Functional significance of Na-K-ATPase in the kidney: effects of ouabain inhibition

Jorge Torretti, Ernesto Hendler, Edward Weinstein, Robert E. Longnecker, and Franklin H. Epstein

MATERIAL AND METHODS

Mongrel dogs of either sex, 16.7–34 kg in weight, were anesthetized with pentobarbital (30/mg per kg body wt ip, supplemented with 30–60 mg iv when required during the experiment). Through an incision in the lower abdomen, polyethylene catheters were placed in each ureter. Catheters were placed in both jugular veins. Blood samples were obtained from a catheter in the right femoral artery. In all experiments, blood pressure was monitored through a catheter placed in the left carotid artery. Occasionally the electrocardiogram was also monitored throughout the experiment.

A catheter introduced through the left femoral artery was placed in either left or right renal artery, and its position was checked by fluoroscopy after the injection of 5–10 ml of 50 % Hypaque (Winthrop Laboratories). After a priming dose, inulin or creatinine was infused at a constant rate through one of the jugular venous catheters. Solutions of different compositions were infused at varying speeds through the other venous catheters to produce the type of diuresis desired. A constant infusion of 0.9 % NaCl at 0.3 ml/min was maintained with a Harvard constant-infusion pump through the catheter in the renal artery. At the appropriate time, saline plus ouabain (Eli Lilly Co.) replaced the saline infusion at the same speed. Then, after 60–120 min, the renal artery infusion was switched back to saline.

After the loading dose of creatinine or inulin was infused, 20–40 min were allowed for equilibration, and then three consecutive 10- to 15-min urine collections were made before starting the infusion of ouabain. During the period of ouabain administration and thereafter until the end of the experiment, urine collections were continued every 10–15 min. Blood samples were taken at intervals of 10–30 min. All experiments were ended 60 min after the infusion of ouabain had been completed. The total dose of ouabain infused varied from 60 to 150 μg/kg. In most experiments, however, 72–96 μg/kg were given over the course of 60 min. Three dogs that received doses of ouabain of 120–150 μg/kg were also treated with diphenylhydantoin, (Dilantin, Parke, Davis & Co.) to suppress cardiac arrhythmias that might have been elicited by this large amount of ouabain. Dilantin, 300 mg, was given just before, and 2.5 mg/min were given during and after the infusion of ouabain,

through the end of the experiment, 60 min after discontinuing
the ouabain infusion.

In five dogs ouabain-3H (New England Nuclear Corp.)
was added to the ouabain infused so as to produce a final
specific activity of 5.3-22.5 μc/μmole. Tritium was mea-
sured with a Packard Tri-Carb liquid scintillation spectrom-
er in samples of plasma, urine, and kidney, the latter
pre pared as described below.

At the end of the experiment, both kidneys were rapidly
removed and placed in ice-cold saline. After removing the
capsule they were weighed and sliced, separating cortex and
outer (red) medulla, and homogenates were prepared for
measurement of Na- K-ATPase activity. Cortical and
outer medullary kidney tissue was homogenized in a
20/1 (v/w) solution containing 0.25 M sucrose, 5 mM Na
EDTA, 30 mM imidazole, and 9.4 mM sodium deoxycholate
(added immediately before use) at pH 6.8. In order to
measure total ATPase activity, 0.1 ml of this homogenate
was incubated in 5 ml of a reaction mixture of pH 7.8
prewarmed at 37 °C and with the following composition:
NaCl 100 mM, KCl 20 mM, imidazole buffer 10 mM. The
reaction was started by the addition of MgCl2 and disodium
adenosine triphosphate to give a 6 mM final concentration,
supplied after 15 min by the addition of 1 ml of ice-cold
35% (w/v) trichloroacetic acid. A parallel determination of
Mg-activated ATPase (Mg-ATPase) was carried in a
potassium-free medium of otherwise equal composition. All
determinations were carried in duplicate. Na- K-ATPase
was calculated from the difference of total and Mg-ATPase.
Details of the procedure have been reported elsewhere
(7, 10). Enzyme activity is expressed as micromoles of
inorganic phosphate released per milligram of protein per
hour (μmole Pi/mg protein per hour). The average Na-
K-ATPase activity determined at the same time in our
laboratory in normal dog cortex is 9.6 ± 0.6 (n = 8)
(mean ± se) and in red medulla is 40.8 ± 2.1 (n = 8)
μmole Pi/mg protein per hour. The average Mg-ATPase
in these normal animals was, in the cortex, 37.5 ± 1.6, and,
in the outer medulla, 49.5 ± 2.8, μmole Pi/mg protein
per hour (7). In urine and plasma samples, inulin (21)
and creatinine (6) were measured, and their clearances
were determined 1:4 by 1:20 in a medium containing sucrose (250 mM), imidazole
(30 mM), and EDTA (5 mM) so as to measure Na- K-
ATPase activity as described above. Aliquots of the concen-
trated 1:4 homogenate were placed in vials, warmed
to 37 °C for 30-60 min with N-chlorosuccinimide solubilizer
(Amersham/Searle Corp.), and after adding 17 ml of
Bray’s solution (2), tritium concentration was measured in
a Packard Tri-Carb liquid scintillation spectrometer. Whencalculating the distribution of ouabain in the kidney, it was
estimated that the cortex represented 90% and the outer
medulla represented 10% of the whole organ. The water
contents of cortex and medulla were assumed to be 75.6
and 82.9%, respectively, as documented in a previous
report from this laboratory (12).

RESULTS

General effects of ouabain infusion. In most dogs, the intra-
renal infusion of ouabain was well tolerated. When doses of
ouabain above 100 μg/kg were given, ventricular extras-
ystoles appeared. Two dogs that had not received diphenyl-
hydantoin died suddenly 30 and 50 min after ouabain was
discontinued.

Changes in blood pressure during the course of the
experiment were variable, the blood pressure ranging from
60 to 120% of control while ouabain was infused. When
ouabain was discontinued, blood pressure usually remained
at approximately the same level observed during the
infusion; in two dogs, it rose. While ouabain was infused into
the renal artery, glomerular filtration rate declined by
approximately 50%. In one-fourth of the experiments, this
reduction was partially reversed after the ouabain infusion
had been completed. In the contralateral kidney, a decrease
in filtration rate of 25-50% was also observed.

Fractional sodium excretion, as discussed in detail below,
increased in all experiments both in the infused and the
contralateral kidneys, though to a much smaller extent in
the latter. The natriuretic effect developed during the
ouabain infusion and was maximal within the 60 min of
observation that followed discontinuation of the ouabain
infusion.

Na- K-ATPase was inhibited in the infused kidney after
ouabain both in outer medulla, to the range of 31.0-4.4
μmole P1/mg protein per hour (22-89% inhibition) and
in cortex, to the range of 4.8-0.4 μmole P1/mg protein

1 Creatinine method as applied for use in Technicon AutoAnalyzer.
TABLE 1. Intrarenal distribution of infused ouabain

<table>
<thead>
<tr>
<th>Type of Diuresis</th>
<th>Body Wt</th>
<th>Ouabain Dose</th>
<th>Infused Kidney</th>
<th>Contralateral Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
<td>µg</td>
<td>Kidney wt</td>
<td>Na- K-ATPase</td>
</tr>
<tr>
<td>Dehydrated Water</td>
<td>14.6</td>
<td>876</td>
<td>42.5</td>
<td>30.4</td>
</tr>
<tr>
<td>Mannitol</td>
<td>20.7</td>
<td>1,490</td>
<td>48.4</td>
<td>42.6</td>
</tr>
<tr>
<td>Mannitol</td>
<td>16.7</td>
<td>1,202</td>
<td>55.1</td>
<td>32.3</td>
</tr>
<tr>
<td>Mannitol</td>
<td>20.3</td>
<td>1,725</td>
<td>45.5</td>
<td>60.2</td>
</tr>
<tr>
<td></td>
<td>17.6</td>
<td>1,760</td>
<td>46.3</td>
<td>62.7</td>
</tr>
</tbody>
</table>

* C = cortex, RM = red medulla, prot = protein.

 Fate of infused ouabain (Table I, Fig. 1). Five dogs received ouabain-³H (one dehydrated, one undergoing water diuresis, and three during mannitol diuresis). Measurements of tritium concentration in kidney cortex and medulla were made in all; in three, the concentration of ouabain in kidney tissue, in plasma, and in urine was calculated from the measurement of radioactivity and the specific activity of the infused ouabain.

