Urine Dilution and Concentration After Digoxin Infusion into the Renal Artery of Dogs[1] (34898)

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(Introduced by H. Brown)

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Previous studies have demonstrated that cardiac glycosides are diuretic in a variety of species (1–7). The sites of action in the nephron and the mechanisms underlying this effect, however, remain largely unknown.

We have recently shown that digoxin inhibits renal concentrating capacity and that this change is associated with a significant reduction of Na^+, K^+–ATPase activity in the medulla (8). In order to determine further the effects of digoxin on medullary sodium transport, we have investigated its action on renal diluting capacity (C_{H_2O}). We have also extended our observations of the effect of the glycoside on renal concentrating capacity.

Materials and Methods. Experiments were performed on 25 mongrel dogs of either sex. Surgical, analytical, and clearance methods have been previously described in detail (9). Digoxin (0.01 mg/ml) was infused at 1 ml/min into the left renal artery. During control periods saline was infused at the same rate. Animals received an average of 0.9 mg of digoxin as a total dose, which produced a diuresis in all animals. Studies were performed in two groups of dogs.

Group 1. Water diuresis studies (12 dogs). Food was removed at least 24 hr prior to the experiment. Water diuresis was induced by the oral administration of 30 ml/kg of body weight of water containing 20–40 ml of absolute alcohol 1 hr before the experiment. Eight animals then received 2.5% glucose in water intravenously at varying rates until urine flow and osmolality became stable. In four of these animals distal delivery was then increased by the infusion of hypotonic saline during the infusion of digoxin into the left renal artery. In four additional animals receiving only 2.5% glucose, the dose of digoxin infused was increased to 0.03 mg/ml and administered at 1 ml/min.

Group II. Solute diuresis studies (13 dogs). All animals were fasted and thirsted for at least 24 hr prior to the experiment. On the morning of the study all dogs received 5 U of pitressin tannate in oil intramuscularly 1 hr before commencing. In addition, the animals received 50–60 mU/kg/hr of aqueous pitressin throughout the duration of the experiment. A mannitol solution was infused at a varying rate in each animal in order to examine the effect of digoxin at varying rates of solute excretion.

Osmolar clearance (C_{osm}), free water clearance (C_{H_2O}) and free water reabsorption ($T^c_{H_2O}$) were all calculated by standard formulae.

Calculations and statistical analyses were performed by standard methods by the personnel of the Common Research Computer

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TABLE I. The Effects of Unilateral (Left) Infusion of Digoxin in the Hydropenic Dog.[a]

Time (min)	V (ml/min)		GFR (ml/min)		$U_{Na}V$ (μEq/min)		U_KV (μEq/min)		C_{osm} (ml/min)		$T^c_{H_2O}$ (ml/min)		C_{Na}/100 ml GFR (%)	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L
−90	Pitressin in oil 5 U im; anesthesia and surgical preparation													
−45	Priming dose of Glofil ^{125}I; begin sustaining infusion of Glofil, aqueous pitressin (50 mU/kg/hr) and mannitol (10%) 1 ml/min. Saline 1 ml/min into left renal artery													
0– 10	0.1	0.2	26	27	26	40	14	16	0.5	0.8	0.4	0.7	0.7	1.0
10– 20	0.1	0.2	25	30	41	58	23	24	0.9	1.0	0.8	0.8	1.0	1.3
20– 30	0.2	0.2	28	35	58	64	37	34	1.2	1.1	1.0	0.9	1.0	1.2
30– 35	Change solution infused into left renal artery for one containing digoxin 0.01 mg/ml and infuse at 1.0 ml/min													
35– 50	0.5	0.5	43	44	81	81	53	49	2.2	2.2	1.6	1.7	1.3	1.3
50– 65	0.5	0.5	42	35	77	75	46	33	2.0	1.8	1.5	1.3	1.3	1.5
65– 80	0.5	0.5	33	44	76	81	33	49	1.8	2.2	1.3	1.7	1.6	1.3
80– 95	0.5	0.5	37	35	68	75	31	33	1.8	1.8	1.3	1.3	1.3	1.5
95–110[b]	0.5	0.8	43	30	81	139	53	32	2.2	2.1	1.6	1.3	1.3	3.3
110–125[b]	0.5	1.6	42	30	77	189	47	34	2.0	3.0	1.5	1.4	1.3	5.6
125–140[b]	0.5	2.0	39	25	78	256	45	33	2.0	3.7	1.4	1.7	1.1	7.4

[a] GFR = glomerular filtration rate; $U_{Na}V$ = sodium excretion rate; U_KV = potassium excretion rate; V = urine flow rate; C_{osm} = osmolar clearance; $T^c_{H_2O}$ = solute-free water reabsorption; C_{Na}/100 ml GFR = fractional sodium excretion.

