Long-term Ouabain Administration Produces Hypertension in Rats

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Ouabain has recently been identified as an endogenous Na⁺,K⁺ pump inhibitor. We administered ouabain chronically to normotensive rats with varying degrees of reduced renal mass (RRM) and to normal two-kidney rats to see whether hypertension could be produced. Normal male Wistar rats and rats with 25%, 60%, and 70% RRM received ouabain (13.9 μg/kg per day IT) in normal saline for 4 weeks followed by ouabain (27.8 μg/kg per day IT) for 3 to 4 more weeks. Respective control animals received vehicle only. Blood pressure was recorded weekly by tail plethysmography. Animals received tap water and standard rat chow, except for 70% RRM rats, which received distilled water and sodium-free chow. After 6 to 8 weeks of treatment, with rats under thiobutabarbitral anesthesia, direct blood pressure was determined. Plasma, tissue, and urinary ouabain levels were measured with a specific radioimmunoassay. Animals receiving ouabain developed significant increases in mean blood pressure compared with control animals (70% RRM, 147±4 vs 116±4 mm Hg; 60% RRM, 140±4 vs 107±3 mm Hg; 25% RRM, 131±5 vs 100±2 mm Hg; no RRM, 116±4 vs 98±5 mm Hg). Plasma ouabain levels measured 24 hours after the last ouabain dose were not different in animals receiving ouabain vs those receiving vehicle. However, kidney tissue ouabain levels were significantly greater (6.39±1.17 vs 2.36±0.52 μg/kg, ***p<0.05) in animals receiving ouabain. In conclusion, ouabain, given chronically, is associated with the development of hypertension in RRM rats as well as in normal rats. Blood pressure was greater in animals with greater degrees of RRM for a given ouabain dose. (Hypertension 1993;22:178-187)

KEY WORDS • ouabain • hypertension, renal • Na⁺,K⁺-ATPase • sodium-potassium pump

An endogenous, circulating Na⁺,K⁺-ATPase inhibitor has been proposed to contribute to the development of hypertension in volume- and salt-dependent, low-renin types of hypertension. 1-3 Such an inhibitor has been shown to be present in the plasma of animals and humans after acute volume expansion, 4, 6 in animals with volume- and salt-dependent hypertension, 7-9 and in humans with low-renin essential hypertension. 10, 11 Cardiac glycosides, which have a high affinity for Na⁺,K⁺-ATPase and inhibit Na⁺,K⁺ pump activity, produce vasoconstriction and increased cardiac contractility. 12-16 A physiological state similar to that seen in low-renin volume-dependent hypertension, 17, 18 based on these observations, an endogenous Na⁺-K⁺ pump inhibitor, similar in function (if not in structure) to the cardiac glycosides, is thought to be released in response to volume expansion and to produce vasoconstriction, increased cardiac output, increased blood pressure (particularly if sodium excretory capacity is reduced), and natriuresis and diuresis (in the animal with normal sodium excretory capacity). 19-21

Numerous investigators have tried to isolate and purify endogenous Na⁺-K⁺ pump inhibitors, 21-28 but until recently, except for lipids, only one high-affinity circulating inhibitor had been purified and its structure identified. This compound was resibufogenin, present in the skin and plasma of the toad Bufo marinus. 29, 30 Another endogenous Na⁺,K⁺-ATPase inhibitor has recently been isolated and purified from human plasma and, based on mass spectral analysis and biochemical and immunological criteria, is indistinguishable from the cardiac glycoside ouabain. 31-34 Elevated plasma levels of ouabain have been detected in deoxycorticosterone acetate-salt-treated uninephrectomized rats and in uninephrectomized rats drinking 0.9% saline, 34 both models of volume-expanded hypertension.

Although ouabain is a strong candidate as an endogenous Na⁺-K⁺ pump inhibitor, some previous observations suggest that its physiological and chemical action is not identical to that observed for such an inhibitor. In the rat, Na⁺,K⁺-ATPase is quite insensitive to ouabain. 35 It has been shown that the sensitivity of the endogenous sodium pump to ouabain in the human is approximately 10⁴-fold greater than that for the rat, whereas the difference in sensitivity is much less (approximately threefold) for the endogenous sodium pump inhibitor. 36 Furthermore, it has been shown that exogenous ouabain, given acutely, does not produce a blood pressure increase in the rat 37 but does so in a "sensitive" species such as the guinea pig, 38 nor does it...
appear to be a potent natriuretic/diuretic in the rat. Long-term studies, however, are lacking.