During the period of ouabain infusion, from 18 to 30% of the infused ouabain was recovered in the urine (12-24% from the infused kidney, and 6-7% from the contralateral kidney). During the hour that followed the infusion, 5-6% was recovered in the urine (2-3% from both the infused and contralateral sides). As expected, the highest concentration of ouabain in plasma (0.145-0.44 µM) was found in the samples drawn after 55 min of infusion, i.e., just before stopping the ouabain, and this was also the time at which the excretion of ouabain by the contralateral kidney was maximal. The peak excretion of ouabain by the infused kidney occurred 10-50 min before the end of the infusion. The clearance of ouabain in the last four urine collections (20-60 min after discontinuing ouabain) in three dogs was (mean ± SEM) 54.7 ± 10.8 and 57.9 ± 13.6% of the inulin clearance in the experimental and contralateral sides, respectively.

At the end of the experiment, 8.5-14.4% of the administered ouabain was recovered from the experimental kidney and 2.8-9.8% from the contralateral kidney. Thus, about 25% of the infused ouabain was excreted in the urine or attached to the infused kidney. The fact that one-fourth or more of the ouabain administered did not enter the general circulation explains the low mortality, although we were using doses that are very close to the LDS₀ for systemic administration of ouabain (14).

The amount of ouabain attached to the infused kidney at the end of the experiment, expressed as moles per liter of tissue water, was estimated to vary from 3.4 × 10⁻⁶ to 6.7 × 10⁻⁶ M in the cortex, and from 6.6 × 10⁻⁶ to 18.4 × 10⁻⁶ M in the outer medulla. The quantity of ouabain bound to protein was 2.5-3.75 times greater in the outer medulla than in the cortex. In the contralateral kidney this ratio was 1.8-2.23.

The amount of ouabain bound in vivo to the cortex and outer medulla of the kidney was closely correlated (Fig. 1).
with the inhibition of Na-K-ATPase estimated\(^3\) in the same kidney as measured in vitro \((r = 0.86, P < 0.001)\). It is of special importance that the correlation was apparent at widely different levels of ATPase inhibition and plasma levels of ouabain, since it was seen in the contralateral kidney as well as in the kidney receiving a direct arterial infusion of ouabain. Binding of ouabain by the dog kidney in vivo averaged \(6.25 \times 10^{-12}\) moles of ouabain per unit (\(\mu\)moles/\(\mu\)g protein per hour) of Na-K-ATPase activity inhibited. This value is about 5 times higher than the ratio of bound ouabain to Na-K-ATPase activity obtained by incubation of kidney microsomes with ouabain in vitro \((7)\).

No clear explanation can be offered as yet for this interesting observation. It might conceivably be caused by a higher degree of nonspecific binding of cardiac glycoside when cells are exposed to ouabain in vivo than when microsomes are incubated with ouabain in vitro. More likely, it is due to greater accessibility of ouabain in vivo to its binding site on the kidney enzyme. The close correlation between ouabain-binding and Na-K-ATPase inhibition observed in the present experiments suggests either the farfetched possibility that nonspecific binding is for some obscure reason proportional to enzyme inhibition or that most of the ouabain bound is inhibiting the enzyme and presumably attached to it in stoichiometric fashion.

Effect of ouabain infusion on sodium excretion and concentrating capacity (Tables 2 and 3, Figs. 2 and 3). The capacity of the infused kidney to concentrate urine \(\left(\frac{T_{\text{CH}_2O}}{T_{\text{H}_2O}}\right)\) was sharply reduced by ouabain (Fig. 2). This occurred when glomerular filtration rate was only slightly reduced during ouabain infusion to 80% of the control level, as well as when the reduction in GFR was more pronounced. When the in vitro Na-K-ATPase activity of the outer medulla was reduced to 10 units or less, concentrating ability was almost completely eliminated, with values for \(T_{\text{CH}_2O}\) below 0.1 ml/min, in 7 of 10 of these experiments, the activity of Na-K-ATPase measured in whole homogenates of outer medulla averaged only \(5.6 \pm 1.13\) \(\mu\)moles Pi/\(\mu\)g protein per hour.

When ouabain was infused into the renal artery of dogs volume expanded with saline alone or with mannitol and saline, a considerable increase in fractional sodium excretion occurred, averaging 11.3% of filtered sodium in five saline-loaded dogs and 57.4% in five dogs loaded more heavily with mannitol and saline (Table 2). The highest fractional sodium excretion was obtained in a dog that received ouabain \(100 \mu\)g/kg during mannitol-saline diuresis. In this animal, Na-K-ATPase activity in the infused kidney was \(0.7\) \(\mu\)moles Pi/\(\mu\)g protein per hour in the cortex and \(7.3\) \(\mu\)moles Pi/\(\mu\)g protein per hour in the outer medulla. \(T_{\text{CH}_2O}\) fell from 1.5 to 0.1 ml/min. More than three-quarters of the filtered sodium appeared in the urine; however, \(23.5\%\) continued to be reabsorbed.

In the representative experiment shown in Table 3, Na-K-ATPase activity of the infused kidney was inhibited to approximately 65% of normal controls in the cortex and 70% in the outer medulla. The percentage of filtered sodium that was excreted in the urine increased from 3.6% during mannitol-saline diuresis to 44.7% after ouabain infusion, and \(T_{\text{CH}_2O}\) fell from 0.70 to 0.20 ml/min. Less marked inhibition of Na-K-ATPase, as seen in the contralateral kidney where both cortical and medullary enzyme activity was slightly below half of the average control, produced a smaller increase in fractional sodium excretion from 8.9 to 10.8%, though \(T_{\text{CH}_2O}\) fell markedly from 1.0 to 0.4 ml/min.

When ouabain was infused into the renal artery of three hydropenic dogs (Table 2), Na-K-ATPase was inhibited in the infused kidney to the same level as in the preceding experiments, but the fractional excretion of sodium attained only about half the level reached in dogs that had been prepared by mannitol-saline diuresis. Under these circumstances, the highest fractional excretion of sodium attained was 35% in a dog in which Na-K-ATPase activity in cortex and outer medulla was 1.8 and 4.6 \(\mu\)moles Pi/\(\mu\)g protein per hour, respectively.

Figure 3 summarizes the diuretic effect of ouabain when Na-K-ATPase activity in the cortex was less than 2.4 \(\mu\)moles Pi/\(\mu\)g protein per hour and in medulla less than 10.2 \(\mu\)moles Pi/\(\mu\)g protein per hour under the various preconditioning circumstances found to influence its action. The inhibitory effect of Na-K-ATPase blockade on sodium reabsorption appears to be approximately doubled by preliminary infusion of mannitol or saline.

Effect of ouabain infusion during water diuresis (Table 4). When given during water diuresis, ouabain reduced diuretic capacity while increasing sodium excretion; fractional sodium excretion, however, approached the levels observed in hydropenic dogs, rather than those seen during solute
TABLE 2. Influence of saline and mannitol diuresis upon changes in kidney function that follow ouabain infusion into renal artery

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min)</th>
<th>V (ml/min)</th>
<th>$T_{\text{H}_{2} \text{O}}$ (ml/min)</th>
<th>(CNa/Cr) $\times$ 100</th>
<th>Na-K-ATPase (pmoles P$_i$/mg protein per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Saline diuresis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>44.3 ± 4.0</td>
<td>3.6 ± 1.1</td>
<td>1.01 ± 0.25</td>
<td>8.3 ± 2.4</td>
<td>1.7 ± 0.48</td>
</tr>
<tr>
<td>Exptl</td>
<td>20.1 ± 2.9</td>
<td>6.7 ± 1.3</td>
<td>0.26 ± 0.11</td>
<td>41.3 ± 4.5*</td>
<td>11.1 ± 2.81</td>
</tr>
<tr>
<td><strong>Mannitol-saline diuresis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>15.0 ± 2.6</td>
<td>2.9 ± 0.6</td>
<td>0.79 ± 0.21</td>
<td>13.1 ± 4.9</td>
<td>1.6 ± 0.54</td>
</tr>
<tr>
<td>Exptl</td>
<td>8.8 ± 1.6</td>
<td>5.6 ± 1.0</td>
<td>0.09 ± 0.03</td>
<td>57.4 ± 6.9†</td>
<td>5.0 ± 1.30</td>
</tr>
<tr>
<td><strong>Hydropenia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>27.8 ± 8.2</td>
<td>0.3 ± 0.03</td>
<td>0.67 ± 0.26</td>
<td>0.4 ± 0.1</td>
<td>1.8 ± 0.46</td>
</tr>
<tr>
<td>Exptl</td>
<td>18.0 ± 8.6</td>
<td>4.1 ± 2.0</td>
<td>0.39 ± 0.28</td>
<td>25.0 ± 5.6</td>
<td>10.8 ± 4.77</td>
</tr>
</tbody>
</table>

Values are means ± se. Dose of ouabain: saline diuresis, 89 µg/kg (range 84-90); mannitol-saline, 112 µg/kg (72-150); hydropenia, 84 µg/kg (each experiment). * Saline diuresis vs. hydropenia P < 0.05. † Mannitol-saline diuresis vs. hydropenia P < 0.01.

TABLE 3. Effect of ouabain infusion in the renal artery of a dog undergoing mannitol diuresis

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Experimental kidney</th>
<th>Contralateral kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>GFR (ml/min)</td>
<td>$T_{\text{H}_{2} \text{O}}$ (ml/min)</td>
</tr>
<tr>
<td></td>
<td>µEq/min</td>
<td></td>
</tr>
<tr>
<td>-225 to -165</td>
<td>Mannitol, 5% in 0.9% NaCl, 1,000 ml iv</td>
<td></td>
</tr>
<tr>
<td>-120</td>
<td>NaCl, 0.9% into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>Mannitol, 5% in 0.9% NaCl iv at 10 ml/min</td>
<td></td>
</tr>
<tr>
<td>-110</td>
<td>Inulin, 177 mg in 32 ml of 0.9% NaCl iv</td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>Inulin, 6.8 mg/min, vasopressin 60 munits/min, in 0.9% NaCl, 1 ml/min iv</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td>Ouabain, 1.2 µg/kg per min, added to 0.9% NaCl into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>60-70</td>
<td>Ouabain, 2.3 µg/kg per min, added to 0.9% NaCl into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>Discontinue ouabain, maintain NaCl 0.9% into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>120-130</td>
<td>Ouabain, 2.3 µg/kg per min, added to 0.9% NaCl into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>130-140</td>
<td>Discontinue ouabain, maintain NaCl 0.9% into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
</tbody>
</table>

Na-K-ATPase activity, µmoles P$_i$/mg protein per hour

<table>
<thead>
<tr>
<th>Cortex 3.4</th>
<th>Medulla 8.5</th>
<th>Cortex 5.1</th>
<th>Medulla 22.2</th>
</tr>
</thead>
</table>

Anesthetized dog, 16.7 kg body wt.

diuresis. In the experiment detailed in Table 4, Na-K-ATPase activity was considerably inhibited in cortex and medulla of the infused kidney. Fractional excretion of sodium on that side, however, never exceeded 30%. On the contralateral side, where enzyme inhibition was less than 40% in outer medulla and about 50% in cortex, the increase in fractional sodium excretion was negligible. Free water clearance (C$_{\text{Fw}}$) diminished on the infused side from 3.1 to 1.2 ml/min, despite an increase in solute excretion, attaining its minimal value at the same time that sodium excretion increased to a maximum.

**Effect of systemic administration of ethacrynic acid after infusion of ouabain into renal artery (Tables 5 and 6).** In four experiments (Table 5), 30 min after the completion of an infusion of ouabain (90 µg/kg over 1 hr), ethacrynic acid was infused intravenously at doses that have been shown to produce maximal natriuresis (1). Table 6 summarizes the observations in a representative experiment. In this 24-kg dog undergoing saline diuresis, 30 min after the infusion of ouabain had been completed, 150 mg of ethacrynic acid were given intravenously in 3 min, followed by a sustaining infusion of 1.5 mg/min until the end of the experiment 60 min later. A rise in fractional sodium excretion to 43%, comparable to that seen in other experiments during saline diuresis, was observed in the experimental kidney 30 min after the infusion of ouabain was given.

On the contralateral side, where fractional sodium excretion had risen from 12 to 20% after ouabain, the infusion of ethacrynic acid was followed by a rise in sodium excretion to a level observed in the experimental kidney. Nevertheless, inhibition of Na-K-ATPase in the contralateral kidney was only 30% in the cortex and zero in the outer medulla. This implies that ethacrynic acid produces its natriuretic effect by a mechanism independent of the inhibition of Na-K-ATPase, as suggested by Proverbio,
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Robinson, and Whittembury (17); in spite of this, its action does not appear to add to the natriuretic effect of ouabain.

DISCUSSION

It has been known for many years that infusions of cardiac glycosides directly into the renal circulation produce a diuresis of salt and water, and it has been presumed that this results from inhibition of Na- K-ATPase in renal tubular cells. In the present experiments, an attempt was made to explore the functional significance of Na- K-ATPase in the kidney by 1) trying to approach maximum inhibition of the enzyme in a kidney perfused with ouabain, while avoiding serious systemic toxicity, and 2) determining the quantitative relationship between inhibition of sodium transport and inhibition of Na- K-ATPase by ouabain.

