments, five rats were studied under the same protocol but received no infusion of drug. At the conclusion of all experiments, the kidneys were removed, stripped of perirenal fat and capsule and weighed in a Mettler analytic balance (Mettler Instrument Corp., Princeton, NJ).

Microinjection studies. Microinjection studies were performed in animals receiving 5% mannitol in isotonic saline at a rate of 12.0 ml/hr. Inulin was not infused systemically. After preparation for study, separate groups of animals received either diluent infusion or a bolus infusion of pyrazinoic acid of 0.40,

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0.80, or 1.6 m*M*/kg body wt followed by the same dose per hour. An equilibration period of 90 min was permitted to elapse before starting microinjections. Intratubular microinjections were performed with a solution containing [2-^{14}C]urate (50 μCi/ml) and [*methoxy*-^{3}H]inulin (100 μCi/ml) adjusted to a pH of 7.4 with a solution of $NaHCO_3$ (0.357 m*M*/liter). The concentration of uric acid in the final solution was 0.24 m*M*/liter. Triplicate droplets of 12–20 nl were prepared, one of which was utilized for the microinjection and the other two counted directly for total radioactivity. Microinjections were performed into early or late proximal tubular sites over a 60–90 sec interval and total urine collections obtained sequentially from both right and left kidneys. The procedures for microinjection, localization of microinjection sites and the calculations of the recovery rates were identical to those of Kramp, Lassiter and Gottschalk (8) and have been described in detail from this laboratory previously (12, 14).

Droplet studies. Animals were prepared as in the microinjection studies except that 5% mannitol in isotonic saline was infused at rates sufficient to increase the urine flow rate to 100–150 μl/min per kidney. 100 nanoliters of the [2-^{14}C]urate and [*methoxy*-^{3}H]inulin solution were placed upon the surface of the left kidney as a droplet and urine collected sequentially in 15–30 sec aliquots from both right and left kidneys. A sample of the droplet solution was counted directly with each experiment to determine the ratio of ^{14}C counts to ^{3}H counts. Droplet studies were obtained in time control animals and in animals infused with PZA in doses of 0.40, 0.80, or 1.6 m*M*/kg body wt/hr as previously indicated. No attempt was made to quantitate total recoveries.

Analytical methods. Radioactivity of blood, urine, and microinjection and droplet samples was determined in Biofluor (New England Nuclear Corp., Boston, MA) in a Packard Tri-Carb liquid scintillation counter (Packard Instruments Co., Downers Grove, IL) with appropriate corrections for ^{14}C counts appearing in the ^{3}H channel. Counts per min were converted to disintegrations per min, after correction for quench, crossover, and efficiency of counting each isotope. The urate concentrations of the serum and urine were determined by a uricase method using the polarographic sensor in a glucose analyzer (Beckman Instruments, Fullerton, CA) as previously described (12). The clearances of inulin (C_{inulin}) and urate (C_{urate}) are expressed as μl/min/g kidney wt and are calculated from standard formulae.

All data are expressed as the mean ± SE of the mean. *P* values were calculated by the Fisher *t* test or the Student *t* test where appropriate.

Results. Clearance studies (Table I). Following the infusion of PZA in a dose of 0.40 m*M*/kg body wt/hr, there was no change in the glomerular filtration rate, plasma urate concentration or in the clearance of urate. The fractional excretion of urate, therefore, was unchanged and averaged 21.0 ± 1.3 and 24.0 ± 2.3% (P = NS) in control and experimental periods respectively. By contrast, the infusion of PZA in a dose of 0.80 m*M*/kg body wt/hr resulted in significant decreases in urate clearance from 276.0 ± 25.1 to 210.7 ± 20.6 μl/min/g kidney wt ($P < 0.005$) and in the fractional excretion of urate from 24.4 ± 2.6 to 19.4 ± 2.4% ($P < 0.01$). The plasma concentration of urate increased from 58.3 ± 4.2 to 86.8 ± 5.4 μ*M*/liter ($P < 0.001$). The infusion of PZA in a dose of 1.6 m*M*/kg body wt/hr resulted in no change in plasma urate concentration, the glomerular filtration rate, or the clearance of urate.

