Pyrazinoate Excretion in the Chimpanzee

RELATION TO URATE DISPOSITION AND THE ACTIONS OF URICOSURIC DRUGS

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ABSTRACT These experiments were designed to define the renal disposition of pyrazinoic acid in a nonhuman primate that is phylogenetically close to man and to relate this to the effects of pyrazinoate on urate excretion. The renal clearance of pyrazinoate was almost always greater than the simultaneous glomerular filtration rate at plasma concentrations ranging from 1.9 to 960 μg/ml. Some inhibitors of tubular secretion, probenecid, MK-282 (an experimental, potent uricosuric drug), *p*-aminohippurate, iodopyracet, sulfinpyrazone, and mersalyl, reduced clearances of pyrazinoate to values far below filtration rate. Chlorothiazide, allopurinol, and salicylate did not. The clearance of pyrazinoate was not influenced by changes in urine flow. It is concluded that pyrazinoate is actively secreted and actively reabsorbed.

Pyrazinoate had a dual effect on urate excretion. At concentrations in plasma less than 10 μg/ml there was a concentration related fall in urate/inulin clearance ratio, reaching values of 10–20% of control. Over the range of 10-100 μg/ml in plasma, the clearance of urate remained maximally depressed. At higher concentrations of pyrazinoate there was a concentration related increase in urate/inulin clearance ratio such that at pyrazinoate levels above 600 μg/ml a definite uricosuric response was obtained. Prior administration of pyrazinoate to give plasma levels of 20–140 μg/ml completely or almost completely prevented uricosuric respones to probenecid, PAH, chlorothiazide, and sulfinpyrazone. Iodopyracet, mersalyl, salicylate and *N*-acetyl-4-dibutylsulfamoyl-3-trifluoromethylbenzenesulfonamide (MK-282) retained significant uricosuric action, but the activities were probably less than normal. The results are consistent with a model of urate transport involving high rates of bidirectional transtubular flux.

A part of this study was presented at the Fourth Kanematsu Conference on the Kidney, 29 November 1972 at Sydney Hospital, Sydney, Australia.

Received for publication 10 January 1973 and in revised form 26 March 1973.

INTRODUCTION

Pyrazinamide, an antituberculous drug, causes a substantial fall in the renal clearance of urate (1) and eventually hyperuricemia (2). It is thought that the decrease in urate clearance results from selective inhibition of tubular secretion (1, 3). In addition, it has been inferred that the difference in urate excretion before and after pyrazinamide ingestion is a minimal estimate of unidirectional urate secretion (3). On this basis the "pyrazinamide suppression test" has been used in research on the renal defect of gout (4, 5), the mechanisms of uricosuric drugs (6–8), and other phenomena (9–13).

Studies in lower animals (14) and man (1) indicate that pyrazinamide itself may not be the agent active in suppressing urate clearance, but that a metabolite, pyrazinoic acid, is responsible for urate retention. In several experiments with a human subject pyrazinoate appeared in body fluids with sufficient rapidity to account temporally for the effects on urate (14). In this and another study (15) the concentrations of pyrazinoic acid in plasma did not exceed 10 μg/ml suggesting that it is extremely potent in suppressing urate excretion.

Other observations in animals indicate that pyrazinoic acid is both secreted and reabsorbed (14), as is uric acid (16, 17). Moreover, there is evidence suggesting that pyrazinoic acid has a dual effect on the renal tubule, i.e. at low concentrations in plasma the compound may cause urate retention while at higher concentrations it may be uricosuric (14).

Since the foregoing results are potentially relevant for interpretation of the "pyrazinamide suppression test," it

seemed desirable to obtain information on the renal disposition of pyrazinoic acid and on the renal response to various concentrations of this compound in man. Three circumstances make the acquisition of such information difficult: (*a*) pyrazinoic acid is not an official drug and preparations for human use are not available; (*b*) high doses of pyrazinoic acid produce considerable discomfort in humans (1, 14), and (*c*) the in vivo rate of formation of pyrazinoic acid from pyrazinamide is slow (14, 15) and thus only a narrow range of pyrazinoate concentrations can be studied when the amide is given.

In view of recent evidence that the pattern of urate excretion in the chimpanzee is very similar to that in man (18–20), we elected to undertake a collaborative study in the chimpanzee. The results demonstrate that pyrazinoic acid is both secreted and reabsorbed, that it is extremely potent in suppressing urate excretion and that at high concentrations it produces a uricosuric effect. In addition, observations have been made on the interaction of pyrazinoate and other drugs influencing urate excretion.

METHODS

A total of 30 clearance experiments were performed under phencyclidine HCl restraint in seventeen male chimpanzees (*Pan troglodytes verus*) weighing between 30 and 62 kg. Details of experimental technique have been described previously (18). The experimental protocols will be apparent from the results. In all instances, pyrogen-free inulin was used to measure glomerular filtration rate (GFR).[1] The clearance of *p*-aminohippurate was determined in all experiments. Except in those instances in which PAH was used as an inhibitor of secretion or in which Tm_{PAH} was determined, the plasma concentrations of this substance were kept in the range of 1–3 mg/100 ml. Inulin, urate, and PAH were determined as previously described (18). The analytical method for pyrazinoate (polarography) and the chromatographic, electrophoretic, and solvent partitioning procedures were identical to those reported elsewhere (14). Ultrafiltrates of plasma were prepared by the method of Toribara, Terepka, and Dewey (21) at 37°C in an atmosphere of 5% CO_2-95% oxygen.

