The Effect of Ouabain on Renal Tubular Reabsorption and Cortical Concentrations of Several Cations and on Their Association with Subcellular Particles

VICTOR E. NAHMOD AND MACKENZIE WALSER

Department of Pharmacology and Experimental Therapeutics and Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

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SUMMARY

Ouabain was infused into one renal artery at 5 or 10 μg/kg/min for 5 or 20 min, in dogs receiving a constant infusion of ^{137}Cs and ^{85}Sr. Plasma K and Cs rose at 20 min, but plasma Na, Mg, Ca, and Sr were unaffected. Renal cortical K became partially replaced with Na and Mg with Ca, even in the uninfused kidneys. Cortical Cs, however, rose at first and later fell. At the smaller dose, tubular reabsorption of all six cations was simultaneously inhibited in the infused kidney and stimulated in the uninfused kidney. At the larger dose, a disproportionate inhibition of Cs reabsorption occurred, while alkaline earth reabsorption was inhibited relatively little, when compared with Na and K. The extent of association of these cations with four particulate fractions obtained by differential centrifugation of homogenized cortex was measured in the same animals. Changes were seen which could be tentatively correlated with the changes in transport. Addition of comparable amounts of ouabain to normal kidneys during homogenization in the cold had no such effects on subcellular binding.

The results suggest that ouabain in small doses may stimulate tubular transport by increasing cation binding by particles found in the fourth ("light microsomal") sediment; inhibition of cellular accumulation of cations and later of transport may be a result of altered selectivity of particles found in the third ("heavy microsomal") sediment for ions in relation to their size.

INTRODUCTION

At toxic levels, cardiac glycosides have unequivocal effects on the ionic composition of cardiac muscle, characterized by an increase in cellular sodium and calcium and a decrease in potassium (1, 2). Similar changes have been shown to occur in a variety of excised tissues when exposed to high concentrations of glycosides (10^{-6} M or greater) (3). At these dose levels, the rate of transport of sodium (3) and calcium ions (4–6) across epithelial membranes is also inhibited; effects on transepithelial potassium transport are rather unpredictable. At these same levels, the sodium- and potassium-stimulated splitting of ATP by enzyme preparations from such tissues is also inhibited (3, 7). At low levels of drug, however, ATP-splitting is enhanced, and ion transport across epithelial membranes may be facilitated (8–11). Whether positive inotropic effects are accompanied by enhanced potassium concentrations and decreased calcium and sodium concentrations in the heart is as yet uncertain. A mechanism responsible for these actions which has been

suggested (see reference 3) is an interaction between the drug and sites on membranes, leading to altered cation binding. Direct evidence indicating an effect of cardiac glycosides on cation binding by subcellular structures is minimal. In a preliminary report, Luckenbach and Lüllman (12) reported that calcium binding by a membrane-containing preparation from heart muscle was diminished by ouabain. Other studies on heart muscle (13), skeletal muscle (14), and erythrocyte membranes (15) have been negative.

Although emphasis has usually been placed upon changes in sodium, potassium, and calcium metabolism in response to these drugs, renal tubular transport and renal cortical concentrations of other cations are also affected (see below). It is conceivable, therefore, that cardiac glycosides alter the affinity of membrane sites so that some cations are more firmly bound than others; i.e., the steric requirements of ion binding might be changed. Although there is no reliable method for measuring the amount of cation binding by subcellular structures which occurs *in vivo*, a procedure for determining the relative binding of related ions is presented in the accompanying paper (16). In the present study, this procedure was applied to the analysis of renal cortex for six cations following ouabain administration, and an attempt was made to correlate the results with observed changes in tubular transport and tissue concentrations of the same cations.

METHODS

Mongrel dogs of both sexes weighing 15–22 kg were anesthetized with sodium pentobarbital, 30 mg/kg. Body temperature was maintained by means of an electrically heated table. Priming doses and a constant infusion of inulin, ^{137}Cs and ^{85}Sr in 5% glucose was administered at 2 ml/min for an equilibrium period of 2 hr. The isotopes were of high specific activity and the amounts of carrier given amounted to less than 2 μmoles in each dog. During the equilibration period, the abdomen was opened and a specially constructed 22-gauge steel cannula 35 cm long, sheathed in polyethylene and bent at the tip, was inserted into the right femoral artery and passed up the aorta into the left renal artery, using a hand in the abdomen to guide it. The polyethylene sheath was then withdrawn a few centimeters. Both ureters were then cannulated, and the abdomen was closed with clips. The renal artery was perfused through the cannula with 5% glucose at 2 ml/min. An injection of phenol red into the perfusion fluid served to verify the position of the catheter, as the dye appeared in the urine from the left kidney only. In nine control dogs, both kidneys were removed at the end of the equilibration period. In four other control dogs, blood samples were obtained 5 and 20 min later. In 12 experimental dogs, ouabain was added to the perfusion fluid at the end of the equilibration period.

