Renal Function and Renin Secretion after Administration of Ouabain and Ouabain Plus Furosemide in Conscious Sheep

EDWARD H. BLAINE AND MARK B. ZIMMERMAN

SUMMARY The effects of ouabain or ouabain and furosemide on renal function and renin secretion were studied in conscious isovolemic sheep. The sheep received a continuous renal arterial infusion of papaverine, 7 mg/min, throughout the experiment. Ouabain alone (7 \times 10^{-7} M in the renal plasma) produced significant decreases in glomerular filtration rate (GFR) and renal plasma flow (RPF) but not in renal perfusion pressure. Plasma [K+] rose after ouabain administration. Fractional (FE\textsubscript{Na}) and absolute (U\textsubscript{Na}V) Na\textsuperscript{+} excretion were 2.9 ± 1.0% (mean ± SE) and 78 ± 54 µEq/min, respectively, during the papaverine infusion and rose to 19 ± 5.1% (P < 0.05) and 528 ± 116 µEq/min (P < 0.01) after ouabain administration. Despite the large changes in Na\textsuperscript{+} reabsorption, renin secretion was not stimulated. During the control period, renin secretion was 281 ± 131 ng/min and the average renin secretion after ouabain administration was 310 ± 78 ng/min (not significant). A smaller dose of ouabain (2 \times 10^{-7} M) infused into the renal artery with 40 mg of furosemide, iv, did not decrease GFR but RPF was suppressed. FE\textsubscript{Na} and U\textsubscript{Na}V averaged 4.4 ± 1.6% and 121 ± 44 µEq/min, respectively, while papaverine was infused into the renal artery and increased to 18 ± 4.8% (P < 0.05) and 636 ± 209 µEq/min (P < 0.05) after ouabain and furosemide were infused. Renin secretion was 118 ± 62 ng/min during the control period and averaged 240 ± 67 ng/min after ouabain plus furosemide. The difference was not statistically significant. Thus ouabain alone does not stimulate renin secretion in the conscious, isovolemic sheep despite a presumed increase in [NaCl] at the macula densa and inhibition of NaCl transport by the loop of Henle. Ouabain also blocks the normal stimulatory effects of furosemide on renin secretion.

IT HAS BEEN argued that the macula densa plays a significant role in regulating renin secretion. Although it has not been possible to define precisely the variable which is sensed by the macula densa, most investigators have favored a role for Na\textsuperscript{+}. Vander and Carlson\textsuperscript{3} proposed that a decrease in [Na\textsuperscript{+}] in, or Na\textsuperscript{+} transport by, the macula densa cells might stimulate renin secretion. They supported this contention by demonstrating that furosemide stimulated renin secretion even if blood volume was maintained. Also, their data revealed a direct relationship between the degree of inhibition of Na reabsorption in the ascending limb of the loop of Henle and the level of renin secretion. Others\textsuperscript{9-10} have favored a similar interpretation. Alternatively, Thurau et al.\textsuperscript{7} suggested that an increase in renin secretion resulted from an increase in [Na\textsuperscript{+}] at the level of the macula densa segment. This hypothesis was based on the observation that retrograde infusion of a variety of sodium salts into the distal renal tubule produced collapse of the proximal tubule and, more recently, by direct measurement of renin content of individual juxtaglomerular apparatuses.\textsuperscript{8} It also has been suggested that furosemide and ethacrynic acid might stimulate renin secretion by increasing [NaCl] at the level of the macula densa.\textsuperscript{9,10}

Another drug that inhibits NaCl transport in the ascending limb of the loop of Henle is ouabain.\textsuperscript{11-13} Like furosemide, ouabain should increase [NaCl] at the macula densa and, if this is a normal secretory stimulus, renin secretion should rise. If ouabain affects the same ion transport process as furosemide,\textsuperscript{14,15} then ouabain also would be expected to increase renin secretion.

However, recent data presented by Whittambury and Proverbio\textsuperscript{16} indicate that two distinct Na\textsuperscript{+} transport processes may be present within the kidney. These transport processes appear to be differentially susceptible to ouabain and furosemide. By studying the effects of ouabain and furosemide, alone and in combination, it should be possible to gain insight into the mechanism by which renin secretion is regulated, particularly concerning the variable which is sensed by the macula densa cells.

