Chronic Central Versus Peripheral Ouabain, Blood Pressure, and Sympathetic Activity in Rats

Bing S. Huang, Xinfan Huang, Eef Harmsen, Frans H.H. Leenen

Abstract  To assess whether chronic ouabain administration causes hypertension by increasing sympathetic activity, we recorded arterial blood pressure and heart rate at rest and after ganglionic blockade in conscious Wistar rats following 10 to 14 days of central or peripheral administration of ouabain. Intracerebroventricular or intravenous infusion of ouabain (10 μg/d for both) as well as subcutaneous ouabain pellets (releasing 25 μg ouabain/d per pellet) increased mean arterial pressure by 20 to 30 mm Hg and heart rate by 40 to 60 beats per minute. Ouabain pellets increased blood pressure and heart rate in a dose-related manner. After 2 weeks of all ouabain treatments, ouabainlike activity in plasma was not changed but increased significantly in hypothalamus and adrenals. Ouabainlike activity in the adrenals was increased more by intravenous than subcutaneous or intracerebroventricular ouabain treatment, but the different treatment modes caused similar increases in the hypothalamus. Concomitant central infusion of antibody Fab fragments against ouabain prevented the ouabain pellet–induced increases in blood pressure and heart rate. Ganglionic blockade by intravenous hexamethonium normalized blood pressure and heart rate in ouabain-treated rats. These data suggest that in normotensive rats exogenous ouabain, regardless of the mode of administration, may act centrally to cause sympathoexcitation and thus hypertension. (Hypertension. 1994;23[part 2]:1087-1090.)

Key Words  • ouabain • blood pressure • sympathetic nervous system • antibody Fab fragments

The relation between high sodium intake and hypertension in salt-sensitive hypertensive rats is complex and not yet understood. We have postulated that high sodium intake intermittently increases Na⁺ concentrations in the cerebrospinal fluid, inducing increased central levels of ouabainlike activity (OLA) and thereby an increase in sympathetic outflow and blood pressure (BP). Substances with OLA are present peripherally and centrally in both normotensive and hypertensive humans and animals and may be of central origin. Brain OLA content is higher in spontaneously hypertensive rats (SHR) than Wistar-Kyoto (WKY) rats, and high sodium intake further increases brain OLA in SHR. Augmented sympathetic activity has been documented in several forms of sodium-dependent hypertension, including Dahl salt-sensitive (DS) rats and SHR. Brain OLA may play a primary role in mediating the sympathoexcitatory and hypertensive effects of high sodium intake in SHR and DS rats.

In humans, circulating OLA is indistinguishable from the glycoside ouabain in terms of biochemical structure and several physiological effects. Furthermore, receptors for ouabain in, for instance, arterial smooth muscle cells and the central nervous system appear to be identical to those for OLA. If an increase in brain or peripheral OLA contributes to the development of hypertension in sodium-sensitive rats, chronic administration of exogenous ouabain should induce hypertension in normotensive rats as well. In normotensive rats, acute central administration of ouabain or brain extracts containing OLA elicits similar sympathoexcitatory and pressor effects, which are blocked by antibody Fab fragments against ouabain. Whether ouabain or other Na⁺,K⁺-ATPase inhibitors indeed can cause a chronic increase in BP is still controversial. Ouabain injected once a week for 6 weeks or continuous intravenous infusion of ouabain for up to 6 days failed to increase BP of Sprague-Dawley rats. In contrast, long-term (more than 1 week) ouabain administration produced hypertension in Wistar rats with or without reduced renal mass. Because the hypertension was associated with an increase in total peripheral resistance (TPR), it was postulated that inhibition of the Na⁺ pump in arterial smooth muscle cells was one of the mechanisms of ouabain-induced hypertension. However, the involvement of central mechanisms has not been explored.

In the present study we therefore studied in Wistar rats the effects of chronic central or peripheral administration of ouabain on arterial BP, heart rate (HR), and plasma and tissue OLA. To assess the possible involvement of central mechanisms and the sympathetic nervous system in changes in BP and HR, we evaluated the effects of central infusion of antibody Fab fragments against ouabain concomitantly with peripheral ouabain as well as the effects of ganglionic blockade.

Methods

Male Wistar rats weighing 200 to 250 g (Charles River) were housed at constant room temperature, humidity, and light cycle (12-hour light/dark) and fed with regular sodium chow (120 μmol sodium/g). Three days later, rats either continued regular sodium chow or were started on high sodium chow (1370 μmol/g) (Harlan Sprague Dawley Inc). Ouabain admin-
struction was started after 2 days of the dietary period. All surgery was done with rats under halothane/nitrous oxide/oxygen anesthesia, except the intracerebroventricular cannulation, which was done under sodium pentobarbital anesthesia (65 mg/kg IP).

**Intracerebroventricular Ouabain Infusion**

With the use of a stereotaxic frame (Harvard Apparatus), a 23-gauge right-angled stainless steel cannula was implanted into the left lateral cerebral ventricle of the rat (coordinates with respect to bregma: 0.5 mm posterior, 1.4 mm lateral, and 4.0 mm deep from dura), fixed to the skull with acrylic cement, and connected to an osmotic minipump (model 2002, Alza Corp) with polyethylene tubing. The pump was implanted subcutaneously on the back of the animal and filled with 0.9% saline, 166 µg ouabain, 2.8 mg antibody Fab fragments (DigiBind, Burroughs Wellcome Co), or 2.8 mg γ-globulins in saline. Mean pumping rate was 12 μL/d, and doses were 10 μg/d for ouabain and 200 μg/d for the Fab fragments or γ-globulins.

**Intravenous Ouabain Infusion**

PE-60 polyethylene tubing was inserted into the right jugular vein and connected to a subcutaneous osmotic minipump filled with either ouabain solution providing 10 μg/d or 0.9% saline.

**Pellet Implantation**

A small incision was made on the back of the neck and one or three controlled time-release pellets (Innovative Research) containing either ouabain (0.5 mg) or vehicle (placebo) were implanted subcutaneously. These pellets are designed to release a constant amount of ouabain or vehicle over a 21-day period. A 0.5-mg pellet releases approximately 25 μg ouabain/d.

**Blood Pressure and Heart Rate Measurements**

A polyethylene catheter was inserted into the left carotid artery in the morning of the experiment. In the afternoon and subsequent days, mean arterial pressure (MAP) was recorded on a Grass 7E polygraph and HR on a Grass 7P44 tachograph in conscious, undisturbed rats.

**Experimental Protocol 1**

Ouabain or saline was infused intracerebroventricularly in rats on a regular or high Na⁺ diet (n=7 for all). Similarly, ouabain or saline was infused intravenously in rats on either Na⁺ diet (n=7 for all). Rats with pellets were divided into two groups (n=9 for both): high Na⁺ with either one ouabain or one placebo pellet. MAP and HR were measured and a 1-mL arterial blood sample was taken on days 10, 12, and 14. At the end of the experiment on day 14, rats were decapitated and the whole brain and adrenals were removed and stored at −70°C. The hypothalamus was obtained by dissection of the brain at 4°C according to Giowinski and Iverson. An assay for Na⁺, K⁺-ATPase inhibitory activity was used for the quantitation of plasma and tissue OLA.

**Experimental Protocol 2**

Rats on high sodium were housed individually in metabolic cages and divided into three groups (n=8 for each): one or three ouabain pellets or one placebo pellet was implanted, respectively. Body weight, food and water intakes, and urine volume were recorded daily. On day 10 after MAP and HR had been measured over 1 hour, the ganglionic blocker hexamethonium (30 mg/kg) was injected intravenously, and responses were monitored for 5 to 6 minutes. Then the vasopressin antagonist (CH₃)₂Tyr(Me)-arginine vasopressin (AVP-ant, 30 µg/kg) was given intravenously, and responses were observed for another 10 minutes.

**Experimental Protocol 3**

In rats on high Na⁺, an intracerebroventricular cannula and minipump filled with Fab fragments or γ-globulins were placed as described above. Two days after the start of this infusion, either one ouabain or one placebo pellet was implanted. Rats were divided into four groups: Fab fragments plus ouabain (n=9) or placebo (n=7) pellet, and γ-globulins plus ouabain (n=9) or placebo (n=7) pellet. BP and HR were measured on days 10, 11, and 12.

**Statistical Analysis**

Results are expressed as mean±SEM. Differences between groups were evaluated by ANOVA followed by Duncan's multiple range test. The level of significance was set at a value of P<0.05.

**Results**

No differences in body weight, food and water intake, and urine volume were observed between rats with ouabain or placebo pellet (not shown). In the other experiments, body weight was not affected either.

**Responses to Chronic Ouabain Administration**

On days 10, 12, and 14, intracerebroventricular, intravenous, or subcutaneous (pellet) ouabain significantly increased MAP by 20 to 30 mm Hg and HR by 40 to 60 beats per minute (bpm) in rats on either diet compared with controls (Table). In rats on regular Na⁺ with intravenous ouabain, increases in BP and HR started on day 12 (Table). Implantation of three ouabain pellets caused a further increase in MAP and HR compared with rats with one ouabain pellet (Figure).

**Responses to Hexamethonium and AVP-ant**

Intravenous hexamethionium decreased MAP and HR rapidly in rats with both ouabain and placebo pellets (Figure). The extent of decreases was greater in rats with one ouabain pellet versus placebo or with three versus one ouabain pellet. After hexamethionium injection MAP was similar in rats with one versus three ouabain pellets and was only slightly (<5 mm Hg, P=NS) higher than the MAP of control rats. Subsequent administration of AVP-ant caused a similar further decrease in MAP (5 mm Hg) and HR (10 bpm) in all groups.

**Blockade of Central "Ouabain" and Chronic Ouabain Administration**

Combined with intracerebroventricular γ-globulins, a ouabain pellet caused significant increases in MAP and HR compared with γ-globulins alone (after 12 days: 130±4 versus 97±5 mm Hg and 447±7 versus 402±14 bpm, P<0.05 for both). In contrast, combined with intracerebroventricular Fab fragments, ouabain pellets did not increase BP and HR (94±5 versus 93±6 mm Hg and 386±8 versus 387±13 bpm, P=NS) compared with Fab fragments alone.

**Plasma and Tissue OuabainLike Activity**

There were no significant differences in plasma OLA (250 to 270 ng/mL) between ouabain-treated and control groups. OLA in the hypothalamus was similarly increased in rats treated with intracerebroventricular, intravenous, or subcutaneous ouabain (9.6±0.6, 8.9±0.4, and 8.6±0.5 μg/g, respectively) compared with corresponding control groups (3.2±0.7, 2.6±0.3, and
Effects of Ouabain Administration for 10 to 14 Days on Resting Mean Arterial Pressure and Heart Rate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Arterial Pressure, mm Hg</th>
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<td>Day 10</td>
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<td>Regular sodium</td>
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<td>High sodium</td>
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<td><strong>IV ouabain</strong></td>
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<td>High sodium</td>
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<td>Regular sodium</td>
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<td>High sodium</td>
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bpm indicates beats per minute; ICV, intracerebroventricular; and IV, intravenous. Mean arterial pressure and heart rate in ouabain-treated rats (except where noted) are all significantly larger than control rats on high or regular sodium.

*P<.05 vs IV ouabain on high sodium on day 10.

2.9±0.5 μg/g, respectively; P<.05 for all comparisons). Adrenal OLA was significantly higher in rats with intravenous versus subcutaneous or intracerebroventricular ouabain treatment (54±3 versus 42±3 or 26±1 μg/g, P<.05 for both), whereas the levels in all ouabain-treated groups were significantly higher than their corresponding controls (intravenous, 18±1; subcutaneous, 21±2; intracerebroventricular, 17±1 μg/g).