Three groups of four dogs each received the drug. The first group received 10 μg/kg/min for 5 min. The other two received 5 and 10 μg/kg/min, respectively, for 20 min. At the end of these intervals, both kidneys were removed.

In the second two groups, clearances were measured in four consecutive 10-min periods, two preceding and two during drug administration. Blood samples were obtained at the midpoint of each period in these groups, and at the time of removal of the kidneys in all.

Both kidneys were promptly cooled, homogenized in 0.25 M sucrose, separated into four sediments and four supernatants, and analyzed for all six cations by methods presented elsewhere (16). Controls consisted of nine normal dogs; in four both cortices were homogenized together; in the other five 25 μg of ouabain was added to one kidney during homogenization. Statistical methods employed are given by Fisher (17).

RESULTS

Effect of Ouabain on Plasma Cation Concentrations

Average plasma concentrations in control dogs were: Na, 143 mM; K, 3.88 mM; Mg, 0.75 mM; Ca, 2.52 mM. Changes induced

by ouabain were calculated for these four cations and also for the two ions determined isotopically as percentage change in each dog at two intervals after drug administration (5 and 20 min) compared with the average of two control periods. The results are shown in Fig. 1. Significant increases ($P < 0.01$) occurred in the plasma

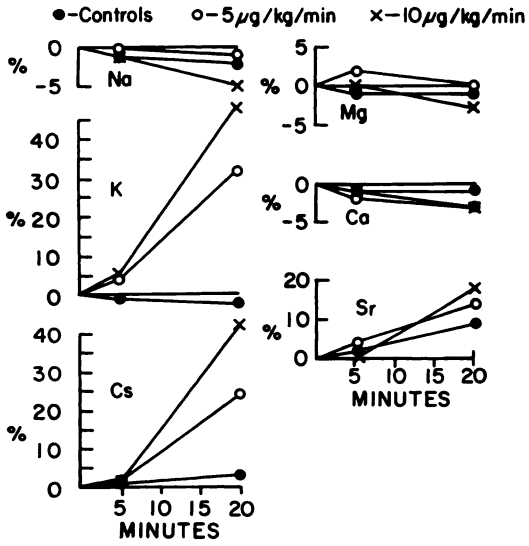

FIG. 1. *Changes in plasma cation concentrations following ouabain*

Per cent change from control values in three groups of four animals each are shown. ^{137}Cs and ^{85}Sr were administered by priming dose and constant infusion for 2 hr before the injections. Only Cs and K concentrations increased significantly in the two groups receiving ouabain, when compared to the control group. Note the different scales employed.

concentrations of potassium and cesium, when compared with values for dogs not given ouabain. Strontium concentration increased somewhat both in drug-infused and control dogs, indicating merely that the size of the priming dose given was somewhat too small in relation to the rate of constant infusion of the isotope. No significant changes occurred in the other cations.

Effect of Ouabain on Renal Clearances

A representative experiment at the smaller dose is shown in Fig. 2. The ex-

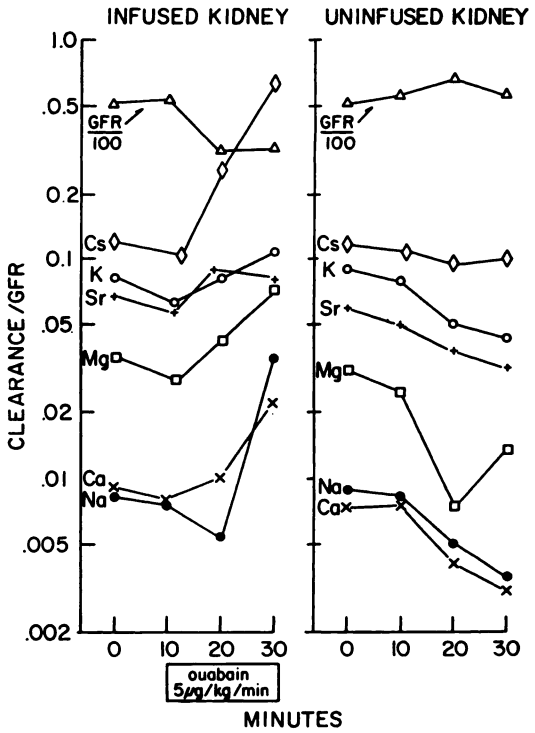

FIG. 2. *Effect of ouabain infusion into one renal artery on cation clearances*

Excreted:filtered ratios of six cations and inulin clearance shown on a logarithmic scale during two control periods and two periods of drug infusion. Reabsorption is inhibited in the injected kidney and stimulated on the contralateral kidney, despite opposite changes in glomerular filtration rate (GFR).

