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Ouabain Changes Arterial Blood Pressure and Vascular Reactivity to Phenylephrine in L-NAME–Induced Hypertension

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Summary: Ouabain is an endogenous compound that has been associated with the genesis and maintenance of hypertension. This compound inhibits the Na^+ pump activity, which leads to an accumulation of intracellular Na^+ and ultimately might increase vascular tone. In nanomolar concentrations, it enhances vasopressor responses to phenylephrine in some vascular beds from normotensive and hypertensive rats. However, it is not known whether this action of ouabain is a common mechanism for all models of hypertension. The aim of this work was to determine whether ouabain can alter pressor responses to phenylephrine in rats with N^{ω}-nitro-L-arginine methyl ester (L-NAME)–induced hypertension. In anesthetized rats, ouabain (0.18 μg/kg, i.v.) increased arterial blood pressure in L-NAME–treated rats but not in controls. Ganglionic blockade by hexamethonium (5 mg/kg, i.v.) prevented the increase in arterial blood pressure produced by ouabain in L-NAME–treated rats. Additional studies using isolated perfused tail artery preparations were performed to investigate which factors are involved in the action of ouabain in L-NAME–treated rats. The effects of 10 n*M* ouabain on the vasoconstrictor actions of phenylephrine were determined on preparations with intact or damaged endothelium or in the presence of tetraethylammonium (a K^+-channel blocker). Ouabain reduced pressor actions of phenylephrine in preparations with an intact endothelium. However, after endothelial damage or infusing tetraethylammonium, the response to phenylephrine was increased after ouabain. In tails from L-NAME–treated rats, the functional activity of the Na^+, K^+-ATPase was reduced, and 10 n*M* ouabain did not produce any further reduction. In conclusion, in this model of hypertension, a low dose of ouabain (0.18 μg/kg) increased arterial blood pressure in vivo probably as a result of increased sympathetic tone. However, this effect was not accompanied by an enhanced action of phenylephrine on the tail vascular bed with an intact endothelium. The results suggest that this was due to the release of an endothelium-derived K^+-channel opener. **Key Words:** EDHF—Endothelium—L-NAME—Nitric oxide—Ouabain—Phenylephrine—Vascular smooth muscle.

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One of the hallmarks of hypertension is an increase in vascular resistance caused by a higher smooth muscle tone. Several mechanisms have been proposed to explain this increment in vascular resistance, such as high intracellular Na^+ concentration (1), endothelial dysfunction (2,3), or increased sympathetic tone (4,5), among others.

Ouabain, an endogenous digitalis compound, has been found in nanomolar concentrations in the plasma of several mammalians (6,7) and was associated with the development of hypertension (8–11). This action is usually explained by its ability to inhibit the Na^+ pump in several tissues. In vascular smooth muscle, the inhibition of the pump results in Na^+ accumulation in the myoplasm, which reduces the activity of the Na^+/Ca^{2+} exchange mechanism and ultimately increases vascular smooth muscle contraction (12–14).

Recent findings in our laboratory suggested that in anesthetized rats, very low doses of ouabain increase arterial blood pressure in spontaneous hypertensive rats (SHR), but not in normotensive rats (15). In the tail vascular bed from normotensive and hypertensive rats, ouabain increased the pressor response to phenylephrine to a greater extent in hypertensive than in normotensive rats (15,16). These results suggest that ouabain in the nanomolar range, which is equivalent to its pathophysiological concentrations (8,10,12,13), might increase the vascular tone by increasing cytosolic free calcium (17) and by sensitizing the vascular smooth muscle to vasopressor agents, which could contribute to the genesis and/or maintenance of hypertension (15,16,18).

Moreover, there are reports showing that the endothelium modulates the actions of ouabain (18–20). In normotensive animals, the endothelium reduces the vasoconstriction caused by ouabain (19) and also reduces the sensitization to vasopressor agents (18). In SHR, different from normotensive rats, the endothelium seems to facilitate the vasopressor sensitization induced by ouabain (19). Recently, we demonstrated that endothelium modulates the effects of nanomolar concentrations of ouabain in the tail vascular bed from normotensive rats (18). This modulation was in part dependent on the release of a K^+-channel opener.

However, it is not known whether enhanced pressor responses and vascular reactivity to phenylephrine are common changes seen in all experimental models of hypertension. There are reports showing that ouabain caused an increased release of nitric oxide (NO) from endothelial cells and induced nitric oxide synthase (NOS) expression and activity in the vascular smooth muscle cell (21,22). Because chronic treatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nonselective inhibitor of NOS isozymes, causes the development of hypertension (23–27), the aim of this work was to determine whether L-NAME treatment alters the pressor response to a low dose of ouabain in the intact animal as well as affecting the actions of a low concentration of ouabain on pressor responses to phenylephrine in the isolated tail artery preparation. Our results show that the pressor actions of a low dose of ouabain are enhanced in L-NAME–hypertensive rats and that this enhancement is not accompanied by an enhancement of the actions of phenylephrine in vascular beds with an intact endothelium. Endothelial damage and K^+-channel blockade with tetraethylammonium (TEA) unmasked an enhancing action of ouabain in tail artery beds from L-NAME hypertensive rats, suggesting that an endothelium-derived K^+-channel opener modulates the effects of ouabain.