All of the drugs used in this study are well known compounds except MK-282, the chemical name of which is *N*-acetyl-4-dibutylsulfamoyl-3-trifluoromethylbenzenesulfonamide. The latter is an experimental drug which is more potent than probenecid as a uricosuric agent and probably shares with probenecid the ability to suppress the secretion of PAH.

RESULTS

Binding of pyrazinoic acid to plasma proteins. Ultrafiltrates were prepared from pooled plasma samples containing from 6 to 380 μg/ml of pyrazinoate. The average concentration ratio, ultrafiltrate/plasma, was 0.98 (range 0.97–1.01). Were pyrazinoate completely ultrafilterable, the expected ratio would be approximately 1.12. This assumes that the volume of water in plasma is 93% of the total and a Donnan factor for a univalent anion of 1.05. Accordingly, in the concentration range studied, approximately 14% of pyrazinoate is bound to proteins. In the following figures and tables, clearances are calculated on the basis of concentrations in whole plasma. Therefore, net reabsorption may be inferred only when clearance ratios of C_{PZ}/GFR fall below 0.86.

Pyrazine compounds excreted during pyrazinoate infusion. All of the polarographically detectable material in urine was a mixture of pyrazinoic acid and 5-hydroxypyrazinoic acid. These identifications are based on polarographic half-wave potentials, high-voltage electrophoresis, paper chromatography and partition between aqueous solutions and various organic solvents. The methods and results are sufficiently similar to those reported for man, *Cebus* monkeys and dogs (14) so as not to warrant detailed presentation. Under the conditions of these experiments (continuous infusion of pyrazinoate) the concentration of 5-hydroxypyrazinoate in urine seldom exceeded 10% of the concentration of pyrazinoate. In most experiments the concentration of 5-hydroxypyrazinoate in plasma was below the limit of detection. This phenomenon is undoubtedly related to the high renal clearance of the latter compound (14). Since it is quite clear that 5-hydroxypyrazinoate is not an important factor in urate transport (14) only the observations on pyrazinoate excretion will be reported in detail.

Renal mechanisms for pyrazinoate excretion. A single experiment, typical of many others in general format, is summarized in Table I. In control periods the clearance of uric acid was approximately 8% of the GFR. The administration of pyrazinoic acid to produce a plasma concentration of approximately 75 μg/ml causes a fall in C_{ur} to about 1% of GFR. The subsequent administration of probenecid in an amount which normally produces an intense uricosuric effect (18, 19) was without substantial effect on the depressed urate clearance. The clearance of pyrazinoate was slightly greater than GFR and was depressed to about 35% of GFR after probenecid administration.

Since in experiments of this type, the second drug (in this case probenecid) was given late in the experiment it seemed necessary to demonstrate the absence of a temporal effect on the clearances of pyrazinoate and urate. In two experiments in which pyrazinoate was infused over the course of 2.5 h, there were only slight variations in the clearance of pyrazinoate and virtually no changes in the depressed urate clearance. The experiment summarized in Table II confirms this point and also demonstrates that the clearances of pyrazinoate and urate are

[1] *Abbreviations used in this paper:* C, clearance; GFR, glomerular filtration rate (clearance of inulin); P, concentration in plasma; PAH, *p*-aminohippurate; Tm, tubular maximum; V, rate of urine flow; the subscripts, ur and PZ, refer to urate and pyrazinoate, respectively.

TABLE I

*Effect of Probenecid on the Renal Clearance of Pyrazinoate; the Lack of Effect on Pyrazinoate-induced Urate Retention**

Time	V	GFR	C_{PAH}/GFR	P_{ur}	C_{ur}/GFR	P_{PZ}	C_{PZ}/GFR
min	*ml/min*	*ml/min*		*μg/ml*		*μg/ml*	
−55	i.v. prime of inulin, 50 mg/kg, and PAH, 8 mg/kg						
−60	Start sustaining infusion to deliver inulin, 45 mg/min, PAH, 15 mg/min in 5% mannitol at 3 ml/min						
0– 20	1.75	80	7.6	33.3	0.081		
20– 43	2.26	84	7.2	36.5	0.078		
43– 60	1.59	78	6.9	33.5	0.081		
61– 69	i.v. prime of pyrazinoic acid, 25 mg/kg						
60	Add pyrazinoic acid to sustaining infusion to deliver 15 mg/kg/h						
90–110	1.20	97	6.9	44.5	0.011	72	1.08
110–130	1.05	93	6.9	42.8	0.009	75	1.09
130–150	1.15	94	6.9	43.2	0.010	82	1.03
151–157	i. v. prime of probenecid, 25 mg/kg						
150	Add probenecid to sustaining infusion to deliver 30 mg/kg/h						
175–195	1.00	94	7.5	43.2	0.015	94	0.34
195–215	0.92	93	7.0	43.2	0.012	103	0.39
215–235	1.10	110	6.8	50.0	0.012	110	0.35

* Chimpanzee La., 53.2 kg, male.

not very responsive to changes in urine flow induced by an osmotic diuretic.

Fig. 1 is a mass plot depicting the relationship of C_{PZ}/GFR and the concentration of pyrazinoate in plasma. The data are only those from control periods, i.e., potential inhibitors of secretion were not present. All but a few of the points are above the line which represents the clearance ratio expected were neither secretion nor reabsorption of pyrazinoate to occur. Net tubular secretion occurs with great frequency. The mass plot does not suggest a strong relationship between clearance ratio and plasma concentration in the range covered. However, in

TABLE II

*Lack of Effect of Osmotic Diuresis on Clearance of Pyrazinoate and on Pyrazinoate-induced Urate Retention**

Time	V	GFR	C_{PAH}/GFR	P_{ur}	C_{ur}/GFR	P_{PZ}	C_{PZ}/GFR
min	*ml/min*	*ml/min*		*μg/ml*		*μg/ml*	
−56	i.v. prime of inulin, 50 mg/kg and PAH, 8 mg/kg						
−55	Start sustaining infusion to deliver inulin, 82 mg/min, and PAH, 15 mg/min, in 5% mannitol, 0.45% NaCl at 3 ml/min						
0– 20	2.40	89	6.7	33.4	0.114		
20– 40	1.95	94	6.5	34.2	0.123		
40– 60	2.15	94	6.4	35.0	0.128		
60– 65	i.v. prime of pyrazinoic acid, 30 mg/kg						
65	Add pyrazinoic acid to sustaining infusion to deliver 15 mg/kg/h						
80–100	1.90	84	7.0	37.0	0.020	72	1.47
100–120	1.60	85	5.2	38.5	0.014	74	1.31
120–140	1.40	70	5.8	36.0	0.014	74	1.26
140	Start an additional infusion of 10% mannitol at 9 ml/min						
140–155	2.87	97	5.7	38.9	0.013	74	1.26
155–170	5.00	80	6.9	38.9	0.015	74	1.43
170–180	7.30	93	5.2	38.0	0.015	72	1.36
180–190	8.40	84	6.4	38.5	0.016	74	1.41
190–200	9.80	86	7.6	38.0	0.016	71	1.45
200–210	11.2	90	6.6	38.9	0.018	71	1.35
210–220	12.2	93	6.4	38.9	0.016	67	1.47

* Chimpanzee To., 54.4 kg, male.

individual experiments in which the concentration of pyrazinoate in plasma was intentionally varied there is a trend toward lower clearance at high concentrations in plasma. This trend is most easily appreciated in the higher range of concentrations. It is obvious from this figure that the substantial decrease in clearance of pyrazinoate after probenecid administration seen in Table I cannot be attributed to minor changes in plasma level.

Table III summarizes the results of fifteen experiments of the type shown in Table I. In each instance after control periods, pyrazinoate was administered and after renal function was assessed, a second drug was given. In every instance pyrazinoate exerted its characteristic inhibition of urate clearance. In most instances the clearance of pyrazinoate exceeded GFR. Several compounds, which are known to inhibit the secretion of other organic anions, probenecid (22), MK-282 (unpublished data), PAH (high concentrations) (23), iodopyracet (24), sulfinpyrazone (25), and mersalyl (26) depressed the clearance of pyrazinoate. Presumably most of these agents are competitive inhibitors of pyrazinoate secretion. A few compounds, chlorothiazide, allopurinol, and salicylate did not depress the renal clearance of pyrazinoate. Two of these, chlorothiazide (27) and salicylate (28) are thought to be competitors for the organic anion (hippurate) secretory mechanism and it is possible that the lack of effect on pyrazinoate secretion was due to insufficient dosage. This point was not pursued.

The depression of pyrazinoate excretion to levels less than the quantities filtered in eight of the experiments constitutes evidence for tubular reabsorption. The fact that the clearance of pyrazinoate is relatively insensitive to rate of urine flow (Table II) even when secretion is inhibited and reabsorption predominates (Table IV) practically eliminates a passive mechanism as the major component of the reabsorptive process. The physical properties of pyrazinoate are such that passive reabsorption would not be expected. The pKa of the compound is 2.92 (29) and it is poorly lipid soluble (14). These results are consistent with those from other species in suggesting that pyrazinoate undergoes bidirectional active transport in the renal tubule (14).

Effects on urate excretion. Several of the compounds tested as potential inhibitors of pyrazinoate secretion (Table III) are potent uricosuric agents in the chimpanzee (18–20, 30). As already demonstrated, the uricosuric effect of probenecid is virtually prevented if pyrazinoate is administered first. Similarly, if probenecid is used first, its uricosuric effect is abolished when pyrazinoate is given subsequently (Table IV). The modest uricosuric action of large doses of PAH (19) is virtually eliminated by prior administration of pyrazinoate. On the other hand, the uricosuric actions of MK-282, iodopyracet, mersalyl, and salicylate (at doses used) were

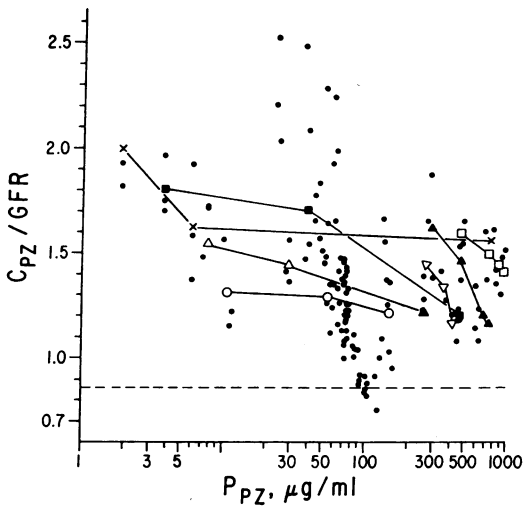

FIGURE 1 A mass plot of C_{PZ}/GFR vs. P_{PZ}. Each point represents a single clearance period, except for the points connected by lines. The latter are means for two to three clearance periods obtained in experiments in which the effects of changes in P_{PZ} were specifically studied. This figure includes 26 experiments.

manifest in spite of the prior administration of pyrazinoate. In these instances we have the impression that the uricosuric responses were smaller than those which obtain in the absence of pyrazinoate but the data are insufficient for precise comparisons. This phenomenon was specifically evaluated with one compound, MK-282. The latter, administered at 10 mg/kg and sustained at 10 mg/kg per h, increased C_{ur}/GFR from 0.12 to 0.70; subsequent administration of pyrazinoate to give a plasma concentration of 141 μg/ml reduced this ratio of 0.48. With sulfinpyrazone (experiment 15 in Table III), it is quite clear that the uricosuric response is blunted by pyrazinoate. This dose normally produces a very substantial effect (19); in experiment 15 the drug merely returned urate clearance to the control value before pyrazinoate. These data plus other results in the chimpanzee (18, 20, 30) indicate that in the presence of ordinarily effective doses of uricosuric drugs, pyrazinoate almost completely eliminates the action of some compounds while allowing others to exert their effects, albeit possibly to smaller than normal extents.