Despite strong evidence suggesting that the endogenous Na-K pump inhibitor may play a role in the mechanism of hypertension, it has not yet been possible to demonstrate that such an inhibitor can cause a sustained elevation of blood pressure in experimental animals. We therefore administered ouabain daily for 6 to 8 weeks to normal two-kidney (2-K) rats and to rats with varying degrees of reduced renal mass (RRM) to test whether chronic hypertension could indeed be produced in these animals. Plasma and tissue ouabain levels were also measured in these animals with a radioimmunoassay specific for ouabain. We used RRM rats because of their reduced capacity for natriuresis. It has been shown that in experimental models of low-renin, volume-expanded hypertension, endogenous Na-K pump levels are elevated only when sodium excretory capacity is greatly reduced. This occurs after surgical reduction in renal mass with or without increased salt intake and/or administration of mineralocorticoids.

Methods

Male Wistar rats weighing 150 to 200 g were purchased from Charles River Laboratories, Wilmington, Mass, and kept in a temperature- and humidity-controlled room with 12-hour light/dark cycles. They were given free access to tap water and standard rat chow. One week after the animals were received from the vendor, systolic blood pressure was determined by tail plethysmography (Natsume KN 209, Tokyo, Japan) to document normotension.

Production of Reduced Renal Mass Rats

For 70% RRM rats, animals weighing 250 to 300 g were anesthetized with ether and prepared for aseptic surgery. Through a midline abdominal incision, the right kidney and both poles of the left kidney were removed. Forty percent of the weight of the left kidney was removed, based on the assumption that the two kidneys are of equal weight. The total renal mass reduction was thus 70%. The poles of the left kidney were excised by encircling each pole with a loop of No. 4-0 silk, then tightening the loop. This method both cuts the tissue and ties off the vessels in the excised area. The abdomen was then closed, and the animals were allowed to recover in clean cages.

For 60% RRM rats, the right kidney was removed as described above. It was weighed, and total kidney weight was estimated. Ten percent of total estimated kidney weight was then removed from the lower pole of the left kidney.

For 25% RRM rats, based on our data for total kidney weight for animals in this weight range (more than 1000 animals), 12% to 13% of total kidney weight was removed from the lower pole of each kidney.

Immediately after surgery, 70% RRM animals were placed on distilled water and a sodium-free diet containing less than 0.05% sodium (Bio-serv, Inc, Frenchtown, NJ) (70% RRM rats maintained on this salt-free diet remain normotensive up to 5 weeks). All other animals were given free access to tap water and standard rat chow. Forty-eight hours after surgery, each RRM group was divided into experimental and control groups. Experimental animals received ouabain, and their respective controls received vehicle, as described below.

Normal Two-Kidney Rats

Normal untouched Wistar rats (2-K) were entered into the study when they reached 350 to 400 g body weight. There were no surgical interventions.

Ouabain Administration

Animals were randomly assigned to receive either ouabain (Sigma Chemical Co, St Louis, Mo) or vehicle (0.9% saline) intraperitoneally daily for 6 to 8 weeks. Ouabain was dissolved in sterile saline at 20 μg/mL and was stored for up to 1 week at 4°C in the dark. Doses were determined based on previously published pharmacokinetic data for ouabain in the dog. Doses administered were estimated to increase average plasma levels 500 to 1000 pM above the physiological level (estimated as 400 to 900 pM).

Stepped-Dose Protocol

Experimental animals in each group (70% RRM, 60% RRM, 25% RRM, 2-K) received ouabain in a stepped-dose protocol. This was carried out as follows: On day 1, 17 μg/kg IP ouabain was administered as a loading dose, followed by 13.9 μg/kg per day IP for 4 weeks (low dose). Then, a loading dose of 34 μg/kg IP was administered, followed by 27.8 μg/kg per day IP for an additional 4 weeks (high dose). Control animals received vehicle only. The 70% RRM animals receiving stepped-dose treatment were killed 1 week early because of the premature death of one animal.

