Effect of Ouabain and Potassium on Isolated Perfused Rabbit Kidney* (33489)

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Numerous reports have appeared in the literature supporting the concept originally suggested in the experimental data of Farber *et al.* (1), that cardiac glycosides produced a diuresis and natriuresis by a direct action on the kidney. These substances affect sodium and potassium transport in several tissues and it is generally believed that this is due to an inhibition of an ion carrier mechanism within the cell, membrane adenosine triphosphatase. Glynn (2) has shown that by increasing the concentration of potassium in the bathing medium of red blood cells, he could block the glycoside produced reduction of sodium efflux from these cells. He concluded that the glycosides compete with potassium for attachments to carrier sites within the cell concerned with the influx of potassium and efflux of sodium. Koch (3), using toxic concentrations of ouabain, first demonstrated that potassium could interfere with the action of ouabain in the dog kidney. Since then, this effect of potassium has been demonstrated with strophanthidin by Orloff *et al.* in the chicken (4) and Cade *et al.* in the dog (5).

The experiments described were performed to determine whether the direct renal effects of the cardiac glycosides observed in the intact animal could be produced in a totally isolated whole blood perfused kidney. If potassium acts directly on the kidney to inhibit the glycoside effect, demonstration of this in the isolated preparation would rule out the possibility of extrarenal factors being involved, such as the effect of potassium on the cardiovascular system or on aldosterone production. The isolated artificial heart–lung whole blood perfused rabbit kidney preparation developed in this laboratory (6, 7) seemed to be an ideal tool for this study. Furthermore, confirmation of these findings would supply additional support for the physiological integrity of this preparation. Our data fully support the concept that the cardiac glycosides exert a direct effect on the kidney to produce a diuresis and natriuresis. However, our data do not support those who have concluded that a high potassium level will inhibit the action of the cardiac glycosides. Recently, Horowicz and Gerber have shown that a high potassium concentration will not reverse the action of strophanthidin on frog striated muscle (8).

Method. A total of 47 experiments were performed using 4–6 lb female Californian rabbits. The weight of the left kidney which was routinely used for the perfusion studies ranged from 5-9 g. The general procedures of the experiment were described in previous publications (6, 7, 9). Five to 10 min after the rabbit was sacrificed, the kidney was connected to the perfusion apparatus. The pressure was set at between 80–90 mm Hg, and the cabinet was closed to allow the temperature and humidity to return to optimal levels. Sixty to 90 min were allowed for the kidney to achieve optimal function, as determined by frequent measurement of urine flow and "observed" renal blood flow (ORBF), rate of blood flow from renal vein into a calibrated 20.0-ml syringe barrel fitted into the circuit. When these functions appeared to be attaining optimal levels, infusion of *p*-aminohippurate (PAH), 150 mg/100 ml, creatinine 175 mg/100 ml, and at times potassium chloride (KCl) 200 mg/100 ml was begun. The priming infusion was given at a rate of 60.0 ml/hr for 3–5 min, followed by the sustaining infusion given at a rate of 3–10 ml/hr. The sus-

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[1] Deceased.

taining infusion rate was determined by the rate of urine flow and was adjusted so that no major fluid imbalance developed. When ORBF and urine flow appeared to be stabilized, clearance measurements were begun. Routinely, ORBF was measured 30 sec before the start of the clearance period, then a urine collection was started and simultaneous samples were obtained from the renal venous system and the arterial system, blood entering the renal arterial cannula. At 5–20 min intervals, the same procedure was followed until 3–6 consecutive control clearance periods are obtained. When potassium chloride was not used in the initial infusion, 50–70 mg may be added followed by an infusion containing KCl (150 mg/100 ml), and 3–4 consecutive clearance periods obtained. After 3–6 control clearance periods and/or 3–4 clearance periods with KCl, 0.05, 0.075, 0.085, or 0.10 mg of ouabain was added to the blood reservoir and 4–8 consecutive clearance periods were obtained. Hematocrit and hemoglobin determinations were made at the start, midway through, and at the end of the experiment. At the termination of the experiment the kidney was sectioned, grossly examined, weighed, and compared to the contralateral nonperfused kidney.

Glomerular filtration rate is estimated by the clearance of creatinine, and renal plasma flow is measured directly (ORBF $\times$ 1—HT). The chemical methods used for the determination of creatinine, PAH, sodium, potassium, and hemoglobin were described previously (6, 9).

Results. In 28 experiments the effect of various doses of ouabain on the isolated perfused kidney was studied. Eight experiments were performed with 0.05 mg of ouabain, 7 experiments with 0.075, 6 experiments with 0.085, and 7 experiments with 0.10 mg of ouabain. Table I lists the mean values, the standard error of the mean, the percentage change and the p value obtained with various doses of ouabain. The values were calculated from the average of 3 control values obtained prior to the addition of ouabain, with the first one or average of two post ouabain values (Post 1) obtained 2–20 min after ouabain and the average of 3–4 values obtained 20–60 min after the addition of ouabain (Post 2).

TABLE I. The Effect of Various Doses of Ouabain on the Function of the Isolated Perfused Kidney.*

N	Conc of ouabain (mg)	Mean kidney wt. (g)	Perfusion pressure (mm Hg)				Renal blood flow (ml/min)				Urine volume (ml/min)				Urine Na (μeq/min)				C_{PAH} (ml/min)				E_{PAH} (%)			
			Mean	SEM	% Δ	p	Mean	SEM	% Δ	p	Mean	SEM	% Δ	p	Mean	SEM	% Δ	p	Mean	SEM	% Δ	p	Mean	SEM	% Δ	p
8	Control		86	±1.7			17	±1.8			0.18	±0.022			25	±3.3			2.2	±0.16			87	±0.8		
	0.05																									
	Post 1	7.1	93	±2.0	+ 8	<.05	15	±1.5	−12	>.1	0.12	±0.022	−33	>.1	17	±3.2	− 32	>.1	1.6	±0.12	− 27	<.05				
	Post 2		82	±1.3	− 5	<.1	19	±1.9	+12	>.1	0.19	±0.043	+ 6	>.1	25	±5.7	0	>.1	1.8	±0.15	−18	<.1	86	±1.6	−1	>.1
7	Control		88	±0.9			16	±2.1			0.13	±0.020			17	±2.8			2.2	±0.27			87	±1.7		
	0.075																									
	Post 1	7.9	98	±1.7	+11	<.01	16	±2.0	−11	>.1	0.09	±0.020	−21	>.1	11	±2.4	−35	>.1	1.6	±0.20	−27	<.1				
	Post 2		83	±1.5	− 6	<.05	18	±2.0	0	>.1	0.20	±0.043	+54	<.1	25	±5.6	+47	>.1	1.9	±0.22	−14	>.1	84	±1.7	−3	>.1
6	Control		84	±1.6			15	±1.4			0.10	±0.012			13	±2.4			2.2	±0.24			87	±0.4		
	0.085																									
	Post 1	7.0	91	±2.2	+ 8	<.05	13	±1.7	−13	>.1	0.08	±0.008	−20	>.1	9	±0.8	−31	>.1	1.8	±0.34	−18	>.1				
	Post 2		85	±1.9	+ 1	>.1	16	±2.0	+ 7	>.1	0.32	±0.062	+220	<.01	41	±8.0	+215	<.02	2.2	±0.21	0	—	85	±0.6	−2	>.1

* The values were calculated from a comparison of the average of 3 control values obtained prior to the addition of ouabain with the first one or average of two postouabain values (Post 1) obtained 2–20 min after the addition of ouabain and the average of 3–4 values obtained 20–60 min after the addition of ouabain (Post 2).

TABLE II. Effect of Ouabain on the Function of the Isolated Perfused Kidney.[a]

	Control	Post 1	%Δ	Post 2	%Δ
BP (mm Hg)	84 ±1.6	91 ±2.2 <.05	+8	85 ±1.9 >.1	+1
RBF (ml/min)	15 ±1.4	13 ±1.7 >.1	−13	16 ±2.0 >.1	+7
C_{CR} (ml/min)	2.2 ±0.24	1.8 ±0.34 >.1	−18	2.2 ±0.21 —	0
UV (ml/min)	0.10 ±0.012	0.08 ±0.008 >.1	−20	0.32 ±0.062 <.01	+220
UNaV (μeq/min)	13 ±2.4	9 ±0.8 >.1	−31	41 ±8.0 <.02	+215
UKV (μeq/min)	5.3 ±0.31	4.4 ±0.60 >.1	−17	8.5 ±1.32 <.1	+60
E_{PAH} (%)	87 ±0.4			85 ±0.6 >.1	−2
Plasma K (meq/liter)	4.6 ±0.29	5.2 ±0.27 >.1	+13	5.2 ±0.27 >.1	+13
Blood pH	7.43 ±0.044			7.48 ±0.006 >.1	
Urine pH	7.12 ±0.387	7.08 ±0.297 >.1		7.47 ±0.169 >.1	

[a] Results of 6 experiments with 0.085 mg of ouabain; mean kidney wt. = 7.0 g; mean values and % change from control period with SEM and p.

bain and the average of 3–4 values obtained 20–60 min after the addition of ouabain (Post 2). In the KCl and ouabain group the values were calculated from a comparison of the average of 3 control values obtained prior to KCl with the average of 2–3 post-KCl values. When ouabain was added after 3–4 KCl clearance periods the percentage change was calculated from a comparison of the post-ouabain values with the pre-KCl, or control values as described above. Those changes which were significant have a p value listed.

A pronounced and consistent diuresis and natriuresis did not occur until a dose of 0.085 mg of ouabain was used. At 0.075 mg of ouabain a variable and inconsistent diuresis and natriuresis was obtained.

In 6 experiments (Table II), immediately after the addition of 0.085 mg of ouabain (Post 1) renal vascular resistance increased causing a reduction in renal blood flow, urine excretion, sodium excretion, creatinine clearance, and an increase in perfusion pressure (8%) $p<0.05$. Plasma potassium always rose

TABLE III. Effect of KCl on the Ouabain Response of the Isolated Perfused Kidney.[a]

	Control	Post-KCl	%Δ	Post 1	%Δ	Post 2	%Δ
BP (mm Hg)	87 ±1.5	82 ±1.5 <.05	−6	92 ±1.8 >.1	+6	83 ±1.8 >.1	−5
RBF (ml/min)	13 ±1.1	14 ±1.0 >.1	+8	11 ±1.2 >.1	−15	14 ±1.3 >.1	+8
C_{CR} (ml/min)	1.9 ±0.36	2.0 ±0.29 >.1	+5	1.2 ±0.20 >.1	−37	1.7 ±0.18 >.1	−11
UV (ml/min)	0.15 ±0.031	0.19 ±0.018 >.1	+27	0.12 ±0.018 >.1	−20	0.37 ±0.031 <.001	+147
UNaV (μeq/min)	21 ±4.3	24 ±3.0 >.1	+14	13 ±1.7 >.1	−38	50 ±4.3 <.01	+138
UKV (μeq/min)	6.6 ±0.99	13.7 ±1.41 <.01	+108	8.6 ±1.29 >.1	+30	10.7 ±0.77 <.01	+62
E_{PAH} (%)	86 ±1.7	84 ±2.1 >.1	−2			82 ±2.4 >.1	−5
Plasma K (meq/liter)	4.9 ±0.21	8.6 ±0.48 <.001	+76	8.1 ±0.46 <.001	+65	7.8 ±0.56 <.01	+59
Blood pH[b]	7.40 ±0.024					7.46 ±0.035 >.1	
Urine pH[c]	7.55 ±0.240	6.86 ±0.328 >.1		6.81 ±0.247 <.1		7.26 ±0.134 >.1	

[a] Results of 9 experiments with KCl + 0.085 mg of ouabain; mean kidney wt. = 7.6 g; mean values and % change from control period with SEM and p.

[b] N = 8.

[c] N = 6.

after the administration of ouabain. At 20–60 min after the administration of ouabain (Post 2) renal vascular resistance fell causing an increase in renal blood flow, urine excretion (+220%) $p<0.01$, sodium excretion (+215%) $p<0.02$, and a return of the creatinine clearance and the perfusion pressure to the preouabain level. Plasma potassium remained elevated.

In 9 experiments (Table III), when KCl was added to the perfusion after 3–4 control periods the plasma K level was raised from an average of 4.9 meq/liter to 8.6 meq/liter and leveled off at 8.1 to 7.8 meq/liter after the addition of ouabain. A diuresis and natriuresis was also produced by the addition of KCl, possibly related to an increase in C_{CR}, but when 0.085 mg of ouabain was added 20–40 min after the addition of KCl an even greater diuresis (+147%) $p<0.001$ and natriuresis (+138%) $p<0.01$ was produced. There was little evidence for a potassium in-

duced inhibition of the ouabain diuresis and natriuresis.