Table V gives the details of a typical experiment testing the relationship between the concentration of pyrazinoate in plasma and the clearance of urate. When the concentration of pyrazinoate in plasma was 1.9 μg/ml, C_{ur}/GFR was reduced to about half the control value; additional depression was observed when the pyrazinoate concentration was raised to 6 μg/ml. On the other hand, a very definite uricosuric response was observed when the concentration of pyrazinoate in plasma was about

TABLE III

Effects of Drugs on Pyrazinoic Clearance: Interaction of Pyrazinoate and Other Drugs in Relation to Urate Transport

Experiment	Condition	Initial dose	Sustaining dose	V	GFR	C_{PAH}/GFR	P_{ur}	C_{ur}/GFR	P_{Pz}	C_{Pz}/GFR
		mg/kg	*mg/kg/h*	*ml/min*	*ml/min*		*μg/ml*		*μg/ml*	
1. Wa., 49.4 kg	Control			0.78	89	3.7	67	0.055		
	Pyrazinoate + probenecid	20	60	1.10	97	3.8	71	0.007	50	2.3
		10	10	2.08	79	4.5	71	0.010	101	1.3
2. La., 53.2 kg	Control			1.87	81	7.2	34	0.080		
	Pyrazinoate + probenecid	25	15	1.13	95	6.9	44	0.010	80	1.1
		10	10	1.01	99	7.1	45	0.013	102	0.36
3. Mac, 30.7 kg	Control			1.85	84	3.1	51	0.133		
	Pyrazinoate + probenecid	30	60	2.10	83	3.3	57	0.010	99	1.0
		25	30	2.34	94	2.9	60	0.030	215	0.30
4. Vi., 56.9 kg	Control			1.04	105	5.5	63	0.071		
	Pyrazinoate + MK-282	20	60	1.55	113	5.4	76	0.011	125	0.98
		10	10	4.05	87	5.2	55	0.298	315	0.28
5. Ch., 45.0 kg	Control			0.95	44	11.3	37	0.099		
	Pyrazinoate + MK-282	10	5	1.44	57	10.0	39	0.018	24	2.2
		3	3	1.87	53	12.4	38	0.310	27	1.2
6. Ed., 56.7 kg	Control			1.77	92	8.6	50	0.108		
	Pyrazinoate + PAH	25	15	1.25	98	9.2	54	0.012	64	1.6
		150	300	6.09	101	3.6	55	0.021	77	0.59
7. Vi., 57.3 kg	Control			0.95	101	4.1	66	0.046		
	Pyrazinoate + PAH	25	30	1.54	107	4.2	70	0.007	107	0.83
		150	300	7.27	95	3.7	71	0.011	168	0.46
8. Po., 52.3 kg	Control			1.58	94	6.1	49	0.116		
	Pyrazinoate + iodopyracet	20	10	3.09	98	5.6	53	0.014	45	1.8
		150	300	4.75	88	4.5	49	0.623	52	0.51
9. Mo., 46.3 kg	Control			1.09	72	6.0	32	0.140		
	Pyrazinoate + mersalyl	25	15	1.31	71	7.5	36	0.015	60	1.6
		1.5*	0	11.1‡	45	6.3	22	0.729	88	0.46
10. Cl., 60.6 kg	Control			1.66	87	8.3	36	0.114		
	Pyrazinoate + allopurinol	50	25	1.70	84	7.0	41	0.014	136	1.4
		10	10	2.11	82	5.7	42	0.019	141	1.6
11. Cl., 60.4 kg	Control			4.12	79	6.7	52	0.078		
	Pyrazinoate + chlorothiazide	20	10	2.52	89	6.8	58	0.007	67	1.2
		10	0	8.10‡	79	7.0	61	0.009	100	1.3
12. Mo., 46.4 kg	Control			1.13	74	5.2	43	0.109		
	Pyrazinoate + chlorothiazide	20	10	1.09	76	5.7	48	0.009	50	1.5
		10	0	4.99‡	68	6.9	52	0.011	54	1.4

TABLE III—(*Continued*)

Experiment	Condition	Initial dose	Sustaining dose	V	GFR	C_{PAH}/GFR	P_{ur}	C_{ur}/GFR	P_{Pz}	C_{Pz}/GFR
		mg/kg	*mg/kg/h*	*ml/min*	*ml/min*		*μg/ml*		*μg/ml*	
13. Cl., 61.8 kg	Control			5.48	87	6.9	43	0.074		
	Pyrazinoate + salicylate	25	15	2.95	92	7.0	46	0.008	77	1.3
		40	20	6.73	83	8.7	43	0.423	78	1.3
14. La., 53.0 kg	Control			4.33	83	5.3	40	0.068		
	Pyrazinoate + salicylate	25	15	1.15	83	6.5	45	0.007	71	1.1
		40	20	2.03	92	5.6	40	0.334	77	0.97
15. Jo., 46.4 kg	Control			2.05	92	7.3	45	0.062		
	Pyrazinoate + sulfinpyrazone	25	15	1.99	94	8.2	47	0.008	56	1.45
		5	10	2.80	106	4.9	48	0.070	73	0.32

Each datum is the mean from two to five consecutive clearance periods.
* As mercury.
‡ In the experiments with diuretics the first period or two after drug administration, during the time when urine flow was changing rapidly, have been excluded in calculating means for all parameters given in the table.

