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Digoxin Transport in the Distal Nephron of Rats during Saline Diuresis (41226)

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Abstract. The nephron segments involved in the renal tubular transport of digoxin and the direction of transport in each segment were evaluated using renal micropuncture techniques in 11 rats made diuretic by i.v. infusion of .85% saline. Tubular fluid was collected from 4 different sites along the nephron: late proximal, early distal, late distal, and ureter. The concentrations of ^{3}H-digoxin and ^{14}C-inulin were measured in each sample and the reabsorption of water and efflux or influx of digoxin were calculated. Water was removed from the lumen along the entire length of the nephron and only 2.53 ± 0.3% of the filtrate was excreted in the urine. Digoxin was also absorbed in the proximal convoluted tubule and in the loop of Henle. About 1/3 of the filtered drug exited in these early nephron segments probably by passive diffusion. In the distal convoluted tubule, digoxin was added to the tubular fluid. The fraction of digoxin present in the lumen increased from 64 ± 3.8% of the filtered load at early distal site to 78.7% ± 4.8% at late distal site indicating that an amount equal to 15% of filtered digoxin entered the tubule. This influx occurred against a concentration gradient of 3-5, suggesting the existence of a carrier mediated or active transport mechanism in this nephron segment. Transport of digoxin beyond the late distal puncture site was negligible. The collecting duct appeared to be relatively impermeable to the drug since a concentration gradient of 30 or greater failed to cause its diffusion out of the tubule. The data indicate bidirectional transport of digoxin in the rat nephron. Efflux occurs primarily in the early nephron segments while net influx is limited to the distal convoluted tubule.

Digoxin is eliminated from the body primarily through the kidneys as unchanged glycoside (1). Its renal excretion is due largely to filtration, at the glomerulus, of the unbound plasma digoxin. In human subjects the digoxin to creatinine clearance ratio tends to be close to unity, strongly suggesting a major role for filtration in the renal handling of this drug. However, there is also evidence for renal tubular secretion as well as reabsorption of digoxin. Steiness (2) observed digoxin to inulin concentration ratios which were greater than 1 in man, suggesting an active tubular secretory mechanism that could be inhibited by spironolactone. Doherty and associates (3) on the other hand, using a stop-flow analysis in dogs found digoxin to creatine concentration ratios of less than 1 in the middle segment of the nephron. This finding suggests that there is tubular reabsorption of the drug.

In our micropuncture experiments (4) in rats made diuretic by iv infusion of hypertonic saline, radiolabeled digoxin was freely filtered at the glomerulus but only 80% of the filtered drug appeared in the final urine. Tubular fluid collected from late proximal convolutions contained 64.1% of the filtered digoxin, indicating reabsorption of the drug along the proximal tubule. Urinary recovery of [^{3}H]digoxin, microinjected into early proximal tubular segments, averaged 62.1%, again implying efflux of luminal digoxin. In the more distal nephron segments, there was an apparent influx of digoxin into the tubule as indicated by surface application of the drug and tubular fluid collection experiments. However, these data did not provide definitive evidence for the transport of this drug in the distal nephron. Urinary recovery of the radiolabeled drug placed on the surface of the kidney provides only indirect evidence for the site and mechanism of transport. The distal tubular fluid samples collected in these experiments (4) were too few to allow clear definition of distal tubular transport processes.

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The present investigations were designed to assess the direction of digoxin transport in distal nephron segments, using free flow micropuncture techniques in rats made diuretic by iv infusion of 0.85% saline.

The results indicate reabsorption of [^{3}H]-digoxin along the proximal convoluted tubule and in the loop of Henle. Digoxin was sequestered in the lumen of the distal convoluted tubules. The influx of the drug in this nephron segment occurred against a concentration gradient. In the collecting ducts, transtubular movement of digoxin was not apparent.