Single-Dose Protocol

Some 70% RRM and 2-K rats were assigned to receive only a single dose of ouabain for 6 weeks. The 70% RRM animals received only the low-dose regimen (17 μg/kg IP loading dose on day 1, followed by 13.9 μg/kg per day IP for 6 weeks), whereas 2-K animals received the high-dose regimen (34 μg/kg IP loading dose on day 1, followed by 27.8 μg/kg per day IP for 6 weeks).

Tail systolic blood pressure was measured weekly in all animals. In some experimental animals on the stepped-dose protocol (70% RRM, 2-K), 4-hour urine collections were performed in metabolic cages weekly. Food, but not water, was withheld during the collections because of the risk of contamination of the urine with substances cross-reactive to the antibody used in our assay. Each milliliter of urine was mixed with 50 μL of 95 mg/mL NaEDTA and 61 mg/mL reduced glutathione (Sigma) dissolved in distilled water and was then stored at ~70°C until assay.

After 6 to 8 weeks of treatment, 24 hours after the last ouabain or vehicle injection, animals were anesthetized with 120 mg/kg IP thiobutabarbital (BYK-Gulden, Germany) for terminal studies. The trachea was cannulated with a PE 240 catheter (Clay-Adams, Parsippany, NJ) for maintenance of free respiration. The left femoral artery was cannulated with a PE 50 catheter for determination of blood pressure, and the right jugular vein was cannulated with a PE 50 catheter for bolus infusion of iced saline for cardiac output determination. A thermocatheter catheter (Cardiomax II, Columbus Instruments, Columbus, Ohio) was placed 3.2 to 3.5 cm into the right carotid artery for cardiac output determi-
Pharmacokinetic Studies

To demonstrate that ouabain was indeed absorbed from the peritoneum, we performed short-term pharmacokinetic studies comparing intravenous with intraperitoneal administration. Normal male Wistar rats (400 to 500 g) were anesthetized with sodium pentobarbital (50 mg/kg IP). The trachea was cannulated with a PE 240 catheter for maintenance of free respiration, and the right jugular vein and carotid artery were cannulated with a PE 50 catheter for intravenous saline infusion and blood withdrawal, respectively. The bladder was cannulated with a PE 90 catheter through a suprapubic incision. After a 20-minute stabilization period, animals received 0.9% saline IV at 0.0206 mL/min, and urine was collected in preweighed tubes for 1 hour. At the end of this control period, 1 mL of blood was withdrawn from the carotid cannula, mixed with 20 µL of EDTA/reduced glutathione, and treated as above for ouabain assay. One milliliter of blood was returned from a similarly prepared donor animal. Animals then received either 17 µg/kg ouabain (IP or IV, three animals per group) or 34 µg/kg ouabain (IP or IV,
FIG 2. Line graph shows tail systolic blood pressure (BP) in 60% reduced renal mass rats treated with stepped-dose ouabain or saline. n=7 animals per group. *P<.05 vs systolic BP in control (Cont) period; **P<.05 vs systolic BP in preceding week; †P<.05 vs systolic BP in saline-treated animals.

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three animals per group) as a bolus injection. Ten minutes later, 1 mL of blood was drawn from each animal and processed as above. Blood volume was replaced with an equal volume of 0.9% saline. Urine was collected hourly for 6 hours in preweighed microcentrifuge tubes, and blood was again withdrawn (and replaced with saline) at 2 and 5 hours after injection of ouabain. Urine volume was determined gravimetrically. Urine and plasma samples were stored at −70°C until ouabain assay.

Assays

Plasma, tissue, and urinary ouabain was measured by a radioimmunoassay using a ouabain-specific antisera that has been previously characterized.41 A prereacted rabbit primary41 and secondary (goat anti-rabbit) antisera (Antibodies Inc, Davis, Calif) were incubated overnight at 23°C in phosphate-buffered saline (total volume, 0.15 mL) containing 50 000 disintegrations per minute of [3H]ouabain (30 to 40 Ci/mmol, Amersham, Skokie, Ill) and C18-extracted plasma, urine, and tissue samples or commercial ouabain standards (Calbiochem Corp, La Jolla, Calif). The binding reaction was terminated by addition of 2 mL ice-cold phosphate-buffered saline followed by rapid filtration and washing using GF/B glass fiber filters (Brandel Inc, Gaithersburg, Md). The filters were allowed to soak for 12 hours in cocktail (No. 3a70B, Research Products International Corp, Mount Prospect, Ill), and the filter-associated [3H]ouabain was determined by liquid scintillation spectrometry (TA 5000, Beckman Instruments, Palo Alto, Calif). The threshold sensitivity of this assay system (>5% displacement of control binding) is 30 fmol ouabain. The intra-assay and interassay coefficients of variation are 4.3% and 10.9%, respectively.

The preparation of plasma extracts has been described elsewhere.41 Urine was extracted as described for plasma. For tissue measurements, freshly defatted tissue was weighed and homogenized in 20 vol of methanol containing 2 mM ascorbic acid. A clear supernatant was obtained by centrifugation (10 000g, 20 minutes) and dried by vacuum centrifugation. The dried residue was reconstituted with 2 vol of water containing 0.1% trifluoroacetic acid and extracted using disposable C18 columns (Bond Elut, Analytichem International, Harbor City, Calif) as described.41 Tissue levels are reported as micrograms of ouabain per kilogram wet weight.

Plasma renin activity was determined with a commercial radioimmunoassay (Baxter-Dade, Cambridge, Mass).

Statistical Analysis

Data are expressed as mean±SEM. Comparisons between two independent groups were made with the Student's t test. Comparisons between more than two independent groups were made with one-way analysis of variance followed by the Tukey Honestly Significant Difference Test extended for unequal sample size. Within-group comparisons over time (repeated measures) were made with the TSRM Test, followed by Duncan's Multiple-Range Test. A value of P<.05 was considered significant.

Results

Except for 70% RRM rats receiving stepped-dose ouabain, animals treated with ouabain appeared healthy and gained weight normally. Body weights were not significantly different between animals receiving ouabain and their respective controls at the time of death (control vs ouabain: 70% RRM, 455±19 vs 441±21 g; 60% RRM, 471±24 vs 478±19 g; 25% RRM, 470±9 vs 448±10 g; 2-K, 506±12 vs 481±18 g). In 70% RRM rats receiving stepped-dose treatment, weight gain plateaued during the period in which they received 27.8 μg/kg per day IP, and one rat died prematurely at week 7.
As shown in Fig 1, ouabain administered intraperitoneally was readily absorbed, and plasma ouabain levels were not significantly different between the intraperitoneal and intravenous groups at 10 minutes after administration. At 5 hours after injection, plasma levels had returned to near baseline; however, urinary excretion of ouabain was still elevated compared with that of the control period (Fig 1). Total urinary excretion ranged from 2% to 14% of the administered dose and did not differ significantly between the animals receiving intraperitoneal or intravenous ouabain.

Tail systolic pressure in 60% and 25% RRM rats receiving ouabain increased in a dose-dependent manner (Figs 2 and 3), whereas 70% and 2-K animals developed increases in tail systolic pressure that were not clearly dose dependent (Figs 4 and 5). All ouabain-treated groups achieved greater tail systolic pressures, compared with their respective saline-treated controls,
during treatment with 27.8 μg/kg per day ouabain. Only 70% and 60% RRM rats showed significantly greater tail systolic pressures vs controls during treatment with 13.9 μg/kg per day ouabain (Figs 2 through 5). The direct mean arterial pressures of rats in each of the four groups were significantly increased when compared with their respective controls (Fig 6, Table 1), confirming our indirect measurements. There was also a significant increase in mean arterial pressure with increasing reduction in renal mass for a given dose of ouabain (Fig 6). The respective control animals remained normotensive throughout the experiment, except for 70% RRM rats. In these vehicle-treated rats, mean arterial pressures were significantly greater than those observed in the 25% RRM and normal 2-K vehicle-treated groups (Fig 6).

The complete hemodynamic data recorded with rats under thiobutabarbital anesthesia at the time of death are shown in Table 1. Mean arterial pressures were significantly elevated in rats receiving ouabain when compared with their respective controls. Cardiac outputs in rats receiving ouabain were not significantly different when compared with rats receiving vehicle. Calculated peripheral vascular resistance was significantly greater in 70% RRM, 60% RRM, and 25% RRM rats when compared with their respective controls. Heart rate was significantly greater in 60% RRM and 25% RRM animals receiving ouabain vs controls. Stroke volume was not significantly different when compared with controls, except for the 60% RRM animals, in which it was significantly depressed in the ouabain-treated group.