700 μg/ml. The large load of pyrazinoate caused an increase in urine flow rate. However, osmotic diuresis itself will not produce a complete reversal of pyrazinoate-induced urate retention. In the experiment given in Table II when pyrazinoate alone was present, osmotic diuresis had no perceptible effect on C_{ur}/GFR. In the presence of pyrazinoate plus probenecid there was a small increase in this ratio with increases in urine flow (Table IV) but this was not sufficient to bring the C_{ur}/GFR up to control values even at the highest flow rate. Thus, most of the uricosuric action of pyrazinoate seems to be the result of a tubular effect. The following observations suggest that the uricosuric effect is specific, i.e. it is not a consequence of general tubular damage induced by the high rate of drug infusion. In a few experiments because of a mismatch between loading and sustaining doses, the plasma level and urinary excretion of pyrazinoate were declining during the periods of observation. The uricosuric effect fell in proportion to the foregoing parameters, i.e. it was rapidly reversible. At the highest doses of pyrazinoate used, there was no fall in GFR or PAH secretion (C_{PAH}), and the absence of both proteinuria and glucosuria. At the very high doses of the drug, there were increases in the excretion of sodium, potassium and chloride, but these were of the same magnitude encountered at comparable increases in urine flow induced by mannitol, presumably a nonspecific osmotic diuretic. Urine pH ranged between 5.5 and 6.0 in all experiments (except with chlorothiazide) and was not changed by any of the doses of pyrazinoate used.

Fig. 2 displays the complex relation between the urate clearance ratio and the concentration of pyrazinoate in plasma. The figure includes the data from experiments of the type summarized in Table V as well as the control observations from experiments of the type given in Table I. Because of the considerable range in the control values for urate clearance ratio (0.05–0.24), the ordinate is in terms of percent of control. Maximal depression of the clearance ratio is achieved at plasma concentrations of pyrazinoate of about 10 μg/ml. This level of depression is maintained over the range of pyrazinoate concentrations up to 100 μg/ml. At higher concentrations of pyrazinoate the clearance ratio of urate increases and at pyrazinoate levels above 600 μg/ml there is a definite uricosuric response.

There is evidence that uricosuric responses are related to concentrations of drugs in tubular fluid rather than in plasma (31–33). For that reason the response of C_{ur}/GFR has been plotted against a parameter of pyrazinoate excretion which presumably is related to the concentration of the drug at its site of action (Fig. 3). The result is consistent with the hypothesis.

Effect of pyrazinoate on PAH secretion. Pyrazinoate is a very poor inhibitor of PAH secretion. In none of

TABLE IV

*The Effect of Osmotic Diuresis on the Clearances of Pyrazinoate and Urate in the Presence of Probenecid**

Time	V	GFR	C_{PAH}/GFR	P_{ur}	C_{ur}/GFR	P_{Pz}	C_{Pz}/GFR
min	*ml/min*	*ml/min*		*μg/ml*		*μg/ml*	
−47	i.v. prime of inulin, 50 mg/kg and PAH, 8 mg/kg						
−45	Start sustaining infusion to deliver inulin, 82 mg/min, and PAH, 15 mg/min in 5% mannitol, 0.45% NaCl at 3 ml/min						
0– 20	1.35	94	6.6	36.6	0.103		
20– 40	1.70	90	6.2	39.9	0.113		
42– 47	i.v. prime of probenecid, 10 mg/kg						
45	Add probenecid to infusion to deliver 10 mg/kg/h						
60– 80	1.45	84	4.7	36.6	0.572		
80–100	2.28	89	5.4	34.1	0.591		
100–104	i.v. prime of pyrazinoic acid, 15 mg/kg						
100	Add pyrazinoic acid to infusion to deliver 7.5 mg/kg/h						
115–135	1.70	78	5.2	37.1	0.025	49	0.55
135–155	1.90	87	5.0	37.6	0.028	51	0.48
155	Start additional infusion of 10% mannitol at 9 ml/min						
155–175	5.50	95	4.4	36.6	0.037	53	0.42
175–190	9.60	88	5.1	36.6	0.042	53	0.52
190–205	12.1	88	5.0	37.1	0.049	53	0.50
205–215	13.9	89	5.4	36.1	0.052	55	0.41
215–225	15.2	88	5.4	37.6	0.065	56	0.53
225–235	15.4	89	4.8	37.6	0.065	58	0.48
235–245	15.2	85	5.2	36.6	0.069	60	0.50

* Chimpanzee To., 54.8 kg, male.