In four additional experiments, kidney and blood donor rabbits were maintained on a high potassium diet for periods of 10–20 days. Serum potassium of these rabbits averaged 5.7 meq/liter as compared to 4.8 meq/liter for normal rabbits. When kidneys from these rabbits were perfused and the plasma potassium raised to levels of 8.2–12.2 meq/liter, the addition of ouabain produced a natriuresis and diuresis similar in magnitude to that produced in the normal perfused kidney.

It was reported that the cardiac aglycone strophanthidin inhibits the transport of PAH across the tubule (10) and therefore invalidates the use of this substance for the estimation of renal plasma flow. The extraction of PAH was noted to be reduced from 1–5% after the administration of ouabain. However, in normal control experiments, the extraction of PAH falls about 5% during comparable periods of time (9). In our studies, a concentration of 4–9 $\times$ 10^{-6} moles/liter of ouabain were used, whereas previous workers (10) using strophanthidin have used concentrations of 3 $\times$ 10^{-4} or 10^{-5} moles/liter.

After the administration of ouabain, the plasma level of potassium rose immediately in all cases (Table II). The rise in plasma potassium was believed to be the result of inhibition of potassium influx into the renal tubule. However, when studies were done with circulated blood and no kidney, it was observed that ouabain caused a similar rise in plasma potassium. This would indicate that the effect of ouabain was to inhibit the movement of potassium into the red blood cell, a response reported by others (2). It was presumed that in this case, the only accountable source of plasma potassium came from the liberation of potassium from red blood cells after hemolysis. To check this point, plasma levels of potassium and hemoglobin were determined in circulating (Fig. 1) and incubated (Fig. 2) blood before and after the addition of ouabain. The rise in plasma potassium levels obtained after ouabain were observed to be directly correlated with the degree of hemolysis (Figs. 1 and 2). Slight

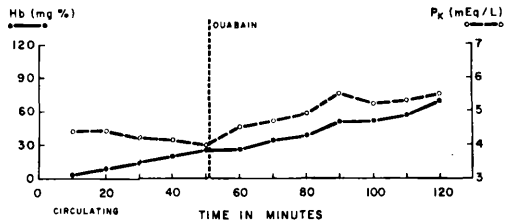

FIG. 1. Effect of ouabain on plasma potassium in circulating blood.

hemolysis was always observed during the course of the normal perfusion experiment. Plasma hemoglobin though absent initially, averaged 39 mg/100 ml at 60–90 min and 81 mg/100 ml at 180–220 min.

Discussion. On the basis of *in vivo* studies in which ouabain or strophanthidin was infused into the renal artery of the dog (5, 3), or into the renal portal vein of the chicken (4), and differences were noted in the function of the normal and the infused kidney, it was concluded that these substances produced diuresis and natriuresis by an independent direct action on the kidney. Our data from the isolated perfused kidney fully support this view. However, our data do not support those who have concluded that potassium loading will inhibit the natriuretic and diuretic effect of the cardiac glycosides.

It was proposed, on the basis of work performed with other tissues, that the passage of potassium into the cell and the passage of sodium out of the cell are loosely linked processes. The rate of sodium transport across the cell membrane depends upon the availability of sodium for transport, the activity of the transport system, and the availability of potassium for exchange with sodium. In the renal tubule, a similar linked Na–K exchange system was suggested as the mechanism for transport of sodium from the cell to the extracellular fluid compartment

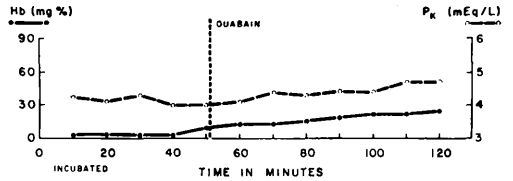

FIG. 2. Effect of ouabain on plasma potassium in incubated (noncirculating) blood.

(11). It was suggested that in the kidney the cardiac glycosides affect sodium transport by interferring with the movement of potassium across the contraluminal border of the renal tubule cell (4).