Materials and Methods. Experiments were performed on male Sprague–Dawley rats weighing 325 ± 13 g. The animals had free access to water but were on a restricted food intake (one-half of normal intake) 12 hr prior to the experiments. They were anesthetized by an intraperitoneal injection of Inactin [ethyl (1-methylpropyl)-malonylthiourea], 100 mg/kg body wt. After tracheostomy, three polyethylene cannulas (PE-10) were placed into the right external jugular vein for drug injections and infusion of radiolabeled markers. Body temperature was maintained at 36–37° by placing the animals on a thermostatically controlled heated board. All of the rats received a continuous iv infusion of 0.85% saline at a rate of 6.0 ml/hr after a priming dose of 10% of body wt. The right common carotid artery was cannulated (PE-50 tubing) to permit recording of blood pressure and to provide blood samples. All of the rats received an iv infusion of [^{3}H]digoxin (40 μCi/ml) and [^{14}C]inulin (20 μCi/ml) throughout the experiment at a rate of 0.6 ml/hr. The priming dose for inulin and digoxin were 8 and 15 μCi, respectively. The specific activity of digoxin was 15 μCi/mg. Therefore, the dose of the drug was 5 μg/kg per hr; a very low dose in rats.

The abdomen was opened with a suprapubic midline incision which was extended laterally from the sternum to expose the left kidney. A small lucite crescent was positioned around the kidney to limit movement. The renal capsule was bathed with mineral oil throughout the experiment to prevent drying and was illuminated by a fiber optic source. All other exposed tissue surfaces were covered with thin parafilm to prevent evaporative water loss and tissue damage. A catheter (PE-50) was placed into the left ureter to permit quantitative urine collection and unilateral urine flow was doubled for clearance calculations. A period of at least 45 min was allowed for equilibration before starting the experiment. Late proximal and early and late distal convoluted tubular segments were identified on the surface of the left kidney following iv injection of 50–75 μl of 5% solution of FD & C dye (Keystone, Chicago, Ill.). Tubular transit times were measured simultaneously and were recorded as the elapsed time between the appearance of the green flush on the surface of the kidney and the arrival of the dye front in each of the selected nephron segments. Tubular fluid samples were obtained by free-flow micropuncture techniques using oil-filled, sharpened glass micropipets with an outer tip diameter of 10–15 μm for proximal, and 7–10 μm for distal tubular puncture. A small oil droplet was injected and kept in place by controlled intermittent suction. An attempt was made to obtain at least one late proximal, early distal, and late distal fluid sample from each rat. Multiple samples collected from similar locations along the nephron were averaged for each animal before calculating mean values.

Urine samples of 20–30 min duration were obtained with each sample of tubular fluid along with a midcollection carotid blood sample of 0.03 ml. Blood pressure was recorded periodically throughout the experiment using a Statham transducer and a Grass polygraph. The volume of tubular fluid samples was determined by measuring the length of a sample after splitting an oil droplet in a calibrated constant bore capillary tubing. Sample size varied from 25 to 200 nl. Aliquots of all blood, urine, and tubular fluid samples were analyzed for ^{3}H and ^{14}C activity in a three-channel liquid scintillation counter with external standardization (Beckman Instruments). The general method of surgical preparation, tubular micropuncture, sample handling, and calculation of data was similar to those described in previous publications from this

TABLE I. SUMMARY OF HEMODYNAMIC AND RENAL CLEARANCE DATA ON DIGOXIN EXCRETION THROUGH THE KIDNEY IN RATS DURING ISOTONIC SALINE DIURESIS

	No. of rats	Results[a]
Mean blood pressure (mm Hg)	11	113 ± 5
Heart rate (beats/min.)	11	334 ± 12
Hematocrit (%)	11	48 ± 1
Urine flow (μl/min per 100 g body wt)	11	26.2 ± 2.9
GFR[b] (μl/min per 100 g body wt)	11	1053 ± 41
Digoxin clearance (μl/min per 100 g body wt)	11	739 ± 44[c]
Net digoxin excretion (% of filtered)	11	71.3 ± 2.9

[a] Data are presented as mean ± SE.
[b] GFR, glomerular filtration rate.
[c] Significantly less than GFR—$P < 0.001$.

laboratory (4, 5). Statistical significance was evaluated using one-way analysis of variance. A P value of less than 0.05 was considered significant.