TABLE V

*Clearance Ratio of Urate at Various Concentrations of Pyrazinoate in Plasma**

Time	V	GFR	C_{PAH}/GFR	P_{ur}	C_{ur}/GFR	P_{Pz}	C_{Pz}/GFR
min	*ml/min*	*ml/min*		*μg/ml*		*μg/ml*	
−53	i.v. prime of inulin, 50 mg/kg, and PAH, 8 mg/kg						
−50	Start sustaining infusion to deliver inulin, 75 mg/min, and PAH, 14 mg/min, in 5% mannitol, 0.85% NaCl at 3 ml/min						
0– 20	1.68	76	6.2	36.3	0.091		
20– 40	1.45	68	6.3	34.7	0.098		
40– 60	1.00	61	6.5	34.7	0.097		
60– 61	i.v. prime of pyrazinoic acid, 0.75 mg/kg						
60	Add pyrazinoic acid to infusion to deliver 0.375 mg/kg/h						
70– 90	1.48	70	6.2	36.3	0.048	1.9	2.14
90–105	1.53	70	6.1	36.8	0.047	1.9	1.93
105–120	1.33	70	6.3	38.2	0.052	1.9	1.82
120–121	i.v. prime of pyrazinoic acid, 1.5 mg/kg						
120	Add pyrazinoic acid to infusion to deliver 0.75 mg/kg/h						
130–145	1.80	71	5.7	39.5	0.026	6.0	1.92
145–160	1.73	74	5.9	40.0	0.032	6.0	1.58
160–175	1.73	71	5.7	39.0	0.033	6.0	1.37
175–196	i.v. prime of pyrazinoic acid, 300 mg/kg						
175	Add pyrazinoic acid to infusion to deliver 100 mg/kg/h						
200–210	9.10	67	6.3	35.4	0.290	840.0	1.42
210–218	8.56	64	5.3	37.2	0.255	729.0	1.55
218–226	6.44	60	6.6	41.0	0.224	699.0	1.60

* Chimpanzee To., 48.2 kg, male.

the experiments in which PAH clearance was estimated was there a consistent decline in C_{PAH}/GFR. In experiments 6 and 7 (Table III) PAH was given in amounts allowing the estimation of Tm_{PAH}. These were 170 and 123 mg/min, respectively. Such values are within the range obtained in this species in the absence of pyrazinoate, 91–193 mg/min, mean 132 (26). This was confirmed in an additional experiment in which Tm_{PAH} as determined before and after administration of pyrazinoate. Control Tm was 74 mg/min; after pyrazinoate sufficient to give a plasma concentration of 115 μg/ml, Tm_{PAH} was 79 mg/min. The lack of potency against PAH secretion contrasts with the great power of pyrazinoate to reduce the excretion of urate.

DISCUSSION

Concentrations of pyrazinoate in plasma comparable to those found in man after the ingestion of pyrazinamide (14, 15) caused substantial decreases in C_{ur}/GFR (Fig. 2). This supports the contention that the urate-retaining action of pyrazinamide is attributable to its metabolite.

The results concerning the renal disposition of pyrazinoate are consistent with those obtained in other animals (14, 34), i.e., there is bidirectional transport. Active secretion of pyrazinoate can be inferred from: (*a*) clearance ratios in excess of unity and (*b*) depression of secretion by other organic anions which are themselves actively secreted. Reabsorption occurs; this is frequently seen after secretion is depressed by presumed competitive inhibitors. In view of the lack of effect of osmotic diuresis on pyrazinoate clearance, it is probable that this reabsorption is also mediated by an active transport process.

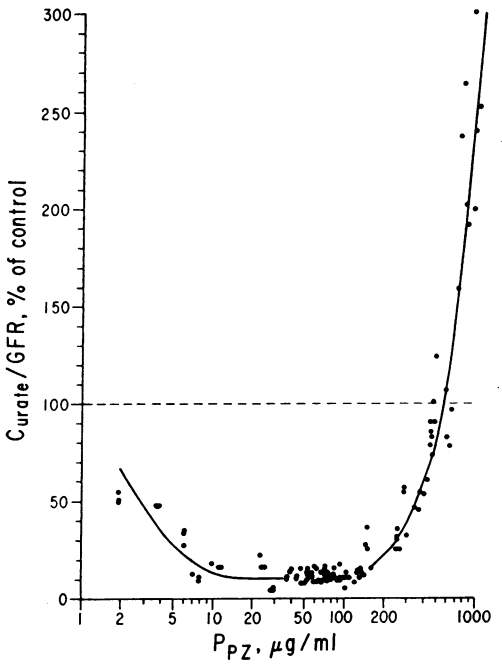

FIGURE 2 C_{urate}/GFR (expressed as percent of control) plotted as a function of P_{PZ}. Each point represents a single clearance period. A few points in the area of greatest density have been omitted. This figure includes 26 experiments.

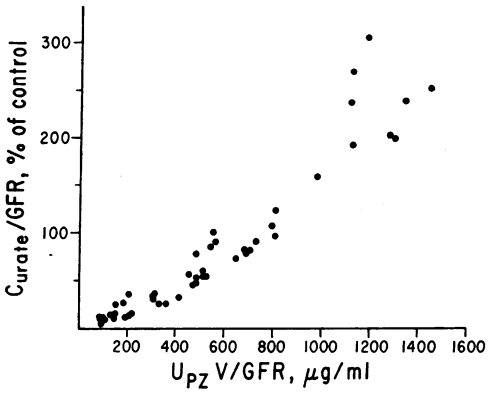

FIGURE 3 C_{urate}/GFR (expressed as percent of control) as a function of pyrazinoate excretion per ml of GFR. Only those data corresponding to $P_{PZ} > 100$ μg/ml are included (*cf.* Fig. 2). Each point represents an individual clearance period. The data are from 12 experiments.

The traditional interpretation of experiments in which the administration of one organic anion depresses the secretion of another is that the anions compete for a common secretory mechanism (24). Accordingly, the inhibition of pyrazinoate secretion by PAH and other compounds (Table III) is a manifestation of competition. The failure of pyrazinoate, at the doses employed, to inhibit PAH secretion may be taken as evidence that pyrazinoate is a poor competitor, i.e., it has a lower affinity for the secretory mechanism than does PAH. Since urate secretion is depressed by pyrazinoate it might be inferred that urate secretion is also mediated by this common mechanism. However, if this were true, PAH, the better of the two exogenous competitors for the common mechanism, should be more potent in suppressing urate secretion than is pyrazinoate. But, PAH does not reduce urate clearance.

