

# Regulation of Blood Pressure During Long-Term Ouabain Infusion in Long-Evans Rats

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We tested whether ouabain, an inhibitor of the sodium pump, can lead to chronic hypertension in Long-Evans rats using sensitive 24-h measurements of blood pressure. After a control week of vehicle isotonic saline infusion (14.4 mL/day), ouabain was infused intravenously at 30  $\mu\text{g}/\text{kg}/\text{day}$  in intact (2K) and uninephrectomized (1K) Long-Evans rats for a total of 4 weeks. Although plasma ouabain concentration rose to  $0.97 \pm 0.15$  nmol/L with ouabain infusion, mean arterial pressure did not

change in either 2K ( $\Delta = -0.6 \pm 1.3$  mm Hg) or 1K ( $\Delta = -1.2 \pm 0.7$  mm Hg) rats. These data suggest that Long-Evans rats are insensitive to the hypertensive effects of ouabain. *Am J Hypertens* 1999;12:423-426 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Hypertension, ouabain, sodium pump, Long-Evans rat.

An endogenous ouabain-like inhibitor of the  $\text{Na}^+, \text{K}^+$ -ATPase pump has been implicated in the pathogenesis of many forms of experimental hypertension, especially in volume-dependent hypertension,<sup>1</sup> through the following mechanism. Inhibition of the sodium pump raises intracellular calcium concentration in vascular smooth muscle cells, via both membrane depolarization and reduction in the  $\text{Na}^+/\text{Ca}^{2+}$  exchange, resulting in vasoconstriction. It has been claimed that endogenous ouabain exhibits physiologic and biochemical activities indistinguishable from plant-derived ouabain.<sup>2</sup> Thus, if endogenous ouabain plays an important role in blood pressure control, administration of exogenous ouabain at a dose that

mimics elevated plasma levels found in hypertension should raise blood pressure.

Indeed, several experimental studies have shown that exogenous ouabain can increase blood pressure in rats.<sup>3-6</sup> Other studies, however, have failed to demonstrate a hypertensive effect of ouabain in rats,<sup>7-9</sup> sheep,<sup>10</sup> dogs,<sup>11</sup> and humans.<sup>12</sup> The diverging results in rats could be linked to differences in the doses and duration of ouabain administration in the strains used or in the methods used for blood pressure measurement. In fact, blood pressure is often measured in rats in conditions of stress, by tail-cuff techniques, or for short recording periods during recovery from surgery and anesthesia. The continuous monitoring of arterial pressure 24 h a day, a technique that is very sensitive to small changes in blood pressure,<sup>13</sup> may help to resolve this issue. Therefore, to further test the role of an endogenous ouabain-like substance in blood pressure control, we infused ouabain in Long-Evans rats at a dose known to elevate blood pressure in some studies<sup>3-6</sup> (30  $\mu\text{g}/\text{kg}/\text{day}$  intravenous [iv] for 28 days) while measuring arterial pressure 24 h a day. Furthermore, we also tested whether reduction in renal mass,

Received May 14, 1998. Accepted October 9, 1998.

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a procedure that reduces the kidney's ability to excrete salt and water, can potentiate the pressor effect of ouabain.

## MATERIALS AND METHODS

**Animal Preparation** Male Long-Evans rats (body weight, 350–450 g; 12–24 weeks old) supplied by the R. Janvier Center (Le Genest-St-Isle, France) were used in this study. All protocols were approved by the State Animal Committee. Using sodium pentobarbital anesthesia and aseptic techniques, the rats were instrumented with arterial and venous catheters inserted into the left jugular vein and the abdominal aorta (via laparotomy), respectively. In some rats, the left kidney was removed during the same procedure. The catheters were tunneled subcutaneously to the scapular region, exteriorized, protected by a flexible coiled steel spring, and connected to a double channel swivel (Instech Laboratories, Plymouth Meeting, PA). The rats were housed in individual metabolic cages, in a room maintained at constant temperature (22°C) with a 12-h light-dark cycle. The venous line was connected to a syringe pump (Kent Scientific, Litchfield, CT). The arterial line was flushed daily, filled with heparin (1000 USP/mL), and connected to a COBE (Lakewood, CO) CDX-III pressure transducer mounted on the cage at animal level. Sodium intake was fixed at about 5 mmol/day using food (25 g rat chow with 0.3% sodium) and isotonic saline intravenous infusion (14.4 mL/day). Free access was available to tap water.

