Localization of Urate and Phosphate Reabsorption in the Mongrel Dog Kidney (37496)

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The precise cellular localization of many of the transport systems responsible for solute reabsorption in the proximal renal tubule remains unknown. Either the peritubular (PTM) or luminal (LM) cell membrane could represent the primary site of activity. Thus, it is believed that sodium is actively reabsorbed by a carrier mediated mechanism at PTM with LM acting simply as a diffusional barrier (1). In contrast, microperfusion experiments have demonstrated carrier sites for both glucose (2) and urate (3) on LM; in addition, LM has been identified as the site of amino acid carrier systems in rabbit kidneys (4).

With these possible models in mind we have investigated reabsorptive urate and phosphate transport in the mongrel dog kidney. Application of the double indicator dilution technique in the presence of suitable transport inhibitors has led to the conclusion that both solutes are reabsorbed initially by mechanisms located at the LM.

Methods. Mongrel dogs weighing 10–22 kg were used. The technique used, double isotope dilution, has been previously described (4) and is similar to that of Silverman, Aganon, and Chinard (5). Briefly, labeled inulin and test substance, ^{14}C-urate or ^{32}P, were injected into the renal artery before and after the administration of one of the compounds described below. Serial urine samples were collected every 15 or 30 sec, depending upon urine flow, for a total of 8–10 min. Significant isotope recirculation was prevented by the collection of total renal venous effluent for 1 min after isotope injection. This volume of blood was replaced by an equal volume of 6% dextran.

Probenecid (200 mg/kg iv or 2 mg ia), PAH (30 mg/kg iv or 60 mg added to ^{14}C-urate injection), and NaCN (0.05 mg/kg/min ia) were administered to alter urate reabsorption. Intravenous doses were added to the venous reservoir minutes before ^{14}C-urate injection. In the phosphate experiments both NaCN (0.05 mg/kg/min) and ouabain (0.05 mg/kg) were administered by constant infusion into the left renal artery. Further details are provided in the legend of the figures.

Results. Figure 1 shows a typical artery-to-ureter transit pattern for urate before and after inhibition by probenecid. The administration of either PAH or NaCN resulted in similar transit patterns. Each of these drugs, as expected, increased the fractional excretion of urate. Table I shows the results of several experiments for each drug. In four experiments probenecid increased labeled

TABLE I. Effects of Inhibitors on Urate Transport.

	FE[a]		$\bar{t}_{Ur}/\bar{t}_{In}$		
	Before	After	Before	After	n
Probenecid	71 ± 2.6	86 ± 1.8	0.99 ± 0.01	0.98 ± 0.01	4
PAH	79 ± 2.5	92 ± 5.0	0.98 ± 0.01	0.98 ± 0.01	3
CN	85 ± 3.8	92 ± 5.1	1.00 ± 0.01	0.99 ± 0.01	3

[a] Percentage fractional excretion of labeled urate.

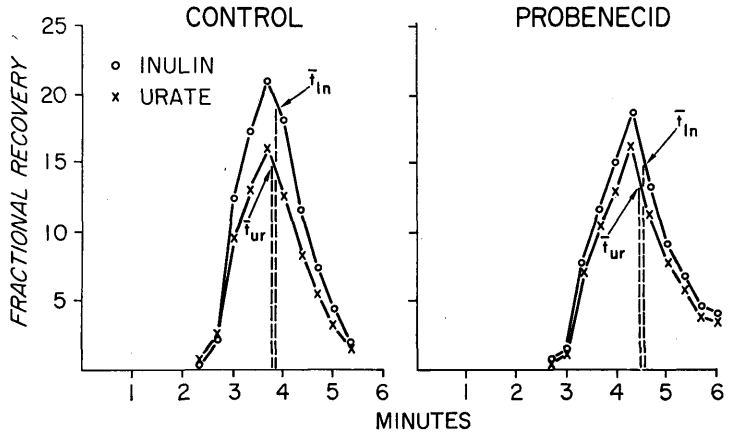

FIG. 1. Effect of probenecid on artery-to-urine transit of urate: Male dog, 16.0 kg; iv infusion: 0.5 ml/kg/min 10% mannitol in 0.9% NaCl containing 20 m*M* creatinine. Period I: renal venous blood flow (RVBF) on left side 215 ml/min; blood pressure 120/64 mm Hg; GFR, 26.4 ml/min (right side), 25.6 ml/min (left side); *V*, 3.1 ml/min (right side), 2.4 (left side); reabsorption of filtered ^{14}C-urate 26%. Period II: RVBF on left side 220 ml/min; BP 112/50 mm Hg; GFR, 16.2 ml/min (right side), 15.6 ml/min (left side); *V*, 2.4 ml/min (right side), 1.9 ml/min (left side); reabsorption of filtered ^{14}C-urate 14%.

urate excretion from 71 ± 2.6% to 86 ± 1.8% of the filtered load. In three experiments a similar increase occurred in fractional excretion of urate from 79 ± 2.5% to 92 ± 5.0% following PAH. Sodium cyanide in three studies also increased urate fractional excretion from 85 ± 3.8% to 92 ± 5.1% of the filtered load. In spite of the significant inhibition of urate reabsorption no alteration in the ratio of mean artery-to-ureter transit time of ^{3}H-inulin ($\bar{t}_{In}$) to that of ^{14}C-urate ($\bar{t}_{Ur}$) could be observed following administration of any of these drugs.