This paradox may be resolved in one of two ways. First, it is possible that there are two mechanisms for organic anion secretion. One of these would be the traditional "hippurate" mechanism which is responsible for PAH secretion and most of pyrazinoate secretion. For this mechanism pyrazinoate would be a weak inhibitor. The other mechanism would be largely responsible for the secretion of urate and it is for this transport system that pyrazinoate would be a strong inhibitor and PAH, a weak one. This hypothesis has already been invoked to

explain a similar discrepancy in the effects of PAH and *m*-hydroxybenzoate on urate excretion (35).

An alternative proposal requires that PAH inhibit both secretion and reabsorption of urate in such a balanced way that there would be little change in urate clearance even though urate secretion were seriously impaired. Since PAH inhibits urate reabsorption in rats (36) and is faintly uricosuric in the chimpanzee (19) this proposal is not entirely unreasonable. However, in view of other evidence, we prefer the hypothesis invoking two secretory mechanisms. At low concentrations in plasma, pyrazinoate prevents mercurial-induced net secretion of urate, while PAH does not (30). If, as we believe, the mercurial effect results from extensive inhibition of urate reabsorption, this should create a situation which would unmask extensive inhibition of secretion by low levels of PAH were it to occur.

The concentration-response curve (Fig. 2) has features in common with the corresponding curve from experiments with salicylate in man (31). However, there are significant differences. First, the suppression of urate clearance by pyrazinoate is almost complete; the clearance is reduced by about 50% with salicylate. And second, there is a much wider separation between urate-retaining and uricosuric concentrations with pyrazinoate than with salicylate. One interpretation of this curve is that urate secretion is almost completely inhibited at low levels of pyrazinoate and that much higher concentrations of the drug are required for even small inhibition of urate reabsorption. This interpretation is in complete harmony with the assumptions underlying the "pyrazinamide suppression test" (3). The new feature, inhibition of urate reabsorption at high levels of pyrazinoate, is irrelevant to the test because of the dose of pyrazinamide usually employed. However, the suppression test carries with it an additional implication, i.e., the secretory and reabsorptive processes are virtually independent. This can only obtain if there is a unidirectional mechanism for the secretion of urate which is distal to the reabsorptive site. If secretion is proximal to or coextensive with reabsorption, it will provide additional urate for reabsorption. In such situations the change in urate excretion after pyrazinamide will be a reflection of how the reabsorptive system responds to a change in urate availability and not a quantitative reflection of the uninhibited secretory rate. Thus, it becomes pertinent to inquire into the appropriateness of a model of the nephron which places secretion of urate distal to reabsorption.

The model involving distal secretion makes it difficult to attribute the action of probenecid and some other drugs to inhibition of the reabsorption of urate. There is no apparent reason why an inhibitor of a small distal secretory mechanism should prevent (Table I) or abolish (Table IV) the action of a drug which inhibits proximal reabsorption of much greater magnitude. In an attempt to resolve a paradox of this type it has been proposed that some uricosuric drugs stimulate secretion of urate (6–8). Accordingly, pyrazinoate would prevent the action of probenecid to enhance secretion. Because there is fairly direct evidence that probenecid inhibits entry of urate into the tubule (37) and limits urate movement out of the tubule (36), it seems somewhat extravagant to propose this adidtional action, enhancement of secretion.

An alternative hypothesis pertinent to this paradox was offered by Amini, Petrakis, Mandel, and Doherty (38) who postulated that pyrazinamide enhanced reabsorption of urate. Accordingly, one could rationalize the failure to observe a uricosuric action with probenecid by assuming that with pyrazinoate pretreatment the enhanced reabsorption of urate is not sufficiently impaired by the usual dose of probenecid. But here again there is fairly direct evidence that pyrazinoate can diminish the ingress (14, 37) and egress (36) of urate from the tubule and the postulate of a third activity is not economical.

Since the only reasonably direct studies on the effects of drugs on transtubular movements of urate suggest inhibition of transport, either secretory or reabsorptive, we will for the rest of this discussion assume that all drugs act on urate movements only by depressing transport.

If secretion of urate is distal to reabsorption, it follows that the maximal rate of urate excretion, i.e., the rate that would obtain after complete blockade of reabsorption, would be equal to but not greater than the sum of the rate of urate filtration and the rate of excretion before blockade. After the administration of the powerfully uricosuric mercurial, mersalyl, one can observe excretory rates in excess of this sum (30).

In summary, on the basis of these pharmacological considerations it is unlikely that urate secretion is distal to urate reabsorption. The older work (39, 40) purporting to demonstrate distal secretion of urate has not been confirmed and some of it has been criticized on technical grounds (37, 41, 42). Accordingly, we will discard the hypothesis of distal secretion in the attempt to evaluate the effects of pyrazinoate and other drugs. This involves denying the validity of the "pyrazinamide suppression test."