Table 2 shows organ weight–body weight ratios. Despite elevated blood pressure in the ouabain-treated rats, ventricular weight–body weight ratio was not significantly increased relative to the respective vehicle-treated controls. Total kidney weight–body weight ratio was not significantly different between groups, nor was significantly elevated in rats receiving ouabain when compared with their respective controls. Cardiac outputs in rats receiving ouabain were not significantly different when compared with rats receiving vehicle. Calculated peripheral vascular resistance was significantly greater in 70% RRM, 60% RRM, and 25% RRM rats when compared with their respective controls. Heart rate was significantly greater in 60% RRM and 25% RRM animals receiving ouabain vs controls. Stroke volume was not significantly different when compared with controls, except for the 60% RRM animals, in which it was significantly depressed in the ouabain-treated group.

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**Fig 5.** Line graph shows tail systolic blood pressure (BP) in normal two-kidney rats treated with stepped-dose ouabain or saline. n=7 animals in vehicle group, 5 animals in ouabain group. *P<.05 vs systolic BP in control (Cont) period; **P<.05 vs systolic BP in preceding week; †P<.05 vs systolic BP in saline-treated animals.

**Fig 6.** Bar graph shows direct mean arterial blood pressure (BP) in normal two-kidney (N1 2K) and reduced renal mass rats receiving stepped-dose ouabain or saline. All rats receiving ouabain had mean arterial pressures significantly (P<.05) greater than their respective controls. *P<.05 vs mean arterial pressure in normal two-kidney rats receiving respective treatment; **P<.05 vs mean arterial pressure in 25% reduced renal mass rats receiving respective treatment.
TABLE 1. Hemodynamic Values at Time of Death

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>HR (bpm)</th>
<th>CO (mL/min)</th>
<th>SV (pL/beat)</th>
<th>PVR ([mm Hg/mL]/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% RRM saline</td>
<td>4</td>
<td>116±4</td>
<td>376±13</td>
<td>105±9</td>
<td>291±24</td>
<td>1.13±0.06</td>
</tr>
<tr>
<td>70% RRM single</td>
<td>5</td>
<td>142±5*</td>
<td>378±8</td>
<td>96±4</td>
<td>279±31</td>
<td>1.52±0.13*</td>
</tr>
<tr>
<td>70% RRM stepped</td>
<td>5</td>
<td>147±4*</td>
<td>410±17</td>
<td>111±3</td>
<td>273±13</td>
<td>1.32±0.04</td>
</tr>
<tr>
<td>60% RRM saline</td>
<td>7</td>
<td>107±3</td>
<td>318±18</td>
<td>99±2</td>
<td>309±11</td>
<td>1.09±0.04</td>
</tr>
<tr>
<td>60% RRM stepped</td>
<td>7</td>
<td>140±4*</td>
<td>366±11*</td>
<td>92±5</td>
<td>251±17*</td>
<td>1.55±0.07*</td>
</tr>
<tr>
<td>25% RRM saline</td>
<td>6</td>
<td>100±2</td>
<td>326±9</td>
<td>108±9</td>
<td>329±30</td>
<td>0.96±0.10</td>
</tr>
<tr>
<td>25% RRM stepped</td>
<td>6</td>
<td>131±5*</td>
<td>362±11*</td>
<td>100±7</td>
<td>278±25</td>
<td>1.36±0.13*</td>
</tr>
<tr>
<td>2-K saline</td>
<td>7</td>
<td>98±5</td>
<td>336±30</td>
<td>92±7</td>
<td>283±26</td>
<td>1.08±0.06</td>
</tr>
<tr>
<td>2-K single</td>
<td>8</td>
<td>116±4*</td>
<td>369±15</td>
<td>99±6</td>
<td>275±22</td>
<td>1.21±0.10</td>
</tr>
<tr>
<td>2-K stepped</td>
<td>5</td>
<td>116±3*</td>
<td>360±8</td>
<td>96±5</td>
<td>268±17</td>
<td>1.22±0.07</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; CO, cardiac output; SV, stroke volume; PVR, peripheral vascular resistance; RRM, reduced renal mass; 2-K, normal two-kidney rats. Values are mean±SEM.