It seems likely from these experiments and others (8, 13–16) that Na- K-ATPase is present in the kidney in considerable excess in the sense that small degrees of inhibition do not result in massive sodium diuresis. With larger doses of ouabain and more complete inhibition, sodium reabsorption is progressively inhibited. The striking finding, however, is that even when the activity of the enzyme is very small or negligible in kidney homogenates, a substantial fraction of the glomerular filtrate continues to be reabsorbed by the tubules. For example, in a dog undergoing mannitol diuresis, in which the in vitro activity of Na- K-ATPase in kidney cortex was only 0.7 μmoles Pi/mg protein per hour and in outer medulla only 7.3 μmoles Pi/mg protein per hour, 23.5% of the sodium filtered was reabsorbed. It is, of course, possible that enzyme activity is less completely inhibited in vivo than in vitro or that the tiny amount of activity persisting is responsible for the remaining reabsorptive activity in the tubules. Nevertheless, this kind of experiment suggests that Na- K-ATPase activity is necessary for only a portion of the total amount of sodium reabsorbed by the nephron. This has also been proposed by Whittembury and Proverbio (22) who pointed out that ouabain and ethacrynic acid blocked different moieties of the sodium extruded by chilled slices of kidney cortex when they were warmed. In our experiments, however, ethacrynic acid did not usually increase sodium excretion in the dog kidney already maximally blocked with ouabain, a fact

![Table 4](http://ajplegacy.physiology.org/doi/abs/10.1152/ajprenal.1977.234.4.1433)

**Table 4. Effect of ouabain infusion into the renal artery of a dog undergoing water diuresis**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Experimental Kidney</th>
<th>Contralateral Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>GFR</td>
</tr>
<tr>
<td>-300 to -270</td>
<td>Dextrose, 2.5% in water, 1,000 ml iv</td>
<td>Dextrose, 2.5% in water iv at 4 ml/min</td>
</tr>
<tr>
<td>-244 to -234</td>
<td>Inulin, 371 mg iv in 53 ml of dextrose 2.5% in water</td>
<td>NaCl, 0.9% into right renal artery at 0.5 ml/min</td>
</tr>
<tr>
<td>-234</td>
<td>Dextrose, 2.5% in water iv at 2 ml/min</td>
<td>Inulin, 7 mg/ml in dextrose, 2.5% in water iv at 1 ml/min</td>
</tr>
<tr>
<td>0-10</td>
<td>3.3</td>
<td>47.1</td>
</tr>
<tr>
<td>10-20</td>
<td>3.7</td>
<td>42.7</td>
</tr>
<tr>
<td>60-70</td>
<td>Oubain 1.2 mg/kg per min, added to 0.9% NaCl into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>8.1</td>
<td>36.0</td>
</tr>
<tr>
<td>80</td>
<td>10.3</td>
<td>33.1</td>
</tr>
<tr>
<td>80-90</td>
<td>Discontinue ouabain, maintain NaCl 0.9% into right renal artery at 0.5 ml/min</td>
<td></td>
</tr>
<tr>
<td>90-130</td>
<td>9.8</td>
<td>29.1</td>
</tr>
<tr>
<td>120-130</td>
<td>8.4</td>
<td>25.2</td>
</tr>
<tr>
<td>130-140</td>
<td>7.9</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Anesthetized dog, 21 kg body wt.
proximal tubule (4), ouabain might be expected to block 
approximately 60% of glomerular filtrate is reabsorbed in the 
between the proximal and distal tubules before giving the 
inhibitor. If the stage were set by hydropenia, when approxi-
by apportioning the reabsorptive load in different ways 
it should be possible to vary the diuretic effect of ouabain 
portion of the tubule. This conclusion is consistent with 
plays a critical role in the reabsorption of sodium in this 
reabsorption in the distal tubule.

stop-flow experiments that ouabain impaired sodium 
concentrating and diluting ability was reported recently 
finite urine was partially eliminated by blocking doses 
of intra-arterial ouabain and that diluting ability was also 
Na-K-ATPase is considerably higher in the red medulla 
than in the cortex (7) and much higher in the ascending 
limb of the loop of Henle and the distal convolution than 
in the proximal convolution. It is not surprising,
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