In order to control for the time course of these experiments, animals studied in identical fashion but not receiving an infusion of PZA, had no significant change in the glomerular filtration rate, the plasma urate concentration, or the clearance of urate.

Microinjection studies (Fig. 1). To assess the effects of varying dosages of PZA on the urate reabsorption process and to localize the nephron site of altered reabsorption, intratubular microinjections were performed into early or late portions of the proximal tubule. Only samples in which inulin recoveries were 95% or greater were included for analysis. Delayed recoveries ranged from 0 to 6% with no significant differences between the groups of animals. Accordingly, the results are expressed as total urate recoveries and are summarized on Fig. 1. Recoveries from early proximal tubule sites averaged 73 ± 2% in

TABLE I. THE EFFECTS OF PZA ON THE CLEARANCE OF URIC ACID.[a]

Dose of PZA infused	C_{inulin} μl/min/g kw		Serum Uric Acid μM/liter		C_{urate} μl/min/g kw		FE_{urate} (%)	
	C	E	C	E	C	E	C	E
No PZA (n = 5)	1016 ± 89.3	953 ± 56.4	67.8 ± 6.5	70.2 ± 5.9	203.0 ± 25.0	217.2 ± 22.7	19.2 ± 1.7	23.1 ± 2.8
P	NS		NS		NS		NS	
0.40 mM/kg/hr (n = 8)	1069 ± 54.7	1007 ± 65.2	70.8 ± 3.0	67.2 ± 3.0	221.6 ± 12.2	237.5 ± 24.7	21.0 ± 1.3	24.0 ± 2.3
P	NS		NS		NS		NS	
0.80 mM/kg/hr (n = 6)	1169 ± 75.8	1175 ± 92.8	58.3 ± 4.2	86.8 ± 5.4	276.0 ± 25.1	210.7 ± 20.6	24.4 ± 2.6	19.4 ± 2.4
P	NS		<0.001		<0.005		<0.01	
1.60 mM/kg/hr (n = 9)	967 ± 36.4	1054 ± 62.9	61.9 ± 4.8	68.4 ± 5.9	230.6 ± 25.7	260.6 ± 27.4	24.6 ± 3.5	25.5 ± 3.0
P	NS		NS		NS		NS	

[a] Values expressed as mean ± SEM. FE_{urate} = fractional excretion of uric acid; C = control periods; E = experimental periods; NS = not significant; (n) = number of animals studied.

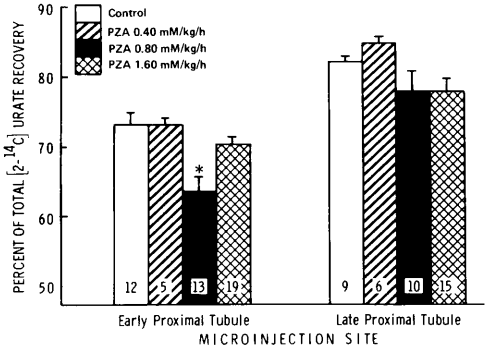

FIG. 1. Per cent of total [2-^{14}C]-urate recovered following microinjections in early and late proximal tubule sites. *$P < 0.01$.

controls. Following infusion of PZA in doses of 0.40, 0.80, or 1.6 mM/kg body wt/hr, recoveries from early proximal tubule sites were 73 ± 1, 64 ± 2, and 71 ± 1% respectively. The urate recoveries after infusion of 0.80 mM/kg body wt/hr PZA (64 ± 2%) were significantly lower than those obtained in controls and in animals infused with PZA in doses of either 0.40 or 1.6 mM/kg body wt/hr. There were no differences in urate recoveries following microinjections in late proximal tubule sites between any of the groups of animals.

Droplet studies (Table II). Urate secretion was considered to be present when the ratio of [2-^{14}C]urate to [*methoxy*-^{3}H]inulin in the first urine sample to contain inulin divided by the ratio of $^{14}C/^{3}H$ in the droplet solution was greater than one. In control animals not receiving PZA, the $^{14}C/^{3}H$ urine-to-droplet ratio of counts averaged 1.79 ± 0.10 in the experimental left kidney and 0.79 ± 0.07 in the contralateral kidney. The infusion of PZA in a dose of 0.40 mM/kg body wt/hr resulted in an 11% decrease in the ratio of counts in the left kidney ($P < 0.05$) and no significant change in the right kidney. Compared to controls, PZA in a dose of 0.80 mM/kg body wt/hr resulted in a significant decrease in the ratio of counts from 1.79 ± 0.04 to 1.19 ± 0.12 ($P < 0.05$) and 0.79 ± 0.04 to 0.57 ± 0.07 ($P < 0.05$) in the left and right kidneys respectively. The largest dose of PZA tested (1.6 mM/kg body wt/hr) resulted in a 38% decrease in the ratio of counts in the experimental left kidney ($P < 0.05$) but no significant change in the right kidney.

Discussion. The presence of active mechanisms for the bidirectional transport of urate by renal tubular cells has made it difficult to assess the individual contribution of urate reabsorption or secretion to the urinary excretion of urate by classical clearance techniques. Pyrazinamide or its active metabolite, pyrazinoic acid (PZA), has been extensively utilized in man and in the intact animal as a pharmacologic aid in assessing the magnitude of each of these transport processes (1–3). The use of PZA in such studies was based upon the observation that, following its administration, the urinary excretion of urate was markedly reduced, an effect ascribed to an inhibition of urate secretion (1–3). More recently, doubt has been cast upon the results of studies utilizing the PZA-induced decrease of urate excretion as an index of urate secretion (4–6).

Prior studies from this and other laboratories have attempted to estimate urate reabsorption and urate secretion utilizing intratubular microinjection and droplet precession techniques, respectively. The rationale behind these techniques has been previously discussed (8, 9, 12–15). PZA in a dose of 0.40 mM/kg body wt/hr did not affect the fractional excretion of urate or the rate of urate

TABLE II. PRECESSION DROPLET STUDIES.[a]

	Left kidney				Right kidney			
	C	E	% Change	P	C	E	% Change	P
No drug ($n = 4$)	1.79 ± 0.10	1.79 ± 0.02	0	N.S.	0.79 ± 0.07	0.79 ± 0.06	0	N.S.
0.40 m*M*/liter ($n = 4$)	1.97 ± 0.07	1.76 ± 0.06	−11%	<0.05	0.79 ± 0.05	0.76 ± 0.04	−4%	N.S.
0.80 m*M*/liter ($n = 4$)	1.79 ± 0.14	1.19 ± 0.12	−34%	<0.05	0.79 ± 0.04	0.57 ± 0.07	−28%	<0.05
1.60 m*M*/liter ($n = 4$)	1.86 ± 0.12	1.16 ± 0.09	−38%	<0.05	0.69 ± 0.02	0.67 ± 0.03	−3%	N.S.

[a] C = control; E = experimental. Values (mean ± SEM) represent the $^{14}C/^{3}H$ urine/droplet ratios of counts in the first urine sample to contain inulin.

recoveries following intratubular microinjections. It did, however, have a small but measurable effect on urate secretion as assessed by the droplet studies. This apparent discrepancy may indicate that either the degree of inhibition of secretion was not physiologically significant, or that it could not be detected by the clearance or microinjection techniques.

By contrast to the 0.40 m*M* dose, PZA in a dose of 0.80 m*M*/kg body wt/hr resulted in a decrease in the fractional excretion of urate. The decrease in urate excretion could be the result of either inhibition of urate secretion, enhancement of urate reabsorption, or a combination of the two. The results of the precession studies confirm that PZA inhibits urate secretion, the degree of inhibition being greater with the 0.80 m*M* dose than with the 0.40 m*M* dose. The intratubular microinjection studies indicate that urate absorption is enhanced. The mechanism by which PZA may enhance urate absorption is unknown, but several possibilities might be considered. On one hand, the decrease in urate recoveries could represent a direct pharmacologic enhancement of urate reabsorption from the proximal tubule. This suggestion has previously been proposed from clearance experiments (6, 7). On the other hand, the decreased fractional recovery of urate following intratubular microinjection in animals receiving PZA in a dose of 0.80 m*M* may be due to an inhibition of peritubular uptake of urate and/or urate secretion alone. It is possible that inhibition of urate uptake at the antiluminal border of the renal tubular cells reduces the cell concentration of urate, thereby creating a more favorable lumen-to-cell gradient for urate. Moreover, inhibition of secretion of urate into the tubular lumen would increase the specific activity of the microinjected [2-^{14}C]urate. Prior studies from this laboratory have indicated that reducing the specific activity of isotopically labeled urate in the tubular lumen does not affect the fractional rate of [2-^{14}C]urate absorption (13). The effect of increasing the specific activity, however, has not been examined directly and, thus, the expected changes in specific activity of [2-^{14}C]urate microinjected into the tubular lumen can not be excluded as a possible mechanism, at the present time. The current studies do not permit us to differentiate between a direct pharmacologic effect of PZA on the urate absorptive mechanisms and an effect of PZA solely on the secretory process with a secondary change in the absorptive process, but the results of studies using PZA in a dose of 1.6 m*M*/kg body wt/hr suggest that the latter is the more likely explanation, namely that PZA in a dose of 0.80 m*M* enhances urate absorption, primarily by inhibition of the secretory process. With the largest dose of PZA tested, fractional urate excretion and fractional urate recoveries following microinjections were similar to control values. This dose of PZA also significantly inhibited urate secretion. It seems likely that PZA, 1.6 m*M*/kg body wt/hr, not only inhibits secretion, but also inhibits reabsorption and, at this dose, secretion and reabsorption were inhibited to an equal extent. When viewed from this perspective, PZA appears to inhibit both urate secretion and urate reabsorption, and the inhibition of these processes is dose-dependent, but not necessarily of equal sensitivity. It was unfortunate that, due to an unacceptably high death rate of the animals, higher doses of PZA could not be examined.

Three previously published studies on the effect of pyrazinamide or PZA on the renal handling of urate in the rat bear directly on the results in the present study. A significant decrease in urate reabsorption has been reported by Kramp *et al.* when single bolus doses of PZA of either 10, 50, or 100 mg/kg body wt/hr were infused (8). The differences between their results and those of the current study can not be readily reconciled. In a series

of clearance studies, Boudry observed a small antiuricosuric effect of PZA, an effect which became more pronounced when the plasma urate concentration was increased (16). In a more recent study by Abramson and Levitt, there was an increase in net reabsorption by the end of the proximal tubule following PZA administration, a result ascribed to inhibition of secretion (11). Also observed in that study was a significant reabsorptive flux of urate in the loop of Henle following PZA infusion. In the current study, recoveries from late proximal tubule sites were lower than controls following PZA administration, but the changes were not statistically significant. Thus, we can not confirm or deny, at this time, an effect of PZA in nephron sites beyond the proximal convoluted tubule.

The use of pyrazinoic acid depression of urate excretion as an index of urate secretion has been based upon the assumptions that PZA inhibits urate secretion and is without effect on urate reabsorption. The results of the present studies confirm that PZA inhibits urate secretion, and thereby may secondarily enhance urate absorption. In high doses, however, PZA has the additional effect of inhibiting urate reabsorption. To the degree that PZA may affect both urate secretion and reabsorption, any conclusions derived from the use of PZA as to the magnitude of the contribution of secreted urate to the urinary excretion of urate can not be considered quantitative.

Summary. These results indicate that urate secretion is inhibited by PZA and that the degree of inhibition is dose dependent. In the highest dose tested (1.6 m*M*/kg body wt/hr), PZA not only inhibits secretion but also inhibits urate absorption. Thus, PZA appears to inhibit both urate secretion and reabsorption. The inhibition of these processes is dose dependent but not necessarily of equal sensitivity.

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