*P<.05 vs respective saline-treated controls.

The findings are remarkable for several reasons. First, clinical experience would suggest that the long-term administration of cardiac glycosides (particularly digoxin) does not produce hypertension. However, ouabain has been used clinically primarily as a short-term, intravenous drug in patients with congestive heart failure or atrial arrhythmias, who may be unable to demonstrate the pressor response. Second, the pressor action of ouabain occurred in normal animals consuming an ordinary diet; reduction in renal mass was not necessary, although pressor response was enhanced with renal mass reduction. Third, based on weight gain, animal behavior, and cardiac rhythm, the pressor effects of ouabain did not appear to be associated with substantial toxicity, except for 70% RRM rats receiving the higher ouabain dose. Harvested tissues from treated rats appeared to be normal. Last, the quantity of ouabain given to each animal daily (approximately 5 to 15 µg) was quite small, emphasizing the potency of ouabain as a pressor agent.

We chose to study the effects of ouabain on RRM rats based on the following reasoning. Animals with 70% RRM, if allowed a regular salt-containing diet (ie, normal rat chow), develop hypertension, extracellular volume expansion, depressed plasma renin activity, increased plasma Na⁺,K⁺-ATPase inhibitory activity, and suppressed cardiovascular muscle Na⁺-K⁺ pump activity.7,9 Rats with 70% RRM on a salt-free diet for 5 weeks and 60% RRM rats on a regular diet do not show significant differences in their hemodynamic values at time of death.

TABLE 2. Organ Weight–Body Weight Ratio at Time of Death

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ventricles</th>
<th>Kidney</th>
<th>Adrenals</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% RRM saline</td>
<td>4</td>
<td>0.234±0.003</td>
<td>0.411±0.024</td>
<td>0.0167±0.0017</td>
</tr>
<tr>
<td>70% RRM single</td>
<td>5</td>
<td>0.242±0.004</td>
<td>0.424±0.019</td>
<td>0.0170±0.0019</td>
</tr>
<tr>
<td>70% RRM stepped</td>
<td>5</td>
<td>0.234±0.005</td>
<td>0.431±0.026</td>
<td>0.0228±0.0016</td>
</tr>
<tr>
<td>60% RRM saline</td>
<td>7</td>
<td>0.220±0.006</td>
<td>0.418±0.015</td>
<td>0.0141±0.0002* (n=3)</td>
</tr>
<tr>
<td>60% RRM stepped</td>
<td>7</td>
<td>0.228±0.006</td>
<td>0.397±0.022</td>
<td>0.0113±0.0007 (n=3)</td>
</tr>
<tr>
<td>25% RRM saline</td>
<td>6</td>
<td>0.217±0.004</td>
<td>0.482±0.013</td>
<td>0.0113±0.0010 (n=5)</td>
</tr>
<tr>
<td>25% RRM stepped</td>
<td>6</td>
<td>0.229±0.006</td>
<td>0.515±0.009</td>
<td>0.0111±0.0010 (n=3)</td>
</tr>
<tr>
<td>2-K saline</td>
<td>7</td>
<td>0.217±0.003</td>
<td>0.628±0.026</td>
<td>0.0062±0.0004</td>
</tr>
<tr>
<td>2-K single</td>
<td>8</td>
<td>0.216±0.005</td>
<td>0.589±0.019</td>
<td>0.0054±0.0002</td>
</tr>
<tr>
<td>2-K stepped</td>
<td>5</td>
<td>0.215±0.007</td>
<td>0.592±0.017</td>
<td>0.0053±0.0003</td>
</tr>
</tbody>
</table>

RRM, reduced renal mass; 2-K, normal two-kidney rats. Values are mean±SEM. Organ weights are expressed as grams per 100 g body weight. Adrenal weight in 2-K animals is weight of left adrenal only; both adrenals were weighed in the other groups.

*P<.05 vs respective saline-treated controls.
develop hypertension but have been used in our laboratory as models of maximally compensated normotension.\textsuperscript{39,42} As would be expected, 25% RRM rats do not develop hypertension when fed a regular diet (unpublished observation). Because of these facts, we hypothesized that normotensive RRM rats might be more sensitive to the effects of long-term, exogenous Na\textsuperscript{+}, K\textsuperscript{-} ATPase inhibition, because they have a decreased capacity for urinary sodium and water excretion\textsuperscript{1,19,42,43} and therefore would be less able to mount natriuresis and diuresis as blood pressure and inhibition of renal Na\textsuperscript{+}, K\textsuperscript{-}ATPase increased.