**Experimental Protocol** After at least 10 days of recovery from surgery followed by a control period of 7 days of saline vehicle infusion, ouabain (Sigma Chemical Corp., St. Louis, MO) was started at 30 µg/kg/day (10 µL/min) in both intact (2K) and uninephrectomized (1K) rats for a total of 4 weeks. All solutions were prepared aseptically and infused through a Millipore filter (0.22 µm, Sterivex-GP, Millipore, Bedford, MA). Additional 2K and 1K rats served as time controls, receiving only the isotonic saline infusion. Daily urine samples and biweekly blood samples (0.7 mL) were collected for creatinine and electrolyte analysis.

**Continuous Hemodynamic Monitoring** The pulsatile arterial pressure signal was sent to an analog-digital converter (CIO-DAS08, ComputerBoards, Mansfield, MA) and analyzed by computer using customized software for beat-to-beat analysis.<sup>13</sup> The analog signal was sampled at 500 Hz, for 5-s periods every 30 s, for 22 h daily (from 10 AM to 8 AM the next day). For each rat, the daily mean arterial pressure and heart rate were thus computed as the average of 2640 sampling periods, and weekly results are reported as the average of 7 consecutive days.

**Analytical Measurements** Sodium, potassium, and creatinine concentrations of urine and blood samples

were measured by flame photometry (model IL 943, Instrumentation Laboratories, Lexington, MA) and by the kinetics of Jaffé (Beckman creatinine analyzer 2, Fullerton, CA), respectively. At the end of the experiments, a 5-mL arterial blood sample was collected in EDTA tubes for measurement of plasma ouabain concentration in duplicate by enzyme immunoassay (DuPont Ouabain EIA kit, DuPont, Boston, MA).

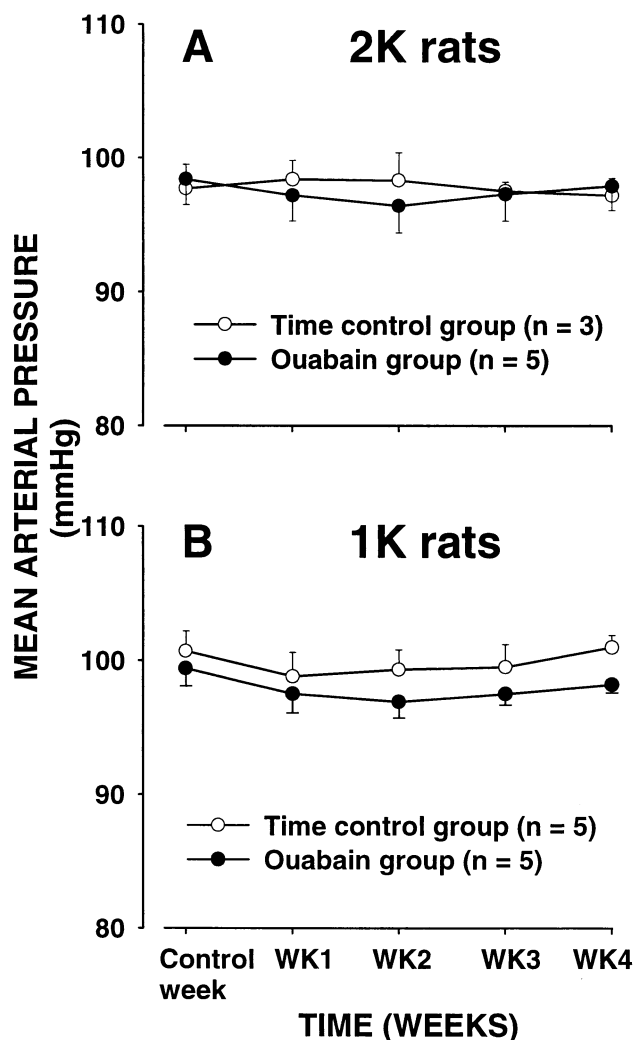
**Statistical Analysis** The data were analyzed by analysis of variance for repeated measurements and Dunnett's multiple comparison *t* test when applicable. Statistical significance was taken as  $P < .05$ . All data are expressed as mean  $\pm$  SEM.