MATERIALS AND METHODS

Animals

Male Wistar rats (200–270 g) were used in all studies. The care and use of the laboratory animals were in accordance with NIH guidelines. All rats had free access to water and were fed with rat chow ad libitum. The rats were divided in two main groups. One group was treated for 7 days with L-NAME, 50 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}, administered in the drinking water. Controls received only water.

Blood pressure measurements

On day six of treatment, systolic blood pressure of conscious rats was measured using a tail-cuff method (IITC Model 29 Pulse Amplifier; IITC Inc., CA, U.S.A.). On the following day, rats ($N = 26$) were anesthetized with urethane (1.2 g/kg, i.p.), supplemented when necessary. The jugular vein and the carotid artery were dissected and cannulated with a polyethylene catheter (PE-50 with heparinized saline) for drug infusion and arterial blood pressure measurements, respectively. Arterial blood pressure was measured with a pressure transducer (Gold P23XL) connected to an MP 100 amplifier (FUNBEC, São Paulo, SP, Brazil) and recorded on a polygraph (RG-300; FUNBEC).

Effect of ouabain on arterial blood pressure in anesthetized animals

After 30 min of stabilization, systolic and diastolic blood pressures were measured before and 60 min after administration of 0.18 μg/kg ouabain, a dose that increases blood pressure in hypertensive but not in normotensive animals (15,28). In additional groups of L-

NAME–treated rats, the effects of ganglionic blockade by hexamethonium (5 mg/kg, i.v.) and the effects of blood pressure reduction by hydralazine (50 μg/kg, i.v.) were investigated. Following anesthesia and stabilization, hexamethonium or hydralazine was administered. Thirty minutes later, a time when the depressor effects of hexamethonium or hydralazine had stabilized, ouabain (0.18 μg/kg, i.v.) was administered. The systolic and diastolic pressures at 60 min after ouabain administration were recorded.

Isolated rat tail vascular bed preparation

Isolated rat tail vascular beds were used in this study as previously reported (29). Briefly, the rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.), and after loss of the righting reflex, heparin (500 UI, i.p.) was administered. Ten minutes after the administration of heparin, a 1-cm strip of the tail artery was dissected free and cannulated with an Intracath (Nipro 24-gauge ¾, Sorocaba, SP, Brazil) near the base of the tail. The vascular bed was flushed with Krebs-Henseleit buffer (KHB; in m*M*: NaCl, 120; KCl, 5.4; $MgCl_2$, 1.2; $CaCl_2$, 1.25; NaH_2PO_4, 2.0; $NaHCO_3$, 27; glucose, 11; and EDTA, 0.03) bubbled with 5% CO_2–95% O_2, at 36 ± 0.5°C. The tail was then severed from the body and placed in a tissue bath and perfused with KHB at a constant flow of 2.5 ml/min with a peristaltic pump (Milan, Colombo, PR, Brazil). After a 30- to 45-min equilibration period, the experimental protocol was initiated. Mean perfusion pressure was measured by using a pressure transducer (TP-200T; Nihon-Kohden), and the data were recorded using an interface and software for computer data acquisition (model MP100A; BIOPAC Systems, Inc., Santa Barbara, CA, U.S.A.) with a sample rate of 500 Hz per channel. Because a constant flow was used, changes in the perfusion pressure represented changes in vascular resistance.

Effects of ouabain on the actions of phenylephrine

After 30–45 min of stabilization, increasing doses of phenylephrine (0.5, 1, 2, 5, and 10 μg, as bolus injections in 100 μl) were administered in the absence and in the presence of 10 n*M* ouabain after a 60-min equilibration period of infusion of KHB containing ouabain. At the end of the experiment, preparations were contracted with continuous infusion of phenylephrine (10^{-7} *M*), and the endothelial function was tested using bolus injection of acetylcholine (ACh; 5 μg in 100 μl). This protocol was performed in preparations from L-NAME–treated rats (N = 7).

Effect of endothelial damage on the effects of ouabain on phenylephrine-induced pressure response

The same protocol as described above was performed in preparations with damaged endothelium (E–). The endothelium was damaged using an 8-mg bolus injection of CHAPS (3-[(3-chloroamidopropyl) dimethylammonio]-1-propane-sulfonate) in 80 μl. Endothelial damage and vascular reactivity to an NO donor were assessed by comparing the responses to bolus injections of ACh (5 μg in 100 μl) and sodium nitroprusside (5 μg in 100 μl) administered before and after endothelial damage, and by histologic evaluation. Responses to phenylephrine were measured in the L-NAME–treated group (N = 8): under control conditions (E+), 30 min after endothelial damage (E–) and 60 min after endothelial damage plus the infusion of 10 n*M* ouabain (E–/OUA).