Based on the above argument, 2-K animals were least likely to show a hypertensive response to long-term ouabain administration. Theoretically, a normal 2-K animal would not be expected to show an increase in blood pressure after administration of an Na\textsuperscript{+}, K\textsuperscript{-} ATPase inhibitor or after a physiological increase in endogenous Na\textsuperscript{+}, K\textsuperscript{-}ATPase inhibitor in response to volume expansion. This is because diuresis and natriuresis would occur, with a decline in endogenous inhibitor production due to decreasing extracellular fluid volume.\textsuperscript{1,19} When given acutely, ouabain does not produce blood pressure elevation in the normal rat.\textsuperscript{37}

By the same token, rats with the greatest renal mass reduction would be expected to be most sensitive to the hypertensive effects of exogenous Na\textsuperscript{+}, K\textsuperscript{-}ATPase inhibitor. This was the case. Mean arterial pressure increased with increasing RRM; mean arterial pressure was significantly greater in 70% RRM rats than in 25% RRM rats treated with the same dose of ouabain. Somewhat surprisingly, normal rats treated with ouabain also became hypertensive, but significantly less so than 25% RRM rats.

Direct blood pressures were more clearly different between treated and untreated animals when compared with differences observed in blood pressure obtained by tail plethysmography. This is most likely due to the increased accuracy of the direct method, especially as the surgical procedures and anesthetic used were identical between treated and untreated groups.

It is noteworthy that for all groups of animals treated with ouabain there was no significant increase in absolute cardiac output compared with controls. Theoretically, increases in Na\textsuperscript{+}, K\textsuperscript{-} ATPase inhibitor would tend to produce an increase in cardiac output by increasing cardiac contractility.\textsuperscript{12-18,22} Stroke volume was unchanged (70% RRM, 25% RRM, 2-K) or decreased (60% RRM), with a compensatory increase in heart rate observed in the 60% RRM animals. Thus, increases in mean arterial pressure induced by ouabain treatment appear to be mediated by increases in peripheral vascular resistance. However, the fact that cardiac output was maintained despite increased afterload suggests either that a relative increase in cardiac contractility occurred or that overall venous return was maintained. Although the increase in vascular resistance may be due to the direct effects of ouabain alone, ouabain may also sensitize the vasculature to other endogenous vasopressors, or it may stimulate centrally mediated sympathetic nervous system activity.\textsuperscript{44-46}

There was no evidence of ventricular hypertrophy in animals receiving ouabain, despite documented hypertension. This is notable, because 70% RRM animals fed a regular diet containing salt develop marked ventricular hypertrophy within 5 weeks of renal mass reduction\textsuperscript{39} as their blood pressure increases. Theoretically, this hypertension is based on volume expansion and Na\textsuperscript{+}, K\textsuperscript{-}ATPase inhibitor production. Therefore, our data suggest that, besides hypertension, some other factor is probably necessary to induce cardiac hypertro-

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**TABLE 3. Plasma and Tissue Ouabain Levels at Time of Death**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Plasma (pM)</th>
<th>Adrenal (µg/kg)</th>
<th>Kidney (µg/kg)</th>
<th>Heart (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain</td>
<td>34</td>
<td>852±102</td>
<td>28.5±4.3</td>
<td>6.39±1.17*</td>
<td>2.71±0.49</td>
</tr>
<tr>
<td>Saline</td>
<td>24</td>
<td>927±122</td>
<td>30.6±4.5</td>
<td>2.36±0.52</td>
<td>4.93±1.15</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

\*P<.05 vs saline-treated controls.