[b] Because of the abrupt unilateral increase in $U_{Na}V$, these periods were taken to represent the action of digoxin and termed Experimental Period II. The preceding periods were termed Experimental Period I.

Facility of the Texas Medical Center in Houston.

Results. A typical experiment depicting the effects of unilateral infusion of digoxin in the hydropenic dog is presented in Table I. Except for a bilateral rise in the glomerular filtration rate (GFR), no change in the parameters measured was noted after digoxin infusion until 60 min had elapsed (period from 95–110 min). After this latent period there was an increase in urine flow rate (V), urine sodium excretion ($U_{Na}V$), osmolar clearance (C_{osm}), and fractional sodium excretion (C_{Na}/100 ml GFR) on the infused side; solute-free water reabsorption ($T^c_{H_2O}$) and urine potassium excretion (U_KV), however, were unchanged.

We have arbitrarily defined Experimental Period 1 as those periods after the beginning of digoxin infusion when the mean of $U_{Na}V$ is not over one and one-half times that of the control values. Periods in which $U_{Na}V$ rose significantly were termed Experimental Period 2.

The results obtained during hydropenia were similar to those during water diuresis and mannitol (10%) infusion.

Effect of Digoxin on GFR, $U_{Na}V$, U_KV, and C_{Na}/100 ml GFR[5]. GFR remained unchanged, rose in three cases by an average of 17%, or dropped an average of 18% (range 0.6–55%) after the infusion of digoxin with no specific pattern to these changes. In all experiments, whether during hydropenia or water diuresis, digoxin induced a massive natriuresis manifested by a 1- to 20-fold increase in $U_{Na}V$ and C_{Na}/100 ml GFR.

During hydropenia urine potassium excretion rose in only six animals despite a consistent increase in $U_{Na}V$.

Figure 1 shows the relationship of U_KV to $U_{Na}V$ during digoxin infusion. As may be seen, at the range of $U_{Na}V$ where these data may be compared, U_KV was lower at any level of $U_{Na}V$ during digoxin effect.

[5] Tables with details of these results are available from the authors upon request.

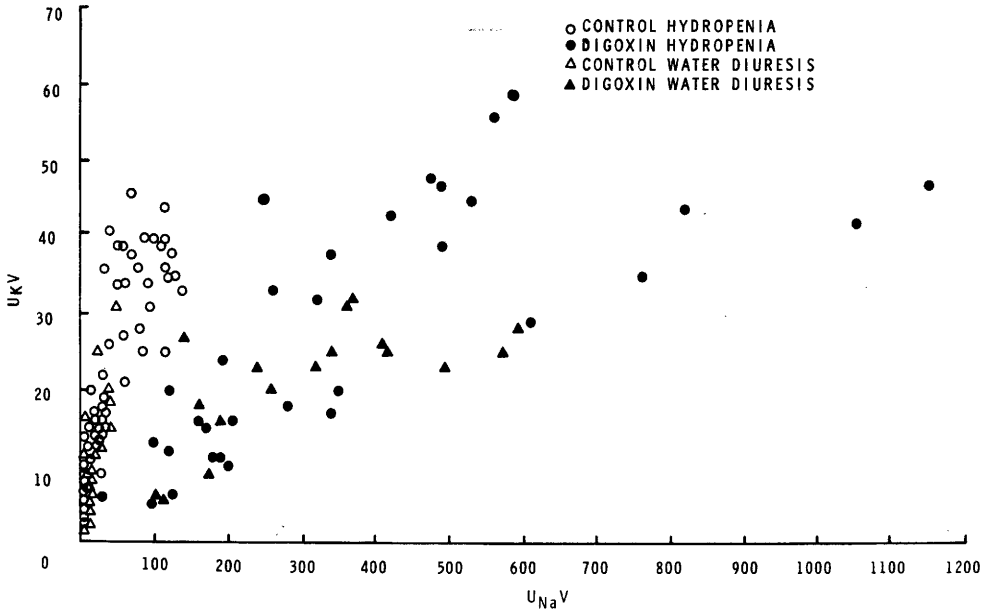

FIG. 1. Relationship between U_KV and $U_{Na}V$ during hydropenia and water diuresis before and after the effects of digoxin.

Higher doses of digoxin (0.03 mg/min) produced greater effect on sodium excretion, but otherwise its action was similar to that of the lower dose (Table II).