Effect of K^+-channel blockade on the effects of ouabain on phenylephrine-induced pressure response

The possibility that ouabain might stimulate the release of a K^+-channel opener was evaluated. In these experiments using L-NAME–treated rats (N = 7), the pressor responses to phenylephrine were determined in the absence and in the presence of 20 m*M* TEA after an equilibration of 30 min and subsequently in the presence of TEA and ouabain after equilibration with 10 n*M* ouabain plus TEA for 60 min. Confirmation of K^+-channel blockade was performed with 5 μg ACh before and during TEA administration.

Functional activity of Na^+, K^+-ATPase

The functional activity of Na^+, K^+-ATPase was measured using K^+-induced relaxation as described by Webb and Bohr (30). Tails obtained from control (N = 6) and L-NAME–treated rats (N = 6) were perfused as described above. After a 30- to 45-min equilibration period in normal KHB, the perfusion buffer was changed to one containing no added potassium. The tails were perfused for 30 min, and then the preparations were precontracted with phenylephrine (10^{-7} *M*). Once a plateau was attained, the concentration of KCl in the perfusate was increased in steps (1, 2, 4, and 6 m*M*), each one with a 5-min duration. This protocol was repeated 60 min after treatment with 10 n*M* ouabain.

Drugs and reagents

Ouabain octahydrate, l-phenylephrine hydrochloride, L-NAME, sodium nitroprusside, acetylcholine chloride, hexamethonium chloride, hydralazine hydrochloride, tetraethylammonium chloride, CHAPS, urethane, and

sodium pentobarbital were purchased from Sigma (St. Louis, MO, U.S.A.), and heparin was purchased from Roche (São Paulo, SP, Brazil).

Data analysis

Results regarding perfusion pressure measurements are presented as changes in the mean perfusion pressure subtracting peak pressure from baseline pressure. Relaxation response to potassium was expressed in percentage of relaxation in preparations precontracted with phenylephrine (10^{-7} *M*). Results are presented as mean ± SEM. Data were analyzed using a t-test and ANOVA. When the ANOVA showed a significant treatment effect, a Tukey post-hoc test was used to compare means; $p < 0.05$ was considered significant.

RESULTS

Treatment with L-NAME (50 mg · kg^{-1} · day^{-1}, p.o.) for 7 days increased the systolic blood pressure (controls: 125.3 ± 1.2 mm Hg vs. L-NAME: 177.6 ± 4.4 mm Hg, $p < 0.001$, unpaired *t* test, measured by the tail-cuff method). However, the treatment did not alter body weight (controls: 249.4 ± 2.7 g vs. L-NAME: 243.9 ± 4.6 g, $p > 0.05$, unpaired *t* test).

Effect of ouabain on arterial blood pressure in anesthetized animals

Anesthesia with urethane reduced systolic and diastolic blood pressures in both groups, similar to results obtained in other studies (15,28). However, both pressures were higher in L-NAME–treated rats as compared with controls (systolic pressure: 99.8 ± 3.4 vs. 133.9 ± 8.5 mm Hg, $p < 0.01$; diastolic pressure: 73.0 ± 4.1 vs. 106.9 ± 9.9 mm Hg, $p < 0.01$, unpaired *t* test, in controls vs. L-NAME–treated rats). In control rats, systolic and diastolic arterial pressures at 60 min after administration of ouabain (0.18 μg/kg, i.v.) were not changed (Fig. 1). In contrast, this treatment significantly increased both systolic and diastolic blood pressures in L-NAME–treated rats (Fig. 1).

Figure 2 shows that treatment with hexamethonium (5 mg/kg, i.v.) significantly reduced systolic and diastolic blood pressures in L-NAME–treated rats ($p < 0.01$) and blocked the pressor effects of ouabain. After ganglionic blockade with hexamethonium, ouabain produced a small but insignificant ($p > 0.05$, paired *t* test) increase in systolic and diastolic pressures. Treatment with hydralazine (50 μg/kg, i.v.) also reduced systolic and diastolic blood pressures in L-NAME–treated rats ($p < 0.01$) to similar levels observed in the group treated with hexamethonium (Fig. 2). However, after reduction in blood pressure of L-NAME–treated rats with hydralazine, ouabain significantly ($p < 0.01$) increased systolic and diastolic blood pressures (Fig. 2).

Effects of ouabain on the actions of phenylephrine in isolated tail vascular bed preparations

Infusion of 10 n*M* ouabain for 60 min did not change baseline mean perfusion pressure (MPP, initial MPP: 67.2 ± 3.5 vs. MPP after ouabain: 63.4 ± 2.7 mm Hg, $p > 0.05$, paired *t* test) in tail artery preparations obtained from L-NAME–hