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EFFECT OF OUABAIN ON RENIN SECRETION IN ANAESTHETIZED DOGS

BY PAUL C. CHURCHILL AND FRANKLIN D. McDONALD
From Wayne State University School of Medicine and Hutzel Hospital, Detroit, Michigan U.S.A.

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SUMMARY

1. Pentobarbitone anaesthetized dogs were used to study the effects on renin secretion of ureteral occlusion and partial aortic clamping before and after intrarenal arterial administration of ouabain.

2. Arterial plasma renin activity (A renin) and renal venous minus arterial plasma renin activity (RV-A renin) were both increased by either ureteral occlusion during mannitol diuresis or partial aortic clamping to reduce renal perfusion pressure. Intrarenal arterial ouabain administration blunted or abolished the effects of these manoeuvres on A and RV-A renins.

3. The observations are not consistent with the hypothesis that renin secretion is uniquely controlled by afferent arteriolar transmural pressure. Both ureteral occlusion and aortic clamping reduce this pressure and increase renin secretion, yet evidence presented herein suggests that ouabain, which also decreases this pressure, decreases renin secretion.

4. The observations are consistent with the hypothesis that renin secretion is controlled by tubular fluid flow and/or composition in the region of macula densa cells. Possibly changes in flow and/or composition are detected as changes in macula densa intracellular sodium concentration.

INTRODUCTION

No single mechanism proposed to control renin secretion satisfactorily explains all the pertinent experimental observations. In the most recent complete review of the subject, Vander (1967) summarized many of these observations in terms of two main hypothetical controlling mechanisms. It is not yet clear whether additional mechanisms must be postulated to account for the effects on renin secretion of the renal nerves and a variety of hormones and drugs, or if these effects are also mediated by one or both of the mechanisms he discussed. According to one, the baroreceptor theory, some haemodynamic parameter, most probably afferent arteriolar pressure

controls renin secretion. Alternatively, according to the macula densa theory, some tubular fluid parameter in the region of macula densa cells serves as the stimulus controlling renin secretion. Vander (1967) marshalled support for the view that renin secretion was controlled by, and inversely related to, the load of sodium delivered in tubular fluid to macula densa cells. He further suggested that macula densa cells might detect changes in tubular load of sodium via changes in their intracellular sodium concentration. Several subsequent experimental observations were interpreted in the context of this hypothesis. Thus, the observations that the natriuretic drugs furosemide and ethacrynic acid stimulate renin secretion (Cooke, Brown, Zacherle & Walker, 1970; Vander & Carlson, 1969) can be explained if one assumes that they reduce intracellular sodium concentration in spite of the increased tubular sodium load resulting from their administration (Vander & Carlson, 1969). Unfortunately, the effects of these drugs on renal intracellular sodium concentration have not yet been clearly established. On the other hand, it is well established that the natriuretic drug ouabain (Bowman, Dolgin & Coulson, 1973; Cade, Shalhoub, Canessa-Fischer & Pitts, 1961; Duarte, Chomety & Giebisch, 1971; Hook, 1969; Nahmod & Walser, 1966; Orloff & Burg, 1960; Wilde & Howard, 1960) increases renal intracellular sodium concentration (Burg & Orloff, 1962; Maude, 1969; Nahmod & Walser, 1966). Accordingly, ouabain should inhibit renin secretion even if tubular sodium load were decreased by some means. The experiments described below were designed to investigate the effects on renin secretion of intrarenal arterial ouabain administration in anaesthetized dogs. Ureteral occlusion and partial aortic clamping were used to decrease tubular sodium load and to stimulate renin secretion.