In experiments in which animals were loaded with potassium, a marked inhibition of the cardiac glycoside induced natriuresis and diuresis occurred. In our studies with an isolated perfused kidney, plasma potassium concentrations as high as 12.0 meq/liter did not inhibit the ouabain induced diuresis and natriuresis. In the experiment of Cade *et al.* (5), complete inhibition of the strophanthidin induced saluresis and diuresis was obtained in the dog by raising plasma potassium levels to 7.0–8.0 meq/liter. However, in this series the animals were maintained on a high potassium diet for 7–10 days before the experiment and then infused with potassium to maintain elevated plasma levels during the experiment. It is known that a high potassium intake will stimulate aldosterone production (12) and it is believed that the cardiac glycosides and aldosterone have antagonistic actions. Is it possible that the strophanthidin inhibition may have resulted from an aldosterone effect rather than a potassium effect? This possibility was considered by these authors (5) who found no difference in response to strophanthidin in bilaterally adrenalectomized animals. It is conceivable that the absence of normal levels of aldosterone will not affect the cardiac glycoside response, whereas the stimulation of increased amounts of aldosterone by potassium loading might inhibit the glycoside effect. In our studies, four experiments were performed using the kidneys and blood from rabbits who were maintained on a high potassium diet for 10–20 days. When kidneys from these rabbits were perfused and the plasma potassium raised to levels of 8.2–12.2 meq/liter, the addition of ouabain produced a natriuresis and diuresis similar in magnitude to that produced in the normal perfused kidney. In studies by Orloff *et al.* (4), potassium loading was shown to depress the effect of strophanthidin in the chicken. In these experiments potassium chloride was infused during the strophanthidin infusion at the peak of the strophanthidin response. Sodium excretion fell from 128.5 to 55.5 μeq/min and urine flow fell from 1.09 to 0.68 ml/min within 5–10 min after the start of potassium infusion. However, in the normal strophanthidin experiment, sodium excretion fell from 114 to 58.8 μeq/min and urine flow fell from 1.23 to 0.82 ml/min during the infusion of strophanthidin at about the same time after the start of the infusion of strophanthidin as when the potassium infusion was begun in the other series. It is difficult to recognize differences between these responses.

In the isolated perfused kidney, potassium loading is ineffective in inhibiting the ouabain response. Is it possible that the potassium loading in the intact animal may exert its cardiac glycoside inhibiting effect via some other mechanism? In the intact animal, the elevation of plasma potassium produced peripheral vascular constriction which was shown to be an indirect effect mediated by stimulation of the adrenal medulla and the sympathetic nervous system (13). Adrenalectomy and sympathetic denervation completely abolish the vasoconstriction produced by hyperpotassemia (14). This may explain why increasing the potassium concentration in isolated perfused organs (15) and in our isolated perfused kidney results in vasodilatation rather than vasoconstriction. Furthermore, if hyperpotassemia stimulates catecholamine production, it may be that the cardiac glycoside inhibition may be related to this factor, since epinephrine and norepinephrine produced a reduction in urine output and sodium excretion in the isolated perfused kidney (9).

In view of some of the questions raised about the evidence supporting the thesis that potassium inhibits the renal action of the cardiac glycosides by insuring a high intracellular content of this ion, it would appear that further work is required to clarify this matter.

Summary. The effect of various doses of ouabain was studied on the isolated perfused kidney. A pronounced and consistent diuresis and natriuresis did not occur until a dose of 0.085 mg of ouabain was used. Within 2–20 min after the administration of ouabain, ren-

al vascular resistance increased causing an increase in perfusion pressure and a reduction in renal blood flow, urine excretion, sodium excretion, and creatinine clearance. Plasma potassium always rose after the administration of ouabain. At 20–60 min after the addition of ouabain renal vascular resistance fell, causing the perfusion pressure to return to normal. There was an increase in renal blood flow, urine excretion (+220%) $p<0.01$ sodium excretion (+215%) $p<0.02$ and the creatinine clearance returned to normal. Plasma potassium remained elevated. When potassium chloride (KCl) was added to the perfusion after 3–4 control periods, the plasma K level was raised from an average of 4.9 meq/liter to 8.6 meq/liter and leveled off at 8.1 to 7.8 meq/liter after the addition of ouabain. A diuresis and natriuresis was also produced by the addition of KCl, but when 0.085 mg of ouabain was added 20–40 min after the addition of KCl, even greater diuresis (+147%) $p<0.001$ and natriuresis (+138%) $p<0.01$ were produced. There was little evidence for a K-induced inhibition of the ouabain diuresis and natriuresis.

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