The present studies compare the effects of ouabain and ouabain plus furosemide on renin secretion. These were studied during papaverine infusion into the renal artery to block the renal vascular receptor for renin secretion.\textsuperscript{17} We have reported previously that furosemide stimulates renin secretion from the kidney of conscious sheep undergoing a similar papaverine infusion in the absence of a change in renal blood flow.\textsuperscript{18}

These studies reveal that, during maintenance of isovolemia and with papaverine continuously administered
into the renal artery, ouabain does not stimulate renin secretion, and that the effects of furosemide on renin secretion are blocked by simultaneous administration of ouabain.

Methods

The general maintenance and surgical preparation of the sheep and analytical procedures have been described previously. Briefly, the sheep underwent a unilateral nephrectomy 3–6 weeks prior to surgical implantation of catheters. One to 3 days before study, catheters were placed in the remaining renal artery and vein and into the ureter during halothane anesthesia (1–3%). A noncannulating electromagnetic flow probe (Carolina Medical Electronics, model EP 400) was placed around the renal artery close to its origin from the aorta. Additional catheters were placed in a carotid artery (via the transverse facial artery) and a jugular or saphenous vein. All catheters were filled with heparinized saline, 1000 U/ml. Plasma renin activity was determined by radioimmunoassay (New England Nuclear Kit).

Experimental Protocol

Six sheep were studied to which ouabain alone was administered and five to which ouabain and furosemide were given. The morning of the experiment, the sheep were connected to the appropriate recording devices (Carolina Electronics blood flow meter, Micron M-15 pressure transducer and Gilson recorder) and a priming solution of inulin (2 g) was administered, intravenously. This was followed immediately by a continuous inulin infusion at approximately 0.5 mg/kg per min. After an equilibration period of 1 hour, during which normal saline was infused into the renal artery at 0.6 ml/min, timed collections of urine were begun. The clearance periods lasted 20 minutes with blood samples being obtained at the midpoint of each clearance period. Blood (6 ml) was withdrawn from the systemic arterial and renal venous catheter simultaneously into chilled plastic tubes containing 50 μl of 10% ethylenediaminetetraacetic acid. The withdrawn blood was replaced immediately with 12 ml of fresh whole sheep blood. Isovolemia was maintained by infusing sterile 0.9% NaCl solution through a variable speed roller pump (Minipuls, Gilson Electronics). The saline reservoir rested on a balance so that the urine volume and amount of saline infused could be compared on a minute-to-minute basis.

During the first 20-minute clearance period, saline was infused into the renal artery. Subsequently, papaverine HCl (7 mg/min) (Sigma Chemical Co.) dissolved in normal saline was infused similarly. From the systemic arterial and renal venous catheter simultaneously into chilled plastic tubes containing 50 μl of 10% ethylenediaminetetraacetic acid. The withdrawn blood was replaced immediately with 12 ml of fresh whole sheep blood. Isovolemia was maintained by infusing sterile 0.9% NaCl solution through a variable speed roller pump (Minipuls, Gilson Electronics). The saline reservoir rested on a balance so that the urine volume and amount of saline infused could be compared on a minute-to-minute basis.

After this second 20-minute clearance period during which only papaverine was infused, ouabain dissolved in the papaverine solution was infused for ten minutes. The ouabain infusion produced an average concentration in the renal plasma of 7 × 10−7 M. The renal arterial infusion was returned to papaverine alone for the duration of the experiment. In the sheep that received ouabain plus furosemide, the furosemide (40 mg) was infused intravenously simultaneously with the renal arterial infusion of ouabain. The amount of ouabain infused in this group was less (2 × 10−7 M in the RPF) but, in combination with the furosemide, resulted in a similar level of inhibition of Na+ reabsorption.

Statistical Analysis

Analysis of the data was based on a pair design wherein each sheep served as its own control. The control period against which the effects of ouabain plus furosemide were compared was the 20-minute period during which only papaverine was infused into the renal artery. Because of inter-animal variation in the time of onset of the effects of ouabain, the data are reported for the individual 20-minute clearance period as well as the average of the four 20-minute periods after diuretic administration.

Results

Effects of Renal Arterial Infusion of Ouabain on Renal Function and Renin Secretion

The data for renal function and renin secretion for the period during which saline was infused are presented in Table 1 along with the average values for all variables for the entire 80-minute period after beginning the ouabain infusion. The changes in renal function on a period-by-period basis are presented in Figure 1.