Effect of Digoxin on V, C_{osm}, $T^c_{H_2O}$ and C_{H_2O}. Digoxin infusion resulted in an increase in V and in C_{osm} in all experiments. In spite of the sharp increases in C_{osm}, $T^c_{H_2O}$ fell, re-

TABLE II. Effect of High Doses of Digoxin on GFR and Electrolyte Excretion During Water Diuresis.[a,b]

Dog no.		GFR (ml/min) R	GFR (ml/min) L	$U_{Na}V$ (μEq/min) R	$U_{Na}V$ (μEq/min) L	U_KV (μEq/min) R	U_KV (μEq/min) L	C_{Na}/100 ml GFR (%) R	C_{Na}/100 ml GFR (%) L
22	Cont.	38	37	11	16	20	19	0.2	0.3
	Exp. 1	38	31	11	15	19	20	0.2	0.4
	Exp. 2	34	27	37	540	26	31	0.4	29.7
23	Cont.	32	29	25	18	12	13	0.6	0.4
	Exp. 1	28	25	24	29	16	18	0.7	0.8
	Exp. 2	26	18	12	623	18	32	0.3	35.6
24	Cont.	34	33	7	10	34	35	0.1	0.2
	Exp. 1	35	33	8	8	44	44	0.2	0.2
	Exp. 2	39	29	43	741	86	53	0.8	24.9
25	Cont.	25	25	21	22	10	10	0.7	0.8
	Exp. 1	24	18	21	28	11	10	0.7	1.3
	Exp. 2	18	14	18	403	13	17	0.8	45.5

[a] All values are the means of three to eight periods.

[b] Since the effect of digoxin was progressive, the maximal value for fractional sodium excretion is given. The animals in this group received 0.03 mg/min of digoxin into the left renal artery.

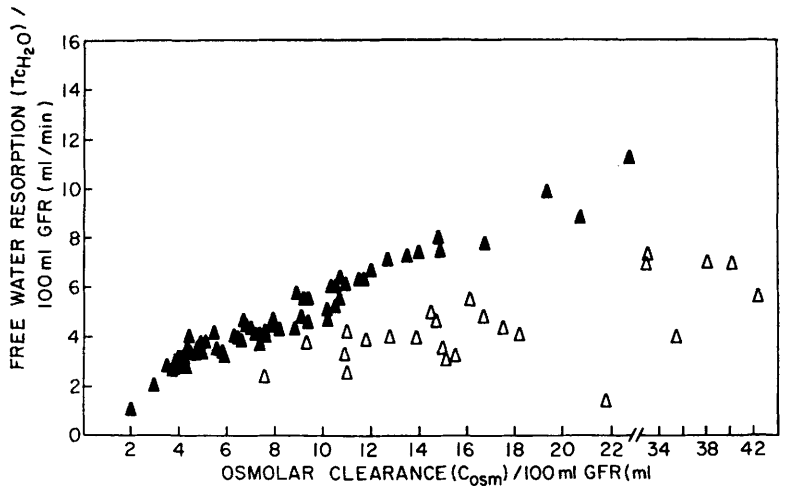

FIG. 2. Relationship between $T^c_{H_2O}$/100 ml GFR and C_{osm}/100 ml GFR. Solid triangles represent control points; open triangles, periods during the infusion of digoxin.

mained unchanged, or rose slightly. However, when the effect of digoxin on the relation of $T^c_{H_2O}$ to C_{osm} was examined, a distinct inhibition of $T^c_{H_2O}$ was seen (Fig. 2). Although both control and experimental points describe a curvilinear relationship between these two functions, $T^c_{H_2O}$ on the experimental side is clearly below the control values throughout the range of C_{osm} examined.

Distinct rises in V above absolute control values occurred in all of the experiments during water diuresis. This was also the case when V was expressed as a fraction of the glomerular filtration rate.

Digoxin resulted in a fall in C_{H_2O} in all experiments. The relationship of C_{H_2O} to V, as distal delivery is increased, is depicted in Fig. 3. Free water clearance during the digoxin effect was distinctly lower than control at all levels of V. In two instances the urine became hypertonic to plasma, resulting in negative values for C_{H_2O}.

Discussion. Infusion of digoxin into one renal artery was followed by a massive natri-

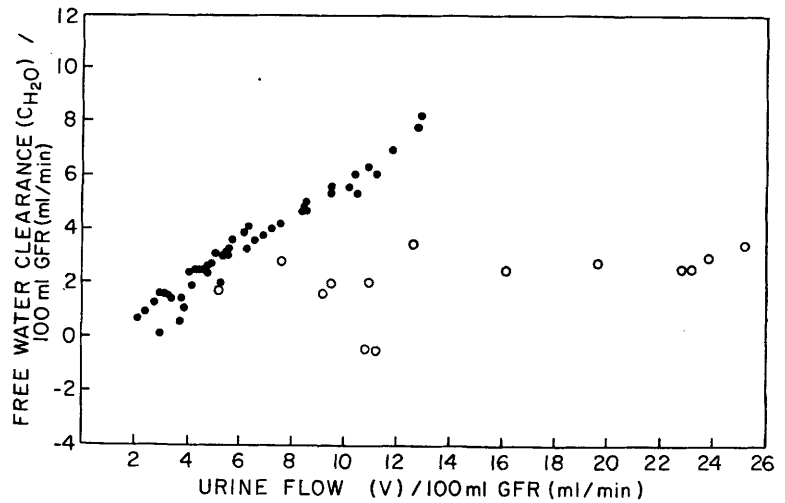

FIG. 3. Relationship between C_{H_2O}/100 ml GFR and V/100 ml GFR. Solid circles represent control periods; open circles, periods during digoxin infusion.