The results of clearance experiments with pyrazinoate and other drugs can be accommodated by models specifying either coextensive secretion and reabsorption or reabsorption distal to secretion. As was appreciated by others, models of these types allow for more transtubular traffic of urate than is easily appreciated from clearance experiments: "It would not be surprising if the unidirectional fluxes, when measured, proved to be quite

high" (41). We will use the coextensive model for illustration because we believe that it is most compatible with published results. A summary of older literature localizing secretion and reabsorption to the proximal tubule is given in reference 42; other pertinent references are 36, 37, 41, 43, and 44. There is one free flow micropuncture study in rats (45) suggesting that net secretion may be proximal to reabsorption, but in fact the two processes are coextensive (46). Obviously at any particular level of the nephron, one or the other unidirectional flux may predominate depending on the relative activities of the two transport processes, extent of fluid reabsorption, etc. There are, in addition, some recent preliminary reports in which the results are interpretable in terms of there being two reabsorptive sites, one coextensive with the proximal secretory site and one more distal (13, 47, 48). The existence of a small distal reabsorptive mechanism would complicate our considerations only slightly and for present purposes need not be considered further.

Since we cannot actually estimate transtubular fluxes, we have for purposes of illustration assumed that the secretory moiety is equal to the filtered load (scheme A, Fig. 4). This is based on the observation that the clearance ratio, C_{ur}/GFR, may be as high as 1.98 after administration of mersalyl (30). Obviously there is no assurance that a given dose of mersalyl inhibits reabsorption completely and does not depress secretion at all; therefore, our estimate of secretion may be low. However, the exact magnitude of this process is not critical for present purposes; it need only be granted that secretion is not small compared to filtration. The magnitude of reabsorption is arbitrarily set at 189 units to have the excretion of urate equal to 11% of the filtered load, a proportion frequently encountered in the chimpanzee. It is now possible to choose values for varying degrees of inhibition of secretion and/or reabsorption that will give values for net excretion conforming to experimental results. It is emphasized that the values chosen here are made to conform with experimental results and they have no meaning other than to illustrate the possibility of rationalizing a great variety of apparently paradoxical phenomena with the model.

Scheme B suggests what might occur in the presence of a dose of pyrazinoate which causes urate retention. As depicted there is partial inhibition of urate secretion, sufficient to allow the reabsorptive system to work at high efficiency. In addition, there might be a small depression of reabsorption but this would not be apparent in the face of the decreased demand on the reabsorptive mechanism. Therefore, it is not necessary to interpret the data of Fig. 2 as indicating complete inhibition of urate secretion at levels of pyrazinoate that have no apparent effect on reabsorption, 10–100 μg/ml.

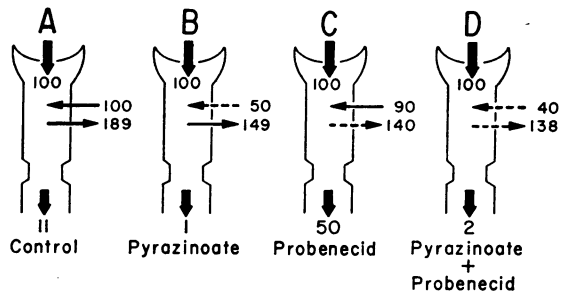

FIGURE 4 Diagrams of proposals for the actions of drugs on urate transport. Solid arrows indicate uninhibited urate transport, dashed arrows, partially inhibited urate transport. See text for discussion.

Scheme C depicts the situation that might obtain after probenecid. A small inhibition of secretion is accompanied by greater inhibition of reabsorption. When the two drugs are given together (scheme D) the depressions of secretion are shown to be additive. (This may or may not be true depending on the shapes of the dose-response curves, but is taken as a first approximation).

It is apparent at this point that a scheme embodying two opposing transport systems of substantial magnitude, allowing for differing susceptibilities of the two systems to various drugs, and further complicated by pharmacokinetic interactions of drugs given in combination (49) can accommodate almost any experimental findings. This is not an exercise in *reductio ad absurdum;* for an economical hypothesis to satisfy the great variety of results with drugs that influence urate excretion, it must be extraordinarily flexible. It would be pointless to continue the arithmetical speculation; the reader can easily satisfy himself that by specifying various extents of inhibition of secretion and/or reabsorption and by including effects of one drug on the disposition of another, the model can be made to fit the following observations only some of which have been illustrated above: (*a*) one uricosuric drug can nullify the action of another; (*b*) the effects of two uricosuric drugs can be additive; (*c*) one uricosuric drug can inhibit the action of another only slightly; (*d*) a single compound can cause urate retention at one dose and uricosuria at another; (*e*) a compound that is weak as a uricosuric agent by itself may nevertheless be able to act in the presence of a suppressor of urate excretion, while a potent uricosuric drug may not (compare salicylate and probenecid in Table III); (*f*) some uricosuric agents have apparent ceilings of activity which are less than the theoretical maximum for complete inhibition of reabsorption. Obviously, considerable effort will be required to prove the appropriateness of the model.

The foregoing neglects the possibility of some passive movement of urate in medullary structures. Something

of the kind might be invoked to explain the concentration of urate in medulla and papilla to levels exceeding that in renal cortex (50, 51). However, there is at present no reason to suspect that the processes involved are large in magnitude or that they are the sites of drug action. An extensive discussion of this phenomenon is given by Mudge, Berndt, and Valtin (52).

ACKNOWLEDGMENTS

The authors dedicate this publication to the late Professor Alexander B. Gutman whose elegant and fundamental studies throughout the years on uric acid have been truly an inspiration to us.

The authors gratefully acknowledge the skilled assistance of Dennis L. Bohn, Sheila S. Reilly, James P. Tinker, and Leiselotta Roth.

The work performed in Syracuse was supported by Research Grant HE-10595 from the National Institutes of Health, U. S. Public Health Service. The authors are indebted to Doctors John E. Baer, Karl H. Beyer, and Clement A. Stone for their support of this project at the Merck Institute for Therapeutic Research.

We are also indebted to Mr. Carl Ziegler and Dr. James M. Sprague for synthesizing and supplying adequate quantities of MK-282 for use in this study.

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