When ouabain was infused for 10 minutes into the renal artery, GFR decreased. This occurred despite the continued administration of papaverine and replacement of urine volume by isotonic saline. The maximum change in GFR occurred 60 minutes after initiating the ouabain infusion and GFR remained depressed for the duration of the experiment. Because of the relatively large variation in the changes in GFR induced by ouabain, only during the two final clearance periods were the differences statistically significant (∗P < 0.05). The average GFR for the entire 80-minute post-ouabain period was approximately 26% below the period for papaverine alone, but the difference was not statistically significant. Arterial blood pressure was not altered after ouabain, but renal resistance was significantly elevated at all time periods. There was a significant fall in RPF which approximated the level observed during saline infusion. It was, however, 27% below the level that was measured during the infusion of papaverine alone (∗∗P < 0.01).

Plasma [Na+] was unchanged throughout the experiment, but plasma [K+] rose. The increase in plasma [K+] was statistically significant at all time periods after ouabain administration (Fig. 2).

The inhibitory effect of ouabain on Na+ transport was clearly demonstrated by the changes in fractional Na+ excretion (FENa) which occurred after beginning the renal arterial infusion of ouabain. During the clearance period in which ouabain was infused, FE Na did not increase significantly. By the end of the next period, FE Na was elevated by approximately 15%. The maximal rise occurred by 80 minutes and was maintained at this level for the final 20-minute period (∗∗P < 0.05).

Figure 3 depicts the changes in renin secretion and absolute Na+ excretion (U Na,V) in this experimental group. When ouabain was administered, U Na,V rose to quite high levels, reaching a maximum of 738 ± 137
Table 1  Average of the Four Clearance Periods after Ouabain Administration Compared to the Periods during which Only Saline or Only Papaverine was Infused into the Renal Artery

<table>
<thead>
<tr>
<th>Renin secretion (ng/min)</th>
<th>Saline</th>
<th>Papaverine</th>
<th>Mean of all periods after ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Na⁺ excretion (μEq/min)</td>
<td>137 ± 45</td>
<td>281 ± 131</td>
<td>310 ± 78</td>
</tr>
<tr>
<td>Fractional Na⁺ excretion (%)</td>
<td>110 ± 41</td>
<td>78 ± 54</td>
<td>528 ± 116*</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
<td>2.1 ± 1.1</td>
<td>2.9 ± 1.0</td>
<td>19 ± 5.1†</td>
</tr>
<tr>
<td>Renal plasma flow (ml/min)</td>
<td>47 ± 7</td>
<td>38 ± 8</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>331 ± 52</td>
<td>455 ± 44</td>
<td>332 ± 47*</td>
</tr>
<tr>
<td>Renal resistance (PRU)</td>
<td>83 ± 5</td>
<td>78 ± 5</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/liter)</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.20 ± 0.03†</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/liter)</td>
<td>146 ± 1</td>
<td>146 ± 1</td>
<td>145 ± 2</td>
</tr>
</tbody>
</table>

PRU = unit of peripheral resistance.

The statistical comparison is between the average for all periods after ouabain and the period during which only papaverine was infused:
* P < 0.01.
† P < 0.05.

μEq/min by 60 minutes after beginning the infusion (P < 0.01). As for \( FE_{Na} \), there was a delay before \( U_{Na}V \) increased, but levels remained elevated for the duration of the experiment. The average \( U_{Na}V \) for the 80-minute post-ouabain period was 528 ± 116 μEq/min, representing approximately a 7-fold increase above the control value (P < 0.01).

Despite the large increase in \( U_{Na}V \), renin secretion was not affected by ouabain (Fig. 3). Thus, at no time after ouabain administration was mean renin secretion significantly elevated above the value for the papaverine alone period. Renin secretion did show a transient rise to 663 ± 236 ng/min 80 minutes after ouabain administration. This increase was due principally to results from a single sheep in which renin secretion rose to 1717 ng/min for this period only, returning to 0 in the subsequent period. It is possible that, in this one sheep, adrenal catecholamine release was triggered inadvertently and caused the transient surge in renin secretion. The average renin secretion for the entire period after ouabain administration was 310 ± 78 ng/min compared to the control level of 281 ± 131. The difference was not statistically significant.