creted:filtered ratios of all six cations and the glomerular filtration rate (GFR) are shown on a uniform logarithmic scale in order to emphasize relative changes. In the infused kidney, tubular reabsorption of all six cations diminished and GFR fell moderately. In the contralateral kidney, reabsorption of all six cations increased; GFR showed little change.

Table 1 summarizes the results in all eight dogs. At the lower dose, increases in flow and in the clearances of all the cations were seen in the infused kidney. GFR fell. Decreased flow and decreased clearances of all six cations were seen contralaterally, and GFR rose. At the larger dose, GFR fell considerably on the infused side, and excreted:filtered ratios rose even more.

TABLE 1

Effect of ouabain on flow, glomerular filtration rate, and excreted:filtered ratios of six cations[a]

Dose rate (μg/kg/min)	Time (min)	Mean per cent change (n = 4)															
		Flow		GFR		Na		K		^{137}Cs		Mg		Ca		^{85}Sr	
		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
5	0–10	−11	−25	−12	+27	−35	−45	−2	−31	+44	−11	+13	−26	+18	−25	+26	−15
5	10–20	+125	−22	−35	+20	+190	−40	+36	−51	+285	−14	+126	−25	+118	−25	+58	−28
10	0–10	−5	+23	−28	+10	+229	−22	−3	−11	+74	−6	−29	−32	−16	−24	−40	−5
10	10–20	+16	+95	−73	−6	+2205	+142	+49	+2	+615	+33	+93	−8	+581	−11	+43	−34

[a] Per cent change from average of two control periods.

The greatest increments were seen in sodium, cesium, and calcium clearance. On the contralateral side, variable responses occurred. Thus biphasic effects on ion transport were obtained simultaneously at the lower dose; intermediate responses occurred on the contralateral side at the larger dose. Since primary alterations in GFR are generally accompanied by changes in excreted:filtered cations in the same direction, the changes in tubular reabsorption seen here cannot be attributed to changes in GFR, but represent a primary tubular effect.

Walser and Trounce (4) reported parallel increments following ouabain in sodium and calcium clearance in the dog, and Vogel *et al.* (5) made similar observations in frogs, but Kupfer and Kosovsky (6) found that dogs given strophanthin or digoxin exhibited increases in calcium and magnesium clearance disproportionately greater than the increase in sodium clearance.

One way to compare relative clearances of two solutes is in terms of a parameter, defined as $k = \log(\text{excreted:filtered A})/\log(\text{excreted:filtered B})$, which expresses in simplified mathematical models the local tubular transport rate constant of A relative to that of B (18–20). When clearances of cations are varied together, as for example by osmotic diuresis, calculated values of k for any pair of cations tend to remain constant. The theoretical basis of this finding is only partially clarified (21); nevertheless this approach provides a useful means for comparing changes in clearance of two or more substances.

Since cardiac glycosides affect sodium–potassium exchange (see reference 3), it is not reasonable to assume that the contribution of distal exchange to excreted sodium and potassium remains constant after ouabain. An estimate of the quantity of sodium unreabsorbed can be made by assuming that all the excreted potassium was derived by secretion in a one-for-one exchange with sodium distally. Then excreted sodium plus excreted potassium is equal to unreabsorbed sodium, i.e., the quantity of sodium delivered to the distal exchange site. Although this assumption is probably incorrect as regards the source of urinary potassium under normal conditions (22), quantitatively it may be close to correct as regards the rate of distal delivery of sodium. This quantity of sodium, divided by the rate of filtration of sodium was used as a referent for the excreted:filtered ratios of the remaining four cations.