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**FIG 7.** Bar graph shows 4-hour urinary ouabain excretion in normal two-kidney rats and in 70% reduced renal mass (RRM) rats receiving long-term intraperitoneal ouabain. n=5 for normal rats; n=6 for 70% RRM rats. \*P<.05 vs ouabain excretion in control (Cont) period; \**P<.05 vs ouabain excretion in preceding week.
phy. Ouabain may actually be cardioprotective. Long-term digoxin administration has been shown to reduce ventricular remodeling and diastolic wall stress in hypertensive two-kidney, one clip diabetic rats. Adrenal atrophy would imply that ouabain (or an analogous substance) is adenally produced. Adrenal levels of ouabain are reported to be relatively high, when compared with other tissues, in the rat, and bovine adrenocortical cells in culture have been shown to produce ouabain. Exogenous administration of ouabain might be expected to suppress production in the gland; however, we found no significant differences in adrenal levels of ouabain in animals treated with the drug vs controls.

Despite the development of hypertension, the plasma ouabain levels of animals receiving ouabain did not significantly differ from controls. This is probably attributable to the timing of our blood sample collection. As shown in the results, we were able to detect increases in plasma ouabain level in rats at 10 minutes after administration, but plasma levels were not significantly different from baseline at 5 hours. A previous study done in dogs and humans found that after intravenous administration, plasma levels at first fell rapidly, reaching an exponential decay phase after approximately 7 hours. Our data suggest that this may differ significantly in the rat, but more extensive studies will be required. Based on our preliminary data, one would not expect plasma levels in the rats to be significantly elevated at 24 hours after the last ouabain dose. In this model, elevated tissue levels may be more predictive of hypertension than plasma levels. Although adrenal and heart ouabain levels were not significantly increased in rats receiving ouabain, renal ouabain content was significantly greater in animals receiving ouabain. We did not measure ouabain levels in vascular tissue, but an increase in vascular levels of ouabain could be associated with the observed increase in peripheral vascular resistance.

Animals in the 70% RRM and 2-K groups receiving ouabain showed increases in urinary excretion of ouabain when compared with baseline. This is consistent with previous studies of the pharmacology of the drug that endogenous Na⁺,K⁺-ATPase inhibitors are excreted in the urine.

Table 4. Plasma Renin Activity at Time of Death

<table>
<thead>
<tr>
<th>Group</th>
<th>70% RRM</th>
<th>60% RRM</th>
<th>25% RRM</th>
<th>2-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain</td>
<td>16.7±4.1*</td>
<td>17.7±2.5*</td>
<td>16.7±0.9*</td>
<td>33.4±3.5</td>
</tr>
<tr>
<td>(n=5)</td>
<td>(n=6)</td>
<td>(n=3)</td>
<td>(n=5)</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>31.8±3.8</td>
<td>29.0±5.0</td>
<td>65.3±23.3</td>
<td>32.1±7.1</td>
</tr>
<tr>
<td>(n=3)</td>
<td>(n=6)</td>
<td>(n=2)</td>
<td>(n=4)</td>
<td></td>
</tr>
</tbody>
</table>

RRM, reduced renal mass; 2-K, normal two-kidney rats. Plasma renin activity is in nanograms per milliliter per hour. Values are mean±SEM. *P<.05 vs saline-treated controls.

Plasma renin activity was significantly less in RRM rats receiving ouabain when compared with their respective controls. This was not the case in normal animals. One possible explanation for this phenomenon is that ouabain directly or indirectly inhibits renin release in rats with RRM. Furthermore, ouabain has been shown to inhibit the release of renin from isolated kidneys, suggesting a direct effect of this steroid. However, other in vivo studies have shown no effect of digoxin administration on plasma renin activity in normal humans. It is also unclear why this effect is observed only in the RRM groups. This phenomenon, as well as the question of volume status in these models, requires further study.

The sustained elevation of blood pressure in response to long-term ouabain administration supports the possibility that ouabain, or a closely related chemical compound, has a pathogenic role in some forms of hypertension that have been attributed to an endogenous Na⁺,K⁺-ATPase inhibitor. Although exogenous ouabain does not produce a hypertension syndrome identical to that seen with low-renin volume-dependent hypertension, many of the features are similar. Chronic hypertension associated with increased peripheral vascular resistance and a normal to low plasma renin activity may be observed in many patients with essential hypertension, suggesting that the mechanisms involved in this new rat model may be clinically relevant. Further study of this model, to include effects of long-term ouabain administration on body fluid volumes, renal function, and other vasoactive hormones, would be of considerable interest and of some clinical relevance.

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References


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C M Yuan, P Manunta, J M Hamlyn, S Chen, E Bohen, J Yeun, F J Haddy and M B Pamnani

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