Figure 2 is a typical $A \rightarrow U$ transit curve for ^{32}P before and after NaCN. Administration of ouabain resulted in a similar pat-

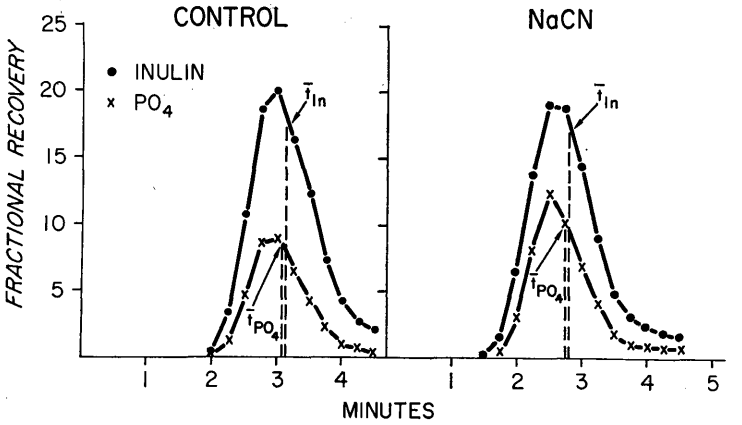

FIG. 2. Effect of NaCN on artery-to-urine transit of PO_4: Male dog, 10.0 kg; iv infusion: 0.5 ml/kg/min 10% mannitol in 0.9% NaCl containing 20 m*M* creatinine. Period I: renal venous blood flow (RVBF) on left side 170 ml/min; GFR, 12.5 ml/min (right side), 13.1 ml/min (left side); *V*, 1.7 ml/min (right side), 2.1 ml/min (left side); reabsorption of ^{32}P 61%; FE_{Na}, 6.4%. Period II: RVBF 205 ml/min; GFR, 11.1 ml/min (right side), 10.7 ml/min (left side); *V*, 1.8 ml/min (right side), 2.8 ml/min (left side); reabsorption of ^{32}P, 47%; FE_{Na}, 16.8%.

TABLE II. Effects of Inhibitors on PO_4 Transport.

	FE[a]		$\bar{t}_P/\bar{t}_{In}$		
	Before	After	Before	After	n
CN	18 ± 3.7	28 ± 6.7	0.95 ± 0.03	0.96 ± 0.02	5
Ouabain	14 ± 5.3	26 ± 5.0	0.97 ± 0.01	0.93 ± 0.03	5

[a] Percentage fractional excretion of labeled phosphate.

tern. Table II shows that in five experiments in which cyanide was used the fractional excretion of ^{32}P increased from 18 ± 3.7% to 28 ± 6.7%. In another series of experiments in which ouabain was used ^{32}P fractional excretion increased from 14 ± 5.3% to 26 ± 5.0%. However, as in the urate experiments, the ratio of mean $A \rightarrow U$ transit times, $\bar{t}_P/\bar{t}_{In}$, remained unchanged.

Discussion. Results of these experiments indicate that both urate and phosphate are reabsorbed by transport mechanisms located at the LM of the tubular cells. This follows from the fact that at constant flow, the volume of distribution, V, of a solute is directly proportional to $\bar{t}$. Equality of mean transit times, therefore, implies that, *e.g.*, $V_{Ur} = V_{In}$. Further, both V_{Ur} and V_{PO_4} must have been limited by the same membrane that limits inulin distribution. This approach has been described in more detail previously (4). With respect to urate, a similar conclusion was reached by Kramp, Lassiter and Gottschalk (3) in their micropuncture study of the rat nephron.

Bidirectional movement of urate has been established previously (6) but the localization of the carrier mediated systems was unknown. With the results of the present experiments and those of Nolan and Foulkes (7) relating to urate secretory transport, the existence of oppositely directed carrier mediated mechanisms located at each of the cellular membranes has been demonstrated. The question of whether these two opposing mechanisms exist in the same cell population or are representative of two separate populations remains to be determined.

It has been suggested that PO_4 reabsorption is mediated by intracellular PO_4 pools (8). While this may indeed occur, our results show that the site of inhibition by various compounds is not at the level of such pools but rather on the cell membrane. This follows from the fact that even in the presence of inhibitors, $\bar{t}_{PO_4} = \bar{t}_{In}$ and thus PO_4 reabsorption is limited by factors associated with LM. This, however, does not exclude a role for intracellular PO_4 in PO_4 reabsorption.

Summary. We have provided evidence that the sites of urate and phosphate reabsorption exist at the luminal cell membrane. This was accomplished by the determination of mean glomerulus to ureter transit times for both compounds in the absence and presence of inhibitors of their reabsorption.

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