Results. Fifteen late proximal, 22 early distal, and 24 late distal tubular fluid samples were collected from 11 rats. Simultaneous clearance data were obtained for all rats. The animals remained in stable condition throughout the experiment as indicated by unaltered GFR, BP, HR, and Hct during the experiment. Urine flow and GFR were similar to those reported previously in rats undergoing saline diuresis (Table I). Mean digoxin clearance was significantly lower than the clearance of inulin (GFR) suggesting reabsorption of filtered digoxin. Fractional excretion of digoxin calculated with the assumption that the drug is freely filtered at the glomerulus, averaged 71.3 ± 2.9%. The micropuncture data are summarized in Table II. Tubular transit time of the green dye from the glomerulus to late distal sites was significantly greater than to early distal sites allowing distinction between these two locations along the nephron. Late proximal transit times were in the expected range for the given experimental condition. Mean tubular fluid-to-plasma inulin concentration ratio (F/P) at the late proximal tubular sampling site was slightly less than 2 indicating that nearly half of the filtrate (46.5%) was reabsorbed by the accessible portion of the proximal convoluted tubules. Digoxin F/P at the same site was always less than that of inulin and averaged 1.43. Mean proximal efflux of digoxin was calculated to be 19.2% of the amount filtered at the glomerulus. At early distal puncture sites inulin F/P increased to 4.99 indicating that the loop of Henle reabsorbed an additional 30% of the filtered water. Cumulative net reabsorption of digoxin in the proximal

TABLE II. MICROPUNCTURE DATA OF TUBULAR WATER AND DIGOXIN TRANSPORT IN DIFFERENT SEGMENTS OF THE RAT NEPHRON DURING ISOTONIC SALINE DIURESIS[a]

	Late proximal (11)[b]		Early distal (11)		Late distal (11)		Ureter (11)
Transit time (sec)	12.6 ± 0.5		36.1 ± 1.6		51.3 ± 2.1		—
P		<0.01		<0.01			
Inulin F/P[c]	1.96 ± 0.13		4.99 ± 0.72		7.63 ± 0.88		50.70 ± 7.47
P		<0.01		<0.05		<0.01	
Digoxin F/P	1.43 ± 0.22		3.28 ± 0.56		5.67 ± 0.41		34.72 ± 4.73
P		<0.01		<0.01		<0.01	
Percentage water reabsorbed	46.5 ± 3.4		75.9 ± 4.2		84.4 ± 6.3		97.5 ± 7.1
P		<0.01		<0.05		<0.01	
Percentage digoxin reabsorbed (net)	19.2 ± 3.2		35.8 ± 4.1		21.3 ± 4.7		28.7 ± 2.1
P		<0.05		<0.05		N.S.	

[a] Data are presented as mean ± SE.
[b] N, number of animals from which data were obtained.
[c] F/P, tubular fluid-to-plasma concentration ratio.

convoluted tubule and loop of Henle averaged 35.8% of filtered load (Table II). In the distal convoluted tubule, inulin F/P increased further, indicating continued water reabsorption. Digoxin F/P also increased from early to late segments of distal convoluted tubule but to a lesser extent than inulin F/P, resulting in a decrease in the calculated cumulative net digoxin reabsorption from early to late distal sites (35.8 vs 21.3%, respectively; see Table II). These results imply addition of digoxin to the tubular fluid along the distal convoluted tubule.

Although water reabsorption was marked in the remainder of the nephron, and urine to plasma inulin concentration ratio increased to 50.7, there was no evidence for net digoxin transport in or out of the collecting ducts. Digoxin concentration in the final urine increased to a similar extent as that of inulin, and cumulative digoxin reabsorption up to late distal sites was not significantly different from that observed at the ureter (Table II). Digoxin concentration 10–35 times higher in the lumen of collecting ducts than in peritubular blood failed to cause efflux of the drug indicating low permeability of this nephron segment to the compound. In Fig. 1, the percentage of the filtered water and digoxin that remained unabsorbed at different sites along the nephron is illustrated. The percentage of unabsorbed water declined along the entire length of the renal tubules and only 2.53 ± 0.3% of filtrate appeared in the urine. The fraction of filtered digoxin that remained in the lumen of the tubules also declined in a nearly linear fashion up to the early distal site. The amount of the drug present in late segments of the distal tubules was significantly greater than at early distal puncture sites indicating the influx of digoxin into the tubule. There is no evidence for transtubular digoxin transport in the collecting tubules in the face of marked water reabsorption.