Discussion

The present study shows that in normotensive rats on either high or regular sodium diet both central and peripheral administration of ouabain for 10 to 12 days increase resting BP and HR. For ouabain pellets, these increases were dose related. The results are consistent with recent observations by Hamlyn and coworkers (see Manunta et al14 and Yuan et al15). In contrast, earlier reports12-13 failed to show hypertensive effects of chronic ouabain. Differences in the experimental protocols can explain these different results. First, in the study by Yasujima et al13 BP was measured for only 2 to 6 days after the initiation of intravenous ouabain infusion. Sekihara et al12 injected ouabain only once a week. In the present study as well as the study by Yuan et al15 in rats on a regular diet BP did not increase until 10 to 12 days after the start of intravenous ouabain infusion. At least 10 to 14 days of continuous ouabain appear to be needed for the development of high BP in rats on a regular sodium intake. Second, the dose used by Yasujima et al13 is 20 to 50 times higher than those used in the present study or that of Yuan et al.15 High doses of ouabain not only may cause cardiotoxic effects, obscuring hemodynamic responses to ouabain, but also may sensitize arterial baroreceptors,17 thereby decreasing sympathetic outflow and possibly preventing BP increase. Indeed, pressor responses to norepinephrine were enhanced by low doses of ouabain18 but attenuated by high doses.13

The mechanisms responsible for chronic ouabain-induced hypertension in normotensive rats are not yet clear. Ouabain-induced hypertension was associated with an increase in TPR,15 and it was postulated19 that an inhibition of Na⁺,K⁺-ATPase in vascular smooth muscle cells is responsible for the TPR increase. However, the present study provides several lines of evidence supporting the involvement of central mechanisms. First, ouabain-treated rats showed significant increases in not only BP but also HR. Tachycardia was...
present in ouabain-treated rats regardless of the mode of administration and is probably not due to an increase in TPR. Second, intracerebroventricular infusion of antibody Fab fragments against ouabain prevented the ouabain pellet-induced hypertension and tachycardia. These Fab fragments have previously been shown to prevent and reverse the binding of ouabain to human erythrocyte receptors in vitro as well as block the pressor responses to intracerebroventricular ouabain in rats in vivo. Third, hypothalamic OLA content was increased to similar levels in rats with intracerebroventricular, intravenous, or subcutaneous administration of ouabain. These findings suggest that ouabain can readily cross the blood-brain barrier and accumulate in brain areas. Fourth, ganglionic blockade with hexamethonium normalized BP and HR in ouabain-treated rats, indicating that sympathetic tone is essential for ouabain-induced elevation of BP and HR. Although TPR was not measured in the present study, one may expect that a ouabain-induced increase in sympathetic tone not only increased HR but also TPR and thus BP. These results are consistent with our previous studies indicating that increased brain OLA may mediate the sympathoexcitatory and hypertensive effects of high sodium intake in SHR and DS rats. The nature of Na⁺,K⁺-ATPase isoforms in different tissues also supports central pressor effects of ouabain. The potency of ouabain in inhibiting the Na⁺ pump depends on the catalytic (α) subunit. ATPase of rat brain tissue has α₁, α₂, and α₃ isoforms, but in peripheral tissues such as kidney, heart, and vascular smooth muscle cells, α₁ is found predominantly and α₂ and α₃ are negligible. Ouabain binds to α₂ and α₃ isoforms with high affinity and binds to α₁ weakly. Thus, the higher binding affinity of ouabain to the brain Na⁺ pump is also in favor of central mechanisms for ouabain-induced hypertension.

Although the data are consistent with a primary role of central mechanisms, the involvement of peripheral mechanisms in ouabain-induced hypertension cannot be ruled out. To strengthen the central hypothesis, further studies will be needed to assess sympathetic nerve activity directly and to find a ouabain dose that will increase BP and HR via the intracerebroventricular but not intravenous route.

In conclusion, in Wistar rats ouabain for 10 to 14 days, regardless of the mode of administration, caused hypertension and accumulation of ouabain in hypothalamic and adrenals but not plasma. Hypertension and tachycardia were prevented by intracerebroventricular antibody Fab fragments against ouabain and reversed by acute ganglionic blockade, suggestive of central effects of ouabain increasing sympathetic tone and inducing hypertension.

Acknowledgments

This work was supported by an operating grant from the Medical Research Council of Canada. F.H.H. Leenen is supported by a career investigatorship from the Heart and Stroke Foundation of Ontario, Canada.

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Hypertension. 1994;23:1087-1090
doi: 10.1161/01.HYP.23.6.1087

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