uresis from that kidney in all experiments, which in some instances amounted to as much as 45% of the filtered load. Previous studies have suggested that the natriuretic action of digitalis is a result of inhibition of sodium reabsorption in the proximal and distal convolutions of the nephron (1, 4, 10). Our results show conclusively that digoxin inhibits sodium reabsorption in the loop of Henle. An effect of digitalis at this site was determined by examining the capacity of the infused kidney to produce free water (C_{H_2O}) during water diuresis and to reabsorb free water (T^cH_2O) during hydropenia and solute diuresis. Free water clearance is principally an indirect measure of sodium reabsorption in the distal nephron, primarily the ascending limb of Henle's loop. On the other hand, T^cH_2O, in addition to its dependence on the maintenance of a hypertonic medullary interstitium as a result of sodium reabsorption in the ascending limb of Henle's loop, also requires the action of ADH on collecting-duct permeability. Therefore, inhibition of sodium transport in the medulla, where the ascending limb is located, should lead to a depression of both renal diluting and concentrating capacity. Digoxin inhibited C_{H_2O} at any level of distal delivery (V) examined (Fig. 3) when the infused kidney was compared to the control organ. Similarly, T^cH_2O was sharply reduced at all levels of C_{osm} when the experimental kidney was compared to the control (Fig. 2). Consequently, these results indicate that digoxin inhibits sodium transport in the ascending limb of the loop of Henle.

Furthermore, the data obtained in animals undergoing water diuresis suggest that proximal tubular reabsorption of salt and water is also depressed by digoxin. Water diuresis leads to relative impermeability of the distal nephron to water; therefore, urine flow (V), except for back diffusion of water into the medullary interstitium, is a close approximation of the amount of fluid leaving the proximal tubule. As a result, any increments in urine flow during water diuresis must represent inhibition of Na^+ reabsorption in the proximal convolution, resulting in increased delivery of Na^+ from that segment to more distal nephronal sites (11). During water diuresis digoxin increased V in all experiments, strongly suggesting that inhibition of the proximal tubule did occur.

In order to determine with more certainty whether digoxin has such an effect, in four experiments during water diuresis the dose of digoxin infused was tripled (0.03 mg/ml infused at 1 ml/min). C_{Na}/100 ml GFR was always above 25% and in one instance almost 50% (Table II). These results are further evidence that digitalis may inhibit proximal tubular reabsorption. Results similar to these have been obtained by Hendler and his collaborators (12).

An action of the glycoside on sodium reabsorption is the distal convolution cannot be determined directly from the present experiments. Although sodium reabsorption in the distal convolution of the dog as measured by micropuncture techniques is small (13), its inhibition may have contributed to the natriuresis observed in the present experiments. Evidence for an effect of digoxin on this segment, however, can be deduced from the effects of the drug on potassium excretion. At any level of sodium excretion, less potassium was excreted in the urine during digitalis action (Fig. 1). Similar results have been reported by Cade *et al.* (1).

In view of our recent demonstration that inhibition of medullary Na^+, K^+–ATPase accompanies the changes in renal concentrating capacity (8), the present experiments advance further evidence that the enzyme may be of major importance in sodium reabsorption by the kidney and plays a fundamental role in the process of concentration and dilution of the urine.

Summary. The present studies extend our observations of the effect of digoxin on renal concentrating capacity and, in addition, investigate its action on renal diluting capacity. Infusion of digoxin into a renal artery of dogs undergoing water diuresis resulted in increases in fractional urine flow (V/100 ml GFR) and fractional sodium clearance (C_{Na}/100 ml GFR) to levels which could not be accounted for simply by distal inhibition, suggesting that the glycoside suppressed

proximal reabsorption as well. Furthermore, C_{H_2O} was inhibited at any level of V/100 ml GFR examined. These effects of digoxin on $T^c_{H_2O}$ and C_{H_2O} indicate that it inhibits sodium transport in the ascending limb of Henle's loop. In view of our previous demonstration that the digoxin inhibition of $T^c_{H_2O}$ is accompanied by significant depression of Na^+, K^+–ATPase activity from medullary renal tissue, the present experiments are further evidence that the enzyme plays a fundamental role in the mechanism of urine concentration and dilution.

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