METHODS

Fifteen mongrel dogs (10·2–28 kg), fasted overnight and anaesthetized with sodium pentobarbitone (30 mg/kg, I.V.) were used. Arterial, venous, left renal venous, and bilateral ureteral catheters were inserted (Churchill & Malvin, 1970). A 24-gauge needle, connected by a polyethylene catheter to a Harvard syringe pump was introduced into the left renal artery near its aortic origin. Fluids at 0·08 ml./min were continuously infused into the artery. A blood flow probe was placed on the left renal artery and flow was measured using an electromagnetic flow meter (Carolina Medical Electronics). Fluids were administered via the jugular vein catheter. Blood was sampled from the arterial and left renal venous catheters. Systemic arterial blood pressure was continuously monitored using a Narco pressure transducer and a multichannel Narco Physiograph.

Following this preparation, ten dogs received a priming solution of inulin and *p*-aminohippuric acid (5 g inulin + 1 g PAH/100 ml. 150 mM-NaCl, 1 ml./kg body wt.) followed by a sustaining infusion (10 g mannitol + 4 mg inulin + 2 mg PAH/100 ml. 150 mM-NaCl, given at 0·5 ml./min.kg body wt.) via the venous catheter. After an equilibration of 30–45 min, a 10 min clearance period was begun. Then, the left

ureteral cannula was completely occluded for 10 min. Five minutes after release of this occlusion, a second 10 min clearance period was begun. During the preceding, 150 mM-NaCl was infused into the left renal artery. In five dogs, 30–45 min later, the sequence of clearance, ureteral occlusion, and clearance periods was repeated while the intrarenal arterial infusion remained the same. In the five remaining dogs, ouabain was incorporated into the intrarenal arterial infusion 30–45 min before repeating the sequence of clearance, ureteral occlusion, and clearance periods. In three of these dogs, ouabain was given at 0·5–1·0 μg/kg.min throughout, and in two dogs at 5 μg/kg.min for 5 min. Between 22 and 30 μg ouabain/kg was given before the beginning of the first clearance period following the ouabain infusion. Blood samples were drawn at the midpoint of each clearance period and just prior to release of the ureteral occlusion.

Another group of five dogs was identically prepared but, in addition, an aortic clamp was positioned above the origins of the renal arteries (Vander & Miller, 1964) and two aortic catheters were inserted via the femoral arteries. The tip of one was positioned above, and the tip of the other below, the aortic clamp. Blood pressure transducers were connected to each of these catheters. Inulin and PAH were given as described above, except that the sustaining infusion consisted of inulin and PAH in 150 mM-NaCl without mannitol, and was given at 0·5 ml./min. After a 30–45 min equilibration, a 10–20 min control clearance period was begun. Then renal perfusion pressure was reduced by tightening the aortic clamp. Ten to fifteen min later, a second 20 min clearance was begun. Following this, ouabain (1 μg/kg.min) was added to the intrarenal arterial infusion and 20–30 min later, the third clearance was begun. Finally, the aortic clamp was completely released and, 10–24 min later, the fourth clearance period was begun while the ouabain administration continued.

Lightly heparinized, ice-cold plastic syringes were used for the collection of blood. Blood was quickly centrifuged at 4° C. Duplicate 0·5 ml. plasma aliquots were frozen after addition of 2 mg EDTA/ml. plasma for later determination of renin activity. For this, the Schwarz/Mann Renin Radioimmunoassay Kit was used (Orangeburg, New York, U.S.A. 10962). Plasma was carefully thawed, the inhibitors of converting enzyme activity were added (8-OH quinoline and dimercaprol), and the samples were incubated at 38° C. Duplicate aliquots were removed after 1 and 2 hr of incubation and angiotensin I concentrations were determined by radioimmunoassay. Plasma renin activity was expressed as nanograms of angiotensin I/ml. plasma hour of incubation (ng A-I/ml. hr). Since the rate of angiotensin I production was linear for 2 hr, renin substrate was not rate-limiting during this incubation.

Sodium and potassium concentrations in plasma and urine were determined using a flame photometer with an internal lithium standard (Instrumentation Laboratories). Inulin in trichloroacetic acid plasma filtrates and urine was determined by the method of Harrison (1942). PAH was determined by the method of Smith, Finkelstein, Aliminosa, Crawford & Graber (1945). Sodium and potassium excretion rates were calculated as the products of the urine concentrations (U_{Na} and U_K) and urine flow rate (V). Inulin clearance was calculated and used as the measure of glomerular filtration rate (GFR). Renal plasma flow (RPF) was determined either from the renal blood flow, measured with the flow probe and meter, and the haematocrit, or from the clearance of PAH corrected by its extraction. Renin secretion rate was calculated as the product of RPF and RV–A renin. The paired t test was used for assessment of statistical significance.

RESULTS

In Table 1 are summarized the results of experiments in which the left ureter was occluded twice, preceded and followed each time by a 10 min clearance period. Isotonic sodium chloride was infused into the left renal artery throughout these experiments. In order to determine the effects of ureteral occlusion, the value of a given parameter during each occlusion

TABLE 1. Renal and systemic effects of ureteral occlusion on two occasions, each preceded and followed by a period for clearance determinations

	Sequence 1			Sequence 2		
	Clearance	Ureteral occlusion	Clearance	Clearance	Ureteral occlusion	Clearance
P_{Na}, m-equiv/l.	137 ± 1	—	137 ± 1	138 ± 1	—	138 ± 1
P_K, m-equiv/l.	2·9 ± 0·4	—	3·2 ± 0·4	3·4 ± 0·5	—	3·9 ± 0·7
Arterial blood pressure, mmHg	114 ± 1	115 ± 4	120 ± 6	121 ± 8	127 ± 6	124 ± 6
A renin, ng A–I/ml./hr	6·7 ± 2·3	12·4 ± 2·2*	6·9 ± 1·0	7·8 ± 1·1	14·3 ± 1·8*	10·3 ± 1·8
RV–A Renin, ng A–I/ml./hr	1·0 ± 1·0	12·1 ± 2·0*	1·0 ± 0·4	0·2 ± 0·8	12·8 ± 1·8*	3·0 ± 1·9
Renin secretion, ng A–I/min.hr	183 ± 118	—	176 ± 75	89 ± 101	—	305 ± 143
RPF, ml./min	215 ± 49	—	226 ± 39	253 ± 65	—	174 ± 31
GFR, ml./min	36·4 ± 4·0	—	38·2 ± 5·2	35·6 ± 4·6	—	30·8 ± 5·1
V, ml./min	4·7 ± 0·8	—	6·4 ± 0·9	6·8 ± 1·0	—	6·6 ± 1·1
$U_{Na}V$, μ-equiv/min	264 ± 72	—	358 ± 78	389 ± 83	—	303 ± 74
U_KV, μ-equiv/min	39 ± 6	—	57 ± 11	67 ± 16	—	79 ± 23

Means ± S.E.M. of mean. $n = 5$ in all cases. * $P < 0.05$, comparing value during ureteral occlusion with average value during preceding and succeeding clearance periods. Renal data are for left kidney only. Abbreviations are: P_{Na} and P_K, plasma sodium and potassium concentrations, respectively; A renin, arterial plasma renin activity; RV–A renin, renal venous minus arterial plasma renin activity; RPF, GFR, and V, renal plasma flow, glomerular filtration rate, and urine flow rate, respectively; $U_{Na}V$ and U_KV, urinary sodium and potassium excretion rates, respectively. Renin secretion was calculated as the product of RPF and RV–A renin.

was compared to the average value of the same parameter during the preceding and succeeding clearance periods. During both periods of ureteral occlusion, increases in A renin and in RV-A renin were observed in each dog. The mean paired increases in both A renin and in RV-A renin were significantly greater than zero ($P < 0.05$) during each occlusion. Since renal plasma flow during mannitol diuresis is unaffected by ureteral occlusion (Kiil, Omvik & Raeder, 1972; Malvin, Wilde & Sullivan, 1958; Table 2) the increased RV-A renin indicates that ureteral occlusion stimulates renin secretion. That it does so reproducibly is suggested by com-

parisons of the two periods of ureteral occlusion. Neither A renin nor RV-A renin during the second period differed significantly ($P > 0{\cdot}05$, paired t test) from the respective values during the first period.

In Table 2 are summarized the results of experiments in which ouabain was added to the intrarenal arterial infusate for the second ureteral occlusion period and its bracketing clearance periods. A renin, RV-A renin, and renin secretion were increased ($P < 0{\cdot}05$) as a result of the first ureteral occlusion. Renal plasma flow was not significantly affected ($P > 0{\cdot}05$). The data during ouabain administration were analysed similarly. Although during the second occlusion A renin, RV-A renin, and renin secretion tended to increase, and RPF to decrease, these changes were not significant ($P > 0{\cdot}05$). The conclusion that ouabain prevented renin secretion from increasing, or at least limited any increase, is further supported by comparing A renin and RV-A renin during the two periods of ureteral occlusion. In every dog, A renin, RV-A renin, and renin secretion during the second occlusion (ouabain) were lower than during the first occlusion, and the mean decreases in each were significant ($P < 0{\cdot}05$). Comparisons of the average values for clearance periods before and after ouabain indicate that mean arterial blood pressure ($P < 0{\cdot}01$), plasma potassium concentration ($P < 0{\cdot}005$), and urinary sodium excretion rate ($P < 0{\cdot}05$) all increased while both RPF ($P < 0{\cdot}005$) and GFR ($P < 0{\cdot}005$) decreased during ouabain administration. The changes in A renin, RV-A renin and RPF (RV-A renin) were not significant ($P > 0{\cdot}05$). Hence, there was no detectable change in renin secretion rate during clearance periods as a result of ouabain administration.

The results of the aortic clamp experiments are presented in Table 3. Glomerular filtration rate, sodium and potassium excretion rates, and urine flow were measured in these dogs but the extremely low urine flow rates suggested that the calculated values were unreliable. Therefore, these data are not included in the table. Renal perfusion pressure was identical to mean arterial pressure before the aortic clamp was tightened. It fell to 82–83 mmHg when the clamp was tightened. Mean systemic arterial pressure ($P < 0{\cdot}025$), A renin ($P < 0{\cdot}025$), RV-A renin ($P < 0{\cdot}025$), and renin secretion ($P < 0{\cdot}05$) all increased as a result. When ouabain was infused into the left renal artery while the aorta remained partially clamped, the changes in all renin parameters were reversed. A renin, RV-A renin, and RPF (RV-A renin) decreased in every dog, and the average decreases were all significant ($P < 0{\cdot}05$). Releasing the clamp while the ouabain administration continued had no further effect on these parameters. Comparisons of initial and final clearance period values confirms some of the effects of ouabain observed in the ureteral occlusion experiments. Plasma potassium increased slightly ($P < 0{\cdot}025$) and RPF

TABLE 2. Renal and systemic effects of ureteral occlusion with and without unilateral intrarenal arterial ouabain. Each ureteral occlusion was preceded and followed by a period for clearance determinations

	Sequence 1			Sequence 2 (Ouabain)		
	Clearance	Ureteral occlusion	Clearance	Clearance	Ureteral occlusion	Clearance
P_{Na}, m-equiv/l.	137 ± 1	—	137 ± 1	136 ± 1	—	136 ± 1
P_K, m-equiv/l.	3·5 ± 0·1	—	4·0 ± 0·2	4·8 ± 0·4	—	5·6 ± 0·3
Arterial blood pressure, mmHg	126 ± 3	133 ± 4	128 ± 1	137 ± 4	137 ± 4	132 ± 5
A renin, ng A–I/ml.hr	5·6 ± 0·9	50·6 ± 25*	6·8 ± 0·4	6·2 ± 0·7	11·0 ± 3·6*	7·9 ± 1
RV–A renin, ng A–I/ml.hr	2 ± 1	220 ± 98*	4 ± 2	5 ± 3	20 ± 8*	9 ± 4
Renin secretion, ng A–I/min.hr	260 ± 132	30,900 ± 13,432*	438 ± 310	290 ± 123	1,750 ± 763*	697 ± 94
RPF, ml./min	183 ± 28	166 ± 22	159 ± 32	117 ± 24	90 ± 26	139 ± 27
GFR, ml./min	32·6 ± 4	—	29·6 ± 3·9	21·6 ± 4·1	—	21·6 ± 3·7
V, ml./min	6·2 ± 0·7	—	6·9 ± 0·5	6·7 ± 0·7	—	8·7 ± 1·3
$U_{Na}V$, μ-equiv/min	437 ± 64	—	415 ± 45	531 ± 69	—	825 ± 162
U_KV, μ-equiv/min	52 ± 8	—	67 ± 6	61 ± 8	—	72 ± 7

Means ± S.E. of mean. $n = 5$ in all cases. * $P < 0.05$ comparing value during ureteral occlusion with average value during preceding and succeeding clearance periods. *not significantly different from average clearance value, but significantly lower than value during first ureteral occlusion $P < 0.05$. Abbreviations are: P_{Na} and P_K, plasma sodium and potassium concentrations, respectively; A renin, arterial plasma renin activity; RV-A renin, renal venous minus arterial plasma renin activity; RPF, GFR, and V, renal plasma flow, glomerular filtration rate, and urine flow rate, respectively: $U_{Na}V$ and U_KV, urinary sodium and potassium excretion rates, respectively. Renin secretion was calculated as the product of RPF and RV-A renin. Renal data are for left kidney only.

decreased ($P < 0.05$). The difference between initial and final RV-A renin was not significant ($P > 0.05$). Ouabain did not detectably affect renin secretion except when renal perfusion pressure was reduced.

Renal venous plasma potassium concentration was measured in all dogs of Tables 2 and 3 in an effort to discover if the increase in plasma potassium concentration was due to potassium leakage from the ouabain-infused kidney, or the blood cells in the blood perfusing this kidney. There was no significant difference ($P > 0.05$) between the RV_K of the left kidney and the P_K during ouabain administration.

TABLE 3. Effects of aortic clamping with and without unilateral intrarenal arterial ouabain

	Aorta clamped		Ouabain infused	
	Control Clearance 1	Clearance 2	Clearance 3	Clearance 4
P_{Na}, m-equiv/l.	143 ± 1	144 ± 1	144 ± 1	143 ± 1
P_K, m-equiv/l.	3·6 ± 0·1	3·8 ± 0·1	4·1 ± 0·2	4·5 ± 0·3
Arterial blood pressure, mmHg	121 ± 4	135 ± 8	141 ± 8	136 ± 12
Perfusion pressure, mmHg	121 ± 4	83 ± 1	82 ± 1	136 ± 12
A renin, ng A–I/ml.hr	12 ± 3	19 ± 1	12 ± 1	9 ± 1
RV-A renin, ng A-I/ml.hr	0·5 ± 0·3	13 ± 5	5 ± 3	3 ± 2
Renin secretion, ng A–I/min.hr	42 ± 19	1045 ± 619	208 ± 98	146 ± 54
RPF, ml./min	113 ± 32	74 ± 16	76 ± 26	62 ± 15

Means ± S.E.M. of mean $n = 5$ in all cases. Renal data are for the left kidney only. A renin and RV–A renin were higher during the second as compared with the first period ($P < 0.025$) and lower during the third as compared with the second ($P < 0.05$). Abbreviations are: P_{Na} and P_K, plasma sodium and potassium concentrations, respectively; A renin, arterial plasma renin activity; RV–A renin, renal venous minus arterial plasma renin activity; RPF, renal plasma flow. Glomerular filtration rate, sodium and potassium excretion rates, and urine flow data are not included; urine flow rates were so low as to make their calculations unreliable. Renin secretion was calculated as the product of RPF and RV–A renin.

DISCUSSION

The renal effects of cardiac glycosides have been described for a variety of species (Bowman *et al.* 1973; Burg & Orloff, 1962; Cade *et al.* 1961; Duarte *et al.* 1971; Hook, 1969; Martinez-Maldonado *et al.* 1969, 1970; Maude, 1969; Nahmod & Walser, 1966; Orloff & Burg, 1960; Vogel & Tervooren, 1965; Wilde & Howard, 1960). Unilateral decreases in renal plasma flow and in glomerular filtration rate are regularly observed after

intrarenal arterial ouabain administration in dogs (Cade *et al.* 1961; Nahmod & Walser, 1966). Evidence has been marshalled suggesting that ouabain increases calcium influx into arteriolar muscle, decreasing afferent arteriolar radius and thereby decreasing both RPF and GFR (Cade *et al.* 1961). Despite reduction of the filtered load of sodium, a dramatic natriuresis and diuresis was regularly observed on the ouabain-infused side (Cade *et al.* 1961; Hook, 1969; Nahmod & Walser, 1966; Wilde & Howard, 1960). The effects on potassium excretion are less straightforward. Although potassium reabsorption is certainly depressed, there appears to be little effect on excretion in many instances (Cade *et al.* 1961; Nahmod & Walser, 1966; Orloff & Burg, 1960; Wilde & Howard, 1960) unless excretion rate is initially elevated, in which case excretion is somewhat depressed by ouabain (Cade *et al.* 1961; Orloff & Burg, 1960). Our observations are consistent with these many previous reports.

The effects of ouabain are spacially generalized within the kidney. Decreased proximal (Maude, 1969), Henle's Loop (Martinez-Maldonado *et al.* 1970), and distal (Cade *et al.* 1961; Duarte *et al.* 1971; Martinez-Maldonado *et al.* 1970; Orloff & Burg, 1960; Wilde & Howard, 1960) ion and water transport have been inferred from clearance (Cade *et al.* 1961; Martinez-Maldonado *et al.* 1969; Orloff & Burg, 1960), stop-flow (Wilde & Howard, 1960), and micropuncture (Duarte *et al.* 1971; Maude, 1969) experiments. Moreover, both renal cortical (Duggan & Noll, 1965; Hook, 1969; Martinez-Maldonado *et al.* 1969) and medullary (Martinez-Maldonado *et al.* 1969) sodium-potassium activated ATPase are inhibited by ouabain.

The mechanism of action on renal transport processes has received much attention. A growing body of evidence supports a model of the renal distal tubular cell in which active sodium efflux and potassium influx occur at the contralumenal membrane. Intracellular potassium and sodium concentrations are maintained high and low, respectively, with respect to interstitial fluid. Permeation of sodium across the lumenal membrane occurs passively, down the electrochemical gradient established and maintained by the pump (Burg & Orloff, 1962; Cade *et al.* 1961; Duarte *et al.* 1971; Maude, 1969; Orloff & Burg, 1960). Three lines of evidence suggest that cardiac glycosides act on the active contralumenal component. First, ouabain diminishes the renal oxygen consumption thought to be necessitated by active Na transport (Whittam & Willis, 1963). Secondly, ouabain inhibits renal sodium-potassium activated ATPase (see above) which histochemical techniques have localized on the contralumenal side of renal cells (Ashworth, Luibel & Stewart, 1963; Spater, Novikoff & Masek, 1958). Thirdly, renal intracellular sodium ion concentration increases after ouabain administration (Burg & Orloff, 1962; Maude, 1969; Nahmod &

Walser, 1966). Although our observations are not intended to provide support for this model, they are entirely consistent with it.