Effects of Renal Arterial Infusion of Ouabain Plus Intravenous Furosemide on Renal Function and Renin Secretion

The data for renal function are presented in Figure 4 and the averaged data for all periods after ouabain plus furosemide are given in Table 2.

Unlike the previously described group, the combination of renal arterial ouabain and intravenous furosemide did not produce any changes in GFR. Mean GFR for each of the four 20-minute clearance periods did not differ by more than 2 ml/min from the papaverine alone control period, and the average GFR for the entire 80 minutes after ouabain plus furosemide was 26 ± 4 ml/min vs. 25 ± 5 ml/min during the papaverine alone period.

Arterial blood pressure also was not affected, and renal resistance was significantly elevated above control only during the clearance period in which ouabain and furosemide were administered (P < 0.01). Also, the average renal resistance for the entire 80-minute period was not different from the papaverine alone control period. Despite these inconsistencies in the changes in renal resistance, RPF was significantly depressed by the combined administration of the two natriuretic agents. The average decrease in RPF for the entire experimental period was 14% (P < 0.01), or approximately one-half that observed with ouabain alone (Table 1).

Plasma [Na⁺] remained unchanged during the period of observation and, in this group, plasma [K⁺] did not increase. This result differs from that observed with ouabain alone and probably reflects the smaller average dose of ouabain administered to this group.

\( FE_{Na} \) increased more promptly than in the ouabain alone group and had doubled during the clearance period in which the drugs were administered. \( FE_{Na} \) reached a maximum of 21.7 ± 5.9% by 80 minutes (P < 0.05), slightly less than the maximal \( FE_{Na} \) which occurred with
RENIN SECRETION IN CONSCIOUS SHEEP/Blaine and Zimmerman

FIGURE 1 Data on renal function and arterial blood pressure for the six conscious sheep which received only ouabain (7 × 10⁻⁴ M in the renal plasma) into the renal artery. During the initial 20-minute period, papaverine (7 mg/min) was infused into the renal artery. The infusion was changed to ouabain dissolved in the papaverine solution and infused for 10 minutes; then the infusion was returned to papaverine alone for the duration of the experiment. The graphic data are the means ± SEM and the numerical data above the points after ouabain administration are the mean differences ± SE of the mean differences from the initial papaverine alone period. *P < 0.05, **P < 0.01.

ouabain alone (Fig. 1). The average increase in FENa for the entire 80-minute experimental period was 17.7 ± 4.8% (P < 0.05), a value quite similar to the average FENa for the ouabain alone group (18.8 ± 5.1%).

Figure 5 illustrates the changes in renin secretion and UNaV which were associated with ouabain plus furosemide administration. UNaV rose rapidly after diuretic administration to reach a maximum of 897 ± 414 µEq/min by 100 minutes. The average increase for the entire postdiuretic period was 636 ± 209 µEq/min (P < 0.05), which represents an approximate 5-fold increase in UNaV over the papaverine alone period.

Renin secretion did not increase significantly after the combined administration of ouabain plus furosemide. Although there was a small, nonsignificant rise in renin secretion (Fig. 5), it was in no way comparable to the response seen with the same dose of furosemide administered without ouabain when maximum renin secretion was 2000 ± 395 ng/min.18

Effects of Epinephrine and Respiratory Arrest on Renin Secretion during Ouabain-Induced Natriuresis

It is possible that ouabain exerts a nonspecific toxic effect on the renin secretory mechanism such that normal stimulatory factors are not effective. The results from the single sheep in which a large transient rise in renin secretion occurred after ouabain administration would argue against such an interpretation. In addition, two other observations also militate against ouabain being a nonspecific toxic inhibitor of renin secretion.

Sheep have quite variable responses to ouabain and, during the course of these experiments, occasionally one would demonstrate a toxic response to the administered ouabain. In one sheep (not included in the above data), respiratory arrest occurred immediately before a renin sample was obtained. Renin secretion was 193 ng/min during the papaverine alone period and increased to 1255

FIGURE 2 Plasma [K⁺] (mean ± SEM) of the six sheep which received only ouabain (•) and the five sheep which received ouabain plus furosemide (○). *P < 0.05.