The results are shown in Table 2. In control periods, tubular transport rates of these

TABLE 2

Effect of ouabain on tubular transport rates of cesium, magnesium, calcium, and strontium expressed in relation to tubular transport rates of sodium plus potassium (means ± SEM)

	Cs		Mg		Ca		Sr	
Control periods	0.47 ± 0.02		0.55 ± 0.03		0.90 ± 0.02		0.51 ± 0.03	
	Left	Right	Left	Right	Left	Right	Left	Right
5 μg/kg/min								
10 min	0.42 ±0.07	0.50 ±0.07	0.55 ±0.01	0.64 ±0.12	0.81 ±0.03	0.91 ±0.06	0.50 ±0.09	0.53 ±0.03
20 min	0.30[c] ±0.09	0.47 ±0.02	0.56 ±0.07	0.57 ±0.08	0.90 ±0.04	0.86 ±0.06	0.61 ±0.08	0.51 ±0.02
10 μg/kg/min								
10 min	0.37[b] ±0.04	0.43 ±0.02	0.68 ±0.07	0.62 ±0.10	1.06[c] ±0.08	0.93 ±0.03	0.69[b] ±0.06	0.58 ±0.07
20 min	0.15[a] ±0.04	0.42[c] ±0.00	0.77[b] ±0.09	0.59 ±0.07	1.17[c] ±0.15	1.00 ±0.08	0.92[a] ±0.03	0.61 ±0.04

[a] $P < 0.01$. [b] $P < 0.02$. [c] $P < 0.05$.

four cations, relative to sodium (plus potassium), were quite constant. Mean values differ only slightly from those reported previously (20, 22). The only significant change which occurred following the smaller dose was a decrease in the relative transport rate of cesium. At the larger dose this decrease became quite pronounced and was seen bilaterally. Significant increases in the relative transport rates of calcium, magnesium, and strontium were seen in the infused kidneys.

It should be reemphasized that these values express reabsorptive rates in relative, not absolute, terms. Thus the pronounced decrease in k for cesium signifies that its clearance increased much more than that of sodium; alkaline earth clearance, on the other hand, increased relatively little.

Effects of Ouabain on Cortical Cation Concentrations

Measurements of cation concentrations per kilogram of wet tissue, including tissue-to-plasma ratios of cesium and strontium, are summarized in Table 3. Within 5 min, sodium rose on the infused side and cesium rose on both sides. This significant effect on the contralateral kidney is remarkable in view of the fact that the total dose was only 50 μg/kg and a substantial portion of this must have been removed during passage through the infused kidney.

After 20 min infusion at the smaller dose, sodium rose on the infused side and potassium fell on both sides. At this dose, tubular reabsorption on the contralateral side was stimulated, as noted above. Magnesium fell on the infused side at both doses. The effects on the contralateral kidney at the larger dose resemble those on the ipsilateral kidney at the smaller dose. Calcium rose at the larger dose, but strontium did not.

As shown in Fig. 3, these effects on the major cations can best be summarized as a progressive replacement of tissue potassium with sodium and tissue magnesium with calcium. Total monovalent cation and total divalent cation remained nearly constant.

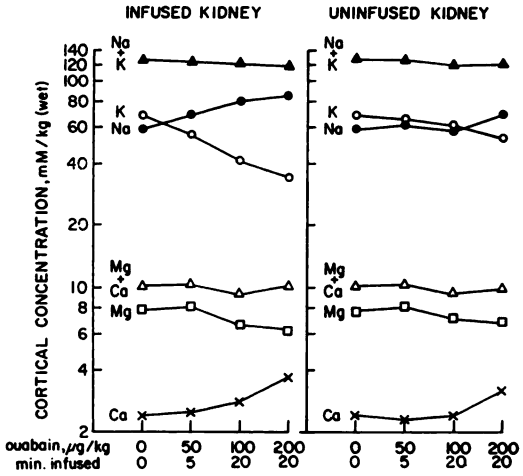

FIG. 3. *Effect of ouabain infusion into one renal artery on cortical cation concentrations*

Mean of four animals each, shown on a logarithmic scale, at two dosage rates and two time intervals.

Changes in Sediment Volumes and Particle Weights

Addition of ouabain to one kidney during homogenization did not alter any of the four sediment volumes or particle weights, when the results were compared with the contralateral control kidney in each of five normal dogs. Intraarterial infusion of ouabain reduced the volume and particle weight of the "nuclear" sediment but did not alter the others. However, there was no significant difference between infused and uninfused kidneys. Consequently estimates of sediment volumes and particle weights in each dog receiving ouabain *in vivo* were made using combined information from analysis of both kidneys. In this way more reliable values were obtained.