Our results are in accord with previous observations of increased renin secretion in response to either ureteral occlusion or reductions in renal perfusion pressure (Cooke *et al.* 1970; Schmid, 1972; Vander & Miller, 1964; Vander, 1967). Although not demonstrated in the present experiments, the renin stimulatory effect of reduced renal perfusion pressure persists for at least several hours (Schmid, 1972; Vander & Miller, 1964; Vander, 1967). Since either ureteral occlusion or aortic clamping reduces both afferent arteriolar transmural pressure and macula densa sodium load, both the baroreceptor and the macula densa theories could explain the increased renin secretion (Vander, 1967). On the other hand, it does not seem likely that the baroreceptor mechanism could explain the renin response to ouabain administration. According to this mechanism, afferent arteriolar transmural pressure must have increased as a result of ouabain administration in order to account for the observed inhibition of renin secretion. Evidence presented above suggests that ouabain actually reduces afferent arteriolar transmural pressure, accounting for the decreases in RPF and GFR which we and others have observed. Thus, ouabain superimposed on ureteral occlusion most probably reduced afferent arteriolar transmural pressure more than ureteral occlusion alone yet renin secretion was stimulated more by ureteral occlusion alone. The renal haemodynamic effects of ouabain in the aortic clamp experiments were not so clear-cut. Low urine flow rates prevented accurate assessment of GFR and thereby prevented drawing conclusions concerning sites of action of ouabain. In any case, it is difficult to interpret the effect of ouabain as supporting the baroreceptor theory. In order to account for the decrease in renin secretion observed between periods two and three (Table 3), ouabain would have to produce an increase in afferent arteriolar transmural pressure. Although an increase in efferent arteriolar resistance would have this effect, it would also have the effect of increasing total renal vascular resistance, and thereby decreasing renal plasma flow. Since plasma flow did not change, interpreting the decreased renin according to the baroreceptor theory requires that ouabain dilates afferent arterioles while constricting efferent arterioles. This seems unlikely but it cannot be excluded with certainty.

Similarly our results are not consistent with the hypothesis that renin secretion and tubular fluid sodium load at the macula densa are inversely related. Distal tubular fluid flow stops shortly after the ureters are occluded during hypertonic mannitol administration (Malvin, Wilde, Vander & Sullivan, 1958; Vander, Malvin, Wilde & Sullivan, 1958). In this circumstance, macula densa sodium load is unchanging and virtually zero whether

or not ouabain is administered. Aortic clamp experiments cannot provide equally convincing evidence against the load theory. Presumably, load decreases during aortic clamping but if ouabain inhibits sodium transport proximal to the macula densa site as suggested above, its administration could increase sodium load despite continued aortic clamping.

When Vander (1967) proposed the macula densa load hypothesis he speculated that perhaps macula densa cells detect changes in tubular sodium load via changes in their intracellular sodium concentration. Renin secretion and intracellular sodium concentration would be inversely related. Since many investigators have measured increases in renal intracellular sodium concentration after ouabain administration, we believe that our results support this hypothesis.

An apparent discrepancy must be mentioned. Ouabain did not detectably decrease the unstimulated level of renin secretion. The most obvious explanation is that factors other than intracellular sodium were operative in controlling renin secretion. On the other hand, we are inclined to believe that variability in initial levels of renin secretion, and in its determination in general, prevented the detection of a real but very small decrease. As support for this belief we cite the many previous studies by others in which the inhibitory effects on renin secretion of osmotic and pharmacologic diuretics, intrarenal arterial potassium infusions, pitressin and adenine compounds could only be demonstrated by first stimulating renin secretion with aortic clamping or acute or chronic sodium depletion (Tagawa & Vander, 1970; Tagawa, Vander, Bonjour & Malvin, 1971; Vander & Miller, 1964; Vander, 1970).

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