## RESULTS

During the 5 weeks of observation, food and water intake, 24-h urinary sodium and potassium excretion, plasma sodium and potassium concentration, and glomerular filtration rate remained stable and were unaffected by the ouabain infusion. As shown in Figure 1, mean arterial pressure did not change throughout the ouabain infusion. Control values were  $98.4 \pm 1.9$  mm Hg in 2K rats ( $n = 5$ ) and  $99.4 \pm 1.3$  mm Hg in 1K rats ( $n = 5$ ), and values during the fourth week of ouabain infusion were  $97.9 \pm 1.8$  mm Hg in 2K rats and  $98.2 \pm 0.6$  mm Hg in 1K rats. Heart rate was also unaffected by the ouabain infusion. In 2K rats heart rate was  $338 \pm 14$  beats/min during the control week and  $331 \pm 9$  beats/min during the fourth week of ouabain infusion. In 1K rats heart rate was  $342 \pm 24$  beats/min during the control week and  $341 \pm 12$  beats/min during the fourth week of ouabain infusion. Plasma ouabain concentrations measured at the end of the experiment were  $0.02 \pm 0.002$  nmol/L in time-control rats (2K+1K),  $0.93 \pm 0.17$  nmol/L in 2K ouabain rats, and  $1.02 \pm 0.26$  nmol/L in 1K ouabain rats (or  $0.97 \pm 0.15$  nmol/L when 2K and 1K data are pooled).

## DISCUSSION

The major finding of the present study is that exogenous ouabain infused for 4 weeks, at a dose that raises plasma ouabain concentration in the nanomolar range, had no effect on systemic blood pressure or heart rate in Long-Evans rats, even under conditions of reduced renal mass and slightly elevated sodium intake ( $\approx 5$  mmol/day). Although endogenous ouabain is structurally indistinguishable from plant-derived ouabain by mass spectrometry,<sup>2</sup> a possible limitation of our study is that endogenous ouabain could be an isomer of ouabain and may produce a more profound inhibition of vascular smooth muscle sodium pump isoforms. However, in preliminary experiments from our laboratory, we have been unable to observe changes in blood pressure with a higher infusion rate of ouabain



**FIGURE 1.** Effects of a 4-week infusion of ouabain (ouabain group) or isotonic saline vehicle (time-control group) on mean arterial pressure in intact 2K rats (A) and in uninephrectomized 1K rats (B) following a first week of control saline infusion (control week). WK, week. Data are expressed as mean  $\pm$  SEM.

(150  $\mu$ g/kg/day for 28 days) in uninephrectomized Long-Evans rats ( $n = 4$ ). A major strength of our study is that arterial pressure was measured 24 h a day in conscious undisturbed animals, a method that provides highly reproducible values from day to day<sup>13</sup> and is thus very sensitive to small changes in blood pressure. Our data therefore strongly suggest that Long-Evans rats are resistant to the hypertensive effects of ouabain.

The role of an endogenous ouabain-like inhibitor of the Na<sup>+</sup>,K<sup>+</sup>-ATPase pump in blood pressure control remains controversial. Several studies have reported an increase in blood pressure with ouabain in different strains of rats. For example, Manunta et al<sup>3</sup> infused ouabain at different doses (3, 10, and 30  $\mu$ g/kg/day subcutaneously for 5 weeks by osmotic minipumps) in

normal Sprague-Dawley rats maintained under a normal salt intake and found a dose-dependent increase in blood pressure measured once a week using the tail-cuff method. Similarly, Kurashina et al<sup>6</sup> injected ouabain (27.8  $\mu$ g/kg/day intraperitoneally for 6 weeks) in Sprague-Dawley rats with a high salt intake and found an elevation of blood pressure measured with an indwelling catheter using anesthesia. An increase in blood pressure was also found in Wistar rats, whether ouabain was injected intraperitoneally<sup>4</sup> (27.8  $\mu$ g/kg/day for 28 days; BP measured by the tail-cuff method), infused<sup>5</sup> (10  $\mu$ g/day), or released from subcutaneously implanted pellets<sup>5</sup> (25–75  $\mu$ g/day for 2 weeks; BP measured by indwelling catheter during recovery from surgery and anesthesia).

In contrast, other studies in Sprague-Dawley rats have reported no change in blood pressure (measured by tail cuff) with ouabain administration.<sup>7–9</sup> Some of these studies<sup>8,9</sup> have been criticized because ouabain was administered at higher doses or for too short a period. High doses of ouabain can cause cardiotoxicity and sensitize arterial baroreceptor,<sup>5</sup> and could therefore attenuate the responses to ouabain. Although this could explain the negative results of Nirasawa et al<sup>9</sup> (ouabain 1, 5, or 10 mg/kg/day intraperitoneally for 26 days) and Yasujima et al<sup>8</sup> (ouabain 1.2 mg/kg/day iv for 6 days), Li et al<sup>7</sup> infused ouabain at much lower doses (10 to 100  $\mu$ g/kg/day intraperitoneally by osmotic minipumps for 28 days) and were unable to detect any change in arterial blood pressure.

Adding to the controversy is the method of blood pressure measurement. All rat studies mentioned here report recordings of blood pressure for short periods only, often in the conditions of possible stress related to tail-cuff measurement, which involves heating and restraint, or measurements with indwelling catheters during or shortly after surgery and anesthesia. This may lead to increased background noise in blood pressure measurements and possibly to conflicting results. In our study the animals were allowed to recover for at least 10 days after surgery, and blood pressure was measured 24 h a day in the conscious undisturbed animal, a method that should detect very small changes in blood pressure.<sup>13</sup>

However, because we used a different strain of rats (Long-Evans), our study does not exclude the possibility that an endogenous ouabain-like substance may play a role in long-term blood pressure control of Wistar or Sprague Dawley rats. Some authors have reported that Long-Evans rats are less sensitive to certain hypertensive agents, such as chronic cold exposure<sup>14</sup> and sucrose loading.<sup>15</sup> However, uninephrectomy increases blood pressure markedly during sucrose loading.<sup>15</sup> Furthermore, Long-Evans rats do not seem to be resistant to mineralocorticoid-induced hypertension, as mineralocorticoid administration

leads to similar increases in blood pressure in Long-Evans rats, compared with Sprague Dawley rats.<sup>16</sup> Nevertheless, we cannot exclude the possibility that Long-Evans rats may have a different distribution of  $\alpha$  isoforms of the sodium pump in comparison to other rat species. Long-Evans rats could have a greater proportion of the  $\alpha 1$  isoform in their vascular smooth muscle, an isoform that is particularly resistant to ouabain.<sup>17</sup> Further experiments remain necessary to test this possibility.

In summary, our data suggest that long-term infusion of ouabain that yields plasma concentrations in the nanomolar range is unable to raise arterial pressure in conscious Long-Evans rats, even under conditions of reduced renal mass and a slight increase in sodium intake. In conclusion, an endogenous ouabain-like inhibitor of the  $\text{Na}^+, \text{K}^+$ -ATPase pump does not seem to play an important role in long-term blood pressure control in Long-Evans rats.

#### ACKNOWLEDGMENTS

This work was supported by a grant from the Swiss National Science Foundation, No. 32-47329.96. We thank M.-F. Baeriswyl for analytical measurements, and L. Monney, A. Gaillard and E. Regli for the general setup of the animal room.

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