FIGURE 3 Absolute Na⁺ excretion (UNaV) and renin secretion for the six sheep receiving only ouabain. See Figure 1 for details.
More recently it has been observed that Cl\textsuperscript{−} is transported actively by the cells of the ascending limb of the loop of Henle, and furosemide inhibits this transport process.\textsuperscript{21} Probably a similar action on the macula densa segment.\textsuperscript{20}

The increases in \( U_{Na}V \) were similar to those observed previously with furosemide alone in conscious sheep, and these were closely correlated with large increases in renin secretion. If this is a normal stimulus to renin secretion, then furosemide should stimulate and ouabain should have no effect.

Many investigators have demonstrated the stimulatory effects of furosemide on renin secretion in a variety of mammalian species,\textsuperscript{1} and we have recently shown that furosemide, at the doses used in this study, markedly increases renin release in conscious, isovolemic sheep.\textsuperscript{18}

Studies on renin secretion using ouabain have provided conflicting results. Haulica et al.\textsuperscript{24} found increased renin release from isolated perfused dog kidneys after ouabain administration, but Churchill and McDonald\textsuperscript{25} reported that ouabain blocked the normal stimulatory effects on renin secretion of ureteral occlusion and partial aortic clamping. In vitro studies also have not provided uniform results. Lyons and Churchill\textsuperscript{26} found that ouabain blocked increased renin release from rat kidney cortex slices which had been stimulated by an elevated [NaCl] in the bathing medium, but Baumbach et al.\textsuperscript{27} reported a small rise in renin release from isolated glomeruli after ouabain treatment.

In the experiments reported here, ouabain did not stimulate renin secretion despite large increases in \( U_{Na}V \). The increases in \( U_{Na}V \) were similar to those observed previously with furosemide alone in conscious sheep, and these were closely correlated with large increases in renin secretion.\textsuperscript{18} It should be emphasized that in both experiments the sheep were maintained isovolemic by replacement of urinary losses with isotonic saline and both had a continuous infusion of papaverine into the renal artery at 7 mg/min.

**Discussion**

To explain how the renal tubules might function to regulate renin secretion, Vander and Carlson\textsuperscript{3} presented a cogent argument in which they suggested that the Na\textsuperscript{+} load delivered to the macula densa segment was important in regulating renin release. The actual variable sensed by the tubular cells could be intracellular [Na\textsuperscript{+}] or, as suggested by Nash et al.,\textsuperscript{4} the rate of Na\textsuperscript{+} transport by the macula densa. Much of the evidence in support of this hypothesis was derived from studies in which various diuretics were administered to alter the Na\textsuperscript{+} load to the macula densa. Furosemide has proven to be a particularly useful diuretic in this regard because it has its principal action in the ascending limb of the loop of Henle\textsuperscript{15} and probably a similar action on the macula densa segment.\textsuperscript{20}

More recently it has been observed that Cl\textsuperscript{−} is transported actively by the cells of the ascending limb of the loop of Henle, and furosemide inhibits this transport process.\textsuperscript{21} Based on these observations and other studies,\textsuperscript{22} it has been suggested that renin secretion might be responsive to changes in the transport rate of Cl\textsuperscript{−} rather than Na\textsuperscript{+}. Thurau\textsuperscript{8} has suggested that both ions may be important in the control of renin secretion.

If it is true that renin secretion occurs secondary to inhibition of Na\textsuperscript{+} or Cl\textsuperscript{−} transport in the macula densa segment, then drugs other than furosemide, for which the principal locus of action is the ascending limb, should also stimulate renin release. Also, Thurau et al.\textsuperscript{7} have suggested that an increase in [NaCl] within the macula densa segment stimulates renin secretion. If Thurau's hypothesis is correct, then inhibition of transport in the ascending limb should increase the intraluminal [NaCl] and stimulate renin secretion.

One such drug, which has not been studied extensively with regard to renin secretion, is ouabain. Ouabain is a potent inhibitor of Na-K ATPase in the renal tubules\textsuperscript{23} and has been shown to exert a major effect on ion transport in the ascending limb of the loop of Henle.\textsuperscript{11-13} We also have observed that, after ouabain administration into the renal artery of conscious sheep, the urine/plasma osmolarity is 1 (unpublished observations).

If ouabain inhibits active Na\textsuperscript{+} transport and furosemide inhibits active Cl\textsuperscript{−} transport in the ascending limb, both drugs should increase the [NaCl] in the macula densa segment. If this is a normal stimulus to renin secretion, then both drugs should produce augmented renin release during isovolemia. If, on the other hand, Cl\textsuperscript{−} transport is the variable that regulates renin secretion, then furosemide should stimulate and ouabain should have no effect.