Absence of Effects of Ouabain Added in Vitro

In five normal dogs, adidtion of 25 μg of ouabain to one kidney during homogenization had no significant effects on the association of cations with subcellular particles. The concentration of ouabain in the whole homogenate was 5×10^{-7} M.

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TABLE 3

The effect of ouabain infusion into the left renal artery on tissue concentrations of Na, K, Mg, and Ca, and tissue:plasma ratios of Cs and Sr

	Na (mmole/kg)		K (mmole/kg)		Cs tissue:plasma		Mg (mmole/kg)		Ca (mmole/kg)		Sr tissue:plasma	
Controls (6–9)	58.8 ± 0.9		68.8 ± 1.1		31.0 ± 4.1		7.82 ± 0.13		2.40 ± 0.04		0.50 ± 0.03	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
10 μg/kg/min for 5 min (4)	68.8[b] ±1.5	61.5 ±1.5	54.8 ±2.7	65.9 ±2.3	50.4[a] ±5.8	49.9[a] ±5.6	8.15 ±0.234	8.07 ±0.074	2.48 ±0.10	2.33 ±0.08	0.53 ±0.03	0.55 ±0.04
5 μg/kg/min for 20 min (4)	79.5[b] ±6.9	57.6 ±2.6	41.3[b] ±4.9	62.4[b] ±1.3	32.6 ±2.6	39.5 ±4.5	6.6[b] ±0.298	7.22 ±0.238	2.81 ±0.08	2.43 ±0.27	0.63 ±0.06	0.59 ±0.05
10 μg/kg/min for 20 min (4)	85.0[b] ±3.2	70.4[a] ±1.2	34.2[b] ±1.0	52.7[b] ±2.5	24.4[c] ±3.2	34.2 ±4.9	6.3[b] ±0.090	6.8[b] ±0.188	3.72[b] ±0.46	3.15[a] ±0.43	0.58 ±0.04	0.55 ±0.04

[a] $P < 0.05$. [b] $P < 0.01$. [c] $P < 0.01$ for paired difference, left vs. right.

Association of Cations with the First ("Nuclear") Particulate Fraction (Table 4)

The percentage of total cortex cations associated per unit weight of these particles was not affected at 5 min, but increased significantly for all the cations except sodium at 20 min. Combined results from all four groups of kidneys in dogs receiving the drug for 20 min show that the average increase is 62% for potassium, 112% for cesium, 39% for magnesium, 77% for calcium, 55% for strontium, and only 28% for sodium.

Association of Cations with the Second ("Mitochondrial") Particulate Fraction (Table 5)

Here again the monovalent cations were increased, even as early as 5 min in the case of potassium. In general, greater increments were seen in the infused kidneys, and again cesium was the most profoundly affected. The fraction of divalent cations associated with these particles was not altered significantly, despite the substantial increase in total tissue calcium and fall in tissue magnesium.

Association of Cations with the Third ("Heavy Microsomal") Particulate Fraction (Table 6)

In this fraction there was a change in the selectivity among cations. In controls there was a slight tendency for increasing binding with atomic size in both groups. After 20 min infusion of ouabain, this trend was reversed and the largest cations of both groups became the least bound. Significant changes are seen in cesium, calcium, and strontium.

Association of Cations with the Fourth ("Light Microsomal") Particulate Fraction (Table 7)

Significant early increases in sodium and potassium and suggestive increases in cesium, magnesium, and calcium were seen in these particles. When the means of the infused kidneys are compared with those of the contralateral kidneys, the former are less than the latter in 15 of the 18 groups, although the paired difference between opposite kidneys is of probable statistical significance only in two groups. The most striking increase (160%) is in sodium in the uninfused kidneys at the intermediate dose. No significant decreases occurred in any of the ions. The mean increase in all the experimental kidneys is 65% for sodium, 64% for potassium, 37% for cesium, but only 6% for magnesium, 2% for calcium, and 1% for strontium. Selectivity within each group changed little.

In summary, the earliest effect of ouabain on the association of cations with these particles was an increase, especially in monovalent cations, in the second and fourth particulate fractions. Later, an increase was seen in all the cations except sodium in the first particulate fraction, and some dimunition, particularly of the larger cations in each group, in the third, particulate fraction.