Discussion. The primary mechanism responsible for the renal elimination of digoxin is filtration by the glomeruli. The drug is also subject partly to reabsorption (6) and secretion (2) by the renal tubules. Although tubular transport of digoxin has been known for some time, the exact nephron

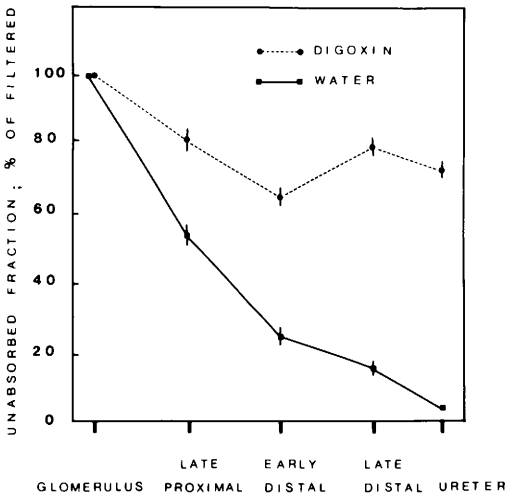

FIG. 1. Percentage of the filtered water and digoxin that remained in the tubular lumen at different sites along the nephron in rats in isotonic saline diuresis.

segments involved were not identified until recently. Roman and Kauker (4), using renal micropuncture techniques, have shown that the drug crosses the tubular epithelium in both directions: reabsorption is predominant in the proximal tubule, and there is a net transtubular influx of digoxin in more distal nephron segments. In the present investigations, the tubular transport of digoxin in the loop of Henle, in the distal convoluted tubule, and in the collecting duct was examined in rats made diuretic by iv infusion of isotonic saline. Reabsorption occurred principally in the proximal convoluted tubules and in the loop of Henle (most likely in the straight portion of the proximal tubule). Together these two nephron segments reabsorbed about 35% of the filtered digoxin. These data are consistent with our previous observations in hypertonic saline diuretic rats (4).

In the distal convoluted tubule digoxin was added to the tubular fluid in an amount equal to 15% of filtered load. This occurred in the face of continued water removal from the lumen of this nephron segment. At early distal puncture site, the concentration of digoxin inside the tubule was nearly four times higher than its estimated concentration in the peritubular blood. At late distal site, the ratio was close to 6. Movement of digoxin against such a high concentration

gradient suggests the operation of a carrier mediated or active transport system. These observations are consistent with an active distal tubular digoxin secretion suggested by Steiness (2), which can be blocked by pretreatment with spironolactone. An attempt to block the distal influx of the drug with spironolactone in these acute experiments was unsuccessful because of the hemodynamic side effects of spironolactone given rapidly in high doses. Passive distal entry of digoxin combined with ion trapping is also compatible with the present data. However, it is unlikely that such a mechanism alone accounts for the generation of the high concentration gradient across the distal epithelium observed in this study.

Under the present experimental conditions digoxin transport was negligible in the terminal portion of the nephron beyond the late distal puncture sites. The digoxin reaching the collecting tubules averaged 78.7 ± 4.8% of its filtered load. This was not significantly different from the percentage of filtered drug excreted in the urine. Although the concentration of digoxin was 34 times higher in the tubular lumen than in the peritubular capillaries, it did not diffuse out of the nephron suggesting that the tubular wall of the collecting duct is impermeable to the drug.

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1. Doherty, J. E., Amer. J. Med. Sci. **255**, 382 (1968).
2. Steiness, E., Circulation, **50**, 103 (1974).
3. Doherty, J. E., Ferrell, C. B., and Towbin, E. J., Amer. J. Med. Sci., **258**, 181 (1969).
4. Roman, R. J., and Kauker, M. L., Circ. Res. **38**, 185 (1976).
5. Kauker, M. L., J. Pharmacol. Exp. Ther. **200**, 81 (1977).
6. Halkin, H., Scheiner, L. B., Peck, C. L., and Melmon, K. L. Clin. Pharmacol. Therap. **17**, 385 (1975).

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