**FIGURE 4** Data on renal function and arterial blood pressure for the five conscious sheep that received ouabain (2 \( \times \) \( 10^{-7} \) M in the renal plasma) plus furosemide (40 mg, iv). See Figure 1 for details.

ng/min after respiratory arrest (Fig. 6). \( U_{Na}V \) was 850 \( \mu \text{Eq/min} \) immediately preceding this renin sample. In another sheep, also illustrated in Figure 6, epinephrine (0.2 \( \mu \text{g/min} \)) was infused into the renal artery after the papaverine infusion had been discontinued but while \( U_{Na}V \) was 1318 \( \mu \text{Eq/min} \). This level of epinephrine induced a renin secretion of 562 ng/min compared to 89 ng/min during the papaverine alone control period.

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TABLE 2  Average of the Four Clearance Periods after Ouabain plus Furosemide Administration Compared to the Periods during which Only Saline or Only Papaverine was Infused into the Renal Artery

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Papaverine</th>
<th>Mean of all periods after ouabain plus furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin secretion (ng/min)</td>
<td>102 ± 52</td>
<td>118 ± 62</td>
<td>240 ± 67</td>
</tr>
<tr>
<td>Absolute Na⁺ excretion (µEq/min)</td>
<td>168 ± 67</td>
<td>121 ± 44</td>
<td>636 ± 209*</td>
</tr>
<tr>
<td>Fractional Na⁺ excretion (%)</td>
<td>4.1 ± 2.1</td>
<td>4.4 ± 1.6</td>
<td>18 ± 4.8*</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
<td>32 ± 4</td>
<td>25 ± 5</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Renal plasma flow (ml/min)</td>
<td>397 ± 59</td>
<td>437 ± 51</td>
<td>374 ± 57†</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>98 ± 7</td>
<td>90 ± 7</td>
<td>89 ± 6</td>
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<tr>
<td>Renal resistance (PRU)</td>
<td>0.16 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/liter)</td>
<td>142 ± 2</td>
<td>141 ± 2</td>
<td>143 ± 2</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/liter)</td>
<td>4.6 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>4.6 ± 0.4</td>
</tr>
</tbody>
</table>

PRU = unit of peripheral resistance.
The statistical comparison is between the average for all periods after ouabain plus furosemide and the period during which only papaverine was infused:
* P < 0.05
† P < 0.01

In previous experiments with furosemide alone, no changes in renal function were detected. However, in the present study, RPF and GFR decreased after ouabain infusion despite the continued administration of papaverine. Such changes have been noted previously in anesthetized animals not undergoing a papaverine infusion.28
Ouabain is well known for its vasoconstrictor activity which may be mediated by activation of adrenergic mechanisms.\(^9\) Based on studies in which adrenergic agonists have been used to stimulate renin secretion,\(^20-32\) and Figure 6, it would be expected that these changes in renal function would have resulted in an increase in renin secretion via activation of the baroreceptor mechanism. In this regard, it is important to note that the vasoconstriction induced by ouabain did not lower the RPF below the levels observed during the period in which only saline was infused (Table 1).

Another factor that must be considered regarding the failure of renin secretion to increase is the rise in plasma [K\(^+\)] after ouabain administration (Fig. 2). Several investigators have reported that an increase in plasma [K\(^+\)]\(^{33,34}\) or a high K\(^+\) diet\(^{35,36}\) can inhibit renin secretion. Conceivably, this could have occurred in the present study. Although the increase in plasma [K\(^+\)] in this group was consistent, it was not large. The average increase for the entire 80-minute period after ouabain was 0.7 ± 0.2 mEq/liter. Increases in plasma [K\(^+\)] of roughly 3 times this magnitude were observed to suppress renin secretion in the studies by Shade et al.\(^33\) and between 2 and 4 times in the studies by Vander.\(^34\) Also, in the group of sheep that received both ouabain and furosemide, plasma [K\(^+\)] did not rise (Fig. 2) and renin secretion also failed to increase. Although a possible inhibitory action of K\(^+\) on renin secretion cannot be entirely discounted in these experiments, the above considerations would argue against these changes being the principal mechanism.