DISCUSSION

Profound alterations in the transport and distribution of all six of these cations were induced by ouabain, even in the uninfused kidneys. Strickler and Kessler (23) found that strophanthidin altered clearances only in the injected kidney, and inferred that most of the drug was largely removed on passage through the kidney. If this was the case in the present experiments, the dose received by the contralateral kidney must have been extremely small. At 10 μg/kg/min, toxic effects on the heart in dogs can be anticipated at about 40 min (24). Thus no cardiac toxicity should have occurred even if our largest dose had been given intravenously. Yet changes in tissue concentrations were seen in the contralateral kidney at half this dose at 20 min. It appears that the sensitivity of the kidney to ouabain in the dog, measured in terms of tissue cation concentrations, far exceeds the sensitivity of the heart to this drug. Ionic alterations can therefore be studied at relatively small doses and early time intervals. Furthermore, the absence of a contractile mechanism, as in the heart, and the presence of an ion transport system whose activity can be quantitated should simplify the interpretation of the results.

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TABLE 4

Effect of ouabain infusion into the left renal artery on the association of cations with the first ("nuclear") particulate fraction

	Fraction of total cortex cation associated per gram of particles per gram of tissue											
	Na		K		Cs		Mg		Ca		Sr	
Controls (5–9)	0.73 ± 0.04		0.82 ± 0.03		0.81 ± 0.15		3.51 ± 0.14		3.14 ± 0.19		2.70 ± 0.16	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1) μg/kg/min for 5 min (4)	0.74 ±0.05	0.68 ±0.06	0.95 ±0.08	0.86 ±0.06	1.09 ±0.12	1.22 ±0.21	3.72 ±0.28	3.64 ±0.25	3.30 ±0.15	2.95 ±0.16	2.95 ±0.30	2.58 ±0.47
5 μg/kg/min for 20 min (3)	0.86 ±0.06	1.06 ±0.14	1.29[a] ±0.13	1.19[a] ±0.01	1.83[a] ±0.17	1.81[a] ±0.21	5.18[a] ±0.63	5.06[a] ±0.33	6.73[a] ±1.03	4.56[a] ±0.44	3.82 ±0.62	4.76[a] ±0.20
10 μg/kg/min for 20 min (4)	0.79 ±0.06	1.04 ±0.30	1.39[a] ±0.19	1.44 ±0.47	1.83[a] ±0.16	1.39 ±0.20	4.84 ±0.95	4.41 ±0.64	5.45[b] ±1.18	5.55 ±1.81	4.58[a,c] ±0.20	3.62[a,c] ±0.21

[a] $P < 0.01$. [b] $P < 0.02$. [c] $P < 0.01$ for paired difference, left minus right.

TABLE 5

Effect of ouabain infusion into the left renal artery on the association of cations with the second ("mitochondrial") particulate fraction

	Fraction of total cortex cation associated per gram of particles per gram of tissue											
	Na		K		Cs		Mg		Ca		Sr	
Controls (5–9)	0.82 ± 0.02		2.63 ± 0.08		6.48 ± 0.64		7.29 ± 0.32		14.73 ± 0.94		28.52 ± 2.57	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
10 μg/kg/min for 5 min (4)	0.84 ±0.08	0.78 ±0.10	3.43[a] ±0.37	3.03 ±0.26	6.83 ±10.00	5.97 ±0.70	7.82 ±0.82	8.05 ±0.93	16.27 ±1.98	16.20 ±1.99	27.65 ±4.52	29.50 ±5.15
5 μg/kg/min for 20 min (3)	1.11[a] ±0.09	0.96[a] ±0.02	3.86[a] ±0.55	3.09[c] ±0.27	9.09[d] ±0.98	7.49[d] 1.24	9.42 ±1.62	9.14 ±1.14	16.21 ±0.20	17.80 ±2.16	32.00 ±3.44	34.63 ±5.52
10 μg/kg/min for 20 min (4)	1.12[b] ±0.10	1.18 ±0.24	3.93[a] ±0.33	3.36[b] ±0.40	7.14 ±1.01	6.59 ±1.02	8.58 ±0.99	8.16 ±0.95	17.28 ±1.70	15.52 ±1.79	28.38 ±4.32	29.78 ±4.67

[a] $P < 0.01$. [b] $P < 0.02$. [c] $P < 0.05$. [d] $P < 0.02$ for paired difference, left minus right.

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TABLE 6

Effect of ouabain infusion into the left renal artery on the association of cations with the third ("heavy microsomal") particulate fraction

	Fraction of total cortex cation associated per gram of particles per gram of tissue											
	Na		K		Cs		Mg		Ca		Sr	
Controls (5–9)	2.67 ± 0.18		2.05 ± 0.20		3.14 ± 0.67		8.98 ± 0.80		10.00 ± 1.58		11.50 ± 2.36	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
10 μg/kg/min for 5 min (4)	2.03[b] ±0.19	2.62 ±0.27	1.51 ±0.24	1.77 ±0.20	3.40 ±0.87	3.11 ±1.40	7.81 ±0.48	8.54 ±0.32	9.17 ±2.09	9.43 ±0.47	6.45 ±2.31	7.48 ±0.54
5 μg/kg/min for 20 min (3)	2.12 ±0.19	3.50 ±0.92	1.68 ±0.16	1.92 ±0.43	0.75[a] ±0.42	2.72 ±0.45	7.82 ±1.46	9.84 ±1.89	6.22[c] ±1.46	11.21[c] ±2.98	8.01 ±2.94	4.71 ±2.18
10 μg/kg/min for 20 min (4)	2.60 ±0.66	2.86 ±0.83	1.83 ±0.24	1.73 ±0.33	1.51 ±0.46	1.49 ±0.17	8.53 ±2.08	8.38 ±1.45	5.22 ±1.52	6.81 ±1.60	3.78[a] ±0.22	4.55 ±0.74

[a] $P < 0.02$. [b] $P < 0.05$. [c] $P < 0.02$ for paired difference, left minus right.

TABLE 7

Effect of ouabain infusion into the left renal artery on the association of cations with the fourth ("light microsomal") particulate fraction

	Fraction of total cortex cation associated per gram of particles per gram of tissue											
	Na		K		Cs		Mg		Ca		Sr	
Controls (5–9)	1.70 ± 0.16		1.17 ± 0.08		2.18 ± 0.23		9.70 ± 0.63		5.51 ± 0.22		2.73 ± 0.54	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
10 μg/kg/min for 5 min (4)	2.36[b] ±0.11	2.02 ±0.23	1.50 ±0.20	1.73[a] ±0.10	2.57 ±0.91	3.34[b] ±0.33	8.86 ±0.49	10.22 ±0.75	6.06 ±0.82	6.60[b] ±0.44	2.57 ±0.12	3.20 ±0.76
5 μg/kg/min for 20 min (3)	2.53[c] ±0.73	4.43[a,c] ±0.92	1.72[a] ±0.17	1.87 ±0.58	3.07 ±1.92	2.13 ±1.49	9.14[c] ±2.94	12.88[c] ±4.93	4.54 ±1.40	5.83 ±0.96	2.52 ±0.90	4.66 ±2.34
10 μg/kg/min for 20 min (4)	2.51 ±0.72	2.94 ±0.93	1.65 ±0.33	1.65 ±0.39	3.01 ±1.14	3.83 ±0.95	10.34 ±2.57	10.38 ±3.05	5.08 ±0.94	5.71 ±1.40	1.28 ±0.46	2.32 ±0.46

[a] $P < 0.01$. [b] $P < 0.05$. [c] $P < 0.05$ for paired difference, left minus right.

In the present studies, the magnitude of the changes seen was considerably beyond our expectations and it is difficult to sort out the primary and secondary effects from such a multitude of alterations.

There are also few guidelines to indicate how cell concentrations might vary with changes in transepithelial transport, or how subcellular binding of cations might vary with either.

In order to establish a working basis for discussion, we shall assume that two distinct transport mechanisms, or "pumps," exist. One maintains the normal ratio of intracellular to extracelluar ionic concentrations, and the other is responsible for transepithelial movement. Ouabain could affect either pump, in a stimulatory or inhibitory fashion. We also assume that the activity of both pumps is reflected in the subcellular cation binding by one or more of these four particulate fractions, when expressed as the fraction of cortex cation associated with each unit weight of particle.

It at once becomes apparent that the former of these two mechanisms, which might be termed the "cell pump," is inhibited at all the doses employed, while the latter mechanism, or "transcellular pump" is at first stimulated and then inhibited. Although it was possible in these experiments to demonstrate simultaneous stimulatory and inhibitory effects of ouabain on cation reabsorption, the changes which occurred in renal cortical cation concentrations were not biphasic, except for cesium. A dose-dependent replacement of tissue potassium and magnesium by sodium and calcium was seen at all levels studied.

The question arises whether the changes seen in whole tissue concentrations reflect those occurring within cells, or are the result of changing quantities of extracellular and tubular fluid. Dry weights of each homogenate were measured, and averaged 0.098 mg/ml in controls and 0.094 mg/ml in ouabain-infused animals. This difference is greater than it appears, because a considerable portion of the dry weights is sucrose. Nevertheless, tissue water cannot have increased more than a few per cent. Pronounced diuresis, which might be associated with distension of the tubules, was seen in only two collection periods. It is therefore doubtful whether more than a small fraction of the substantial increments which were seen in tissue sodium and calcium can be attributed to these sources.