If there are two distinct ion pumps within the macula densa segment, as proposed by Wittembury and Proverbo,\(^16\) then administration of furosemide in combination with ouabain should result in stimulation of renin secretion. That is, inhibition of tranacellular NaCl transport would result in stimulation of renin secretion, and inhibition of Na-K ATPase-dependent homocellular transport would have no effect.

The present experiments tested this hypothesis and it was found that renin secretion did not increase when both drugs were administered simultaneously (Fig. 5). The combined drugs resulted in levels of UNV and FEN\(^8\) similar to those seen with ouabain alone (Tables 1 and 2). Both groups received ouabain in the range of 10\(^{-7}\) M which is adequate to partially inhibit renal Na-K ATPase. However, for the group that received both ouabain and furosemide, the ouabain concentration was 2 × 10\(^{-7}\) M, whereas for the group that received ouabain alone, it was 7 × 10\(^{-7}\) M. This probably accounts for the fact that GFR did not fall significantly and plasma [K\(^+\)] was not elevated in the former group. Because plasma [K\(^+\)] did not change, it is unlikely that K\(^+\) had a suppressive effect on renin secretion. Likewise, the other measured variables (Fig. 4) did not change in a manner which would be expected to suppress the normally stimulatory effect of furosemide on renin secretion. It also would appear likely that the lower concentration would exert less nonspecific inhibitory effects if they exist.

Although a complete understanding of how ouabain might function to inhibit renin release is lacking, a possible mechanism could involve ouabain-induced depolarization of the macula densa segment. In this context, the transport of Na\(^+\) mediated by Na-K-dependent ATPase is not coupled directly to the renin secretory mechanism, but the depolarization of the cells would allow passive redistribution of Cl\(^-\) into the regulatory cells. This could inhibit the normal stimulatory effects of decreased Cl\(^-\) entry\(^{37-39}\) from the tubular lumen which is produced by furosemide.

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Coronary Blood Flow in Experimental Canine Left Ventricular Hypertrophy

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SUMMARY To determine whether left ventricular hypertrophy (LVH) altered total and regional coronary blood flow, we inflated a balloon around the ascending aorta of nine dogs; six acute and six sham-operated dogs were controls. After 6 weeks, all dogs were studied with an open chest under anesthesia; the balloons were deflated. There was moderate LVH as shown by increased left ventricular weight and fiber diameter. At rest there were no major differences of coronary flow or resistance per gram of muscle. With maximal coronary vasodilatation due to adenosine or carbochrome, mean coronary vascular resistance was 84% higher in LVH than in normal hearts; with isoproterenol, resistance was 54% higher in LVH. These changes were similar in right and left ventricles. Minimal coronary resistance at end diastole also was higher in LVH—64% and 94% for the two sets of vasodilators, respectively. There were no significant differences in capillary or large vessel proportional volumes in LVH and control dogs, but arterial capacity could not be estimated. The raised minimal coronary resistance suggests the possibility that, with stress, coronary flow, especially to subendocardial muscle, might be inappropriate and perhaps cause ischemic damage. However, the changes noted might have been due to coronary arterial responses to raised coronary pressures rather than to hypertrophy itself.

LEFT ventricular hypertrophy (LVH) may impair cardiac function but the mechanisms for this change are not clear. Hypertrophy alters excitation-contraction coupling, energy utilization, and contractile proteins, but it is not known whether these changes are primary or secondary to other mechanisms. One of those other mechanisms, myocardial ischemia, could occur in two ways: Roberts and Wear2 showed that in rabbits with left ventricular hypertrophy the capillary-fiber ratio remained at its normal value of 1:1 so that the mean diffusion distance must have increased; these findings have been confirmed in man.3,4 Then Linzbach5 and Woods6 reported that, although the main coronary arteries increased in diameter in people with LVH, this increase in diameter did not match the increased heart weight. They therefore inferred that in hypertrophy there might be a reduced coronary vascular reserve that might impair cardiac function. However, no one has shown that the increased diffusion distance for oxygen does impair cardiac function nor that coronary vascular reserve is reduced in hypertrophy.

We wished to test another hypothesis that involves myocardial blood flow, one which also fits in with the known vulnerability of the subendocardial muscle to is-
Renal function and renin secretion after administration of ouabain and ouabain plus furosemide in conscious sheep.
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