The decrease in renal hemodynamics might also be invoked as an explanation of the alterations in cortical composition. However, significant changes in tissue composition were seen before filtration rate became depressed.

The paradoxical rise of cesium concentration may indicate that this ion is pumped into the cell by the second mechanism but, unlike the other cations, cannot be extruded from the cell at the contraluminal membrane. Increased transport of cesium from tubular fluid to cell might therefore operate to maintain it at a higher intracellular concentration, from which it could diffuse passively into the peritubular fluid. This may also explain why tissue cesium fell as cesium transport diminished at high doses.

In searching for changes in subcellular distribution which reflect these responses, a biphasic effect, with sodium and cesium most strongly affected, should characterize the transcellular pump, while a monotonic change, increasing with dosage and affecting sodium, potassium, calcium and magnesium should characterize the cell pump.

The fourth particulate fraction comes closest to the expected characteristics of the transcellular pump, at least as far as the stimulatory portion of the drug effect. Early increases in binding were seen, even at 5 min. Although it was not possible to measure changes in tubular reabsorption in this time interval, it is probable that stimulation rather than inhibition occurred bilaterally. The greatest increase in binding at 20 min was that of sodium in the contralateral kidney, where reabsorption was clearly stimulated. Binding of potassium alone was increased in the infused kidneys at this dose, and it is entirely possible that potassium reabsorption was stimulated too; potassium clearance was increased only 36%, while sodium clearance increased 190%. At 20 min after the larger dose,

significant increases in binding by this fraction were no longer seen; however, all the means for the monovalent cations are still higher than controls. Thus stimulation of transport can be correlated with increased binding by this fraction, but no diminution in binding was seen which can be correlated with inhibition of transport.

In the search for a subcellular alteration which may reflect the other pump, the second particulate fraction offers some clues. Potassium increased here in percentage of total cortex content, but not in absolute quantities. The ability of these particles (predominantly mitochondria) to exclude sodium and retain potassium may therefore have been inhibited; as sodium entered the cell and potassium left, there may have been a lag in the escape of potassium from the particles. Calcium was not increased significantly in any single group, but did increase in the experimental kidneys when compared as a group with the control ($P < 0.05$). Thus these particles appear to share the disorder of the cell pump, whether or not they are involved directly in it.

The third particulate fraction showed only diminished binding, and the inhibitory action of ouabain may be reflected here. These changes are probably attributable to both the cell pump and the transcellular pump, which may share a component of the transport mechanism inhibited by all doses of the drug.

Finally the striking increase in association of all cations (possibly excepting sodium) with the first particulate fraction requires comment. As noted above, these were the only particles whose weight was altered by the drug. If the results are expressed as percentage of tissue cation, without regard for the weight of the particles, these changes virtually disappear. It seems likely, therefore, that a change in the state of aggregation of these particles rather than an alteration of binding was responsible.

The interpretations given here are obviously tentative, and are offered chiefly as a stimulus to further study. It would appear, however, that pronounced *in vitro* effects of cardiac glycosides on ion association with some microsomal fractions should be demonstrable, and that both stimulatory and inhibitory effects are possible.

Since ouabain had no effect when added to the homogenate in the cold, it would appear that some metabolic events are necessary to induce the changes seen. Ouabain fails to inhibit the ATP-splitting enzyme system in the cold (25). Incubation of fractions of renal tissue with the drug would be of interest. Luckenbach and Lüllman (12) reported that high concentrations of drug (10^{-4} M) were required for *in vitro* effects on calcium binding in cardiac tissue. The quantity added *in vitro* in the present study (5×10^{-7} M) was chosen to approximate the amount present in the infused kidneys, and larger amounts have not been examined.

It is clear from the present work that ouabain modifies the metabolism of cations other than sodium, potassium, and calcium. Transport of magnesium across tubular epithelium and intracellular magnesium concentration were affected as much as calcium. Furthermore, the two trace cations examined were also affected, particularly cesium, which exhibited more significant alterations than any other cation studied. These results tend to support the view that ion transport mechanisms possess selectivity for cations, rather than absolute specificity, and that drug-induced effects on these mechanisms may involve altered selectivity rather than stimulation or inhibition of a specific ion reaction. Investigation of glycoside effects on other cations within these two groups may clarify the relationship between altered selectivity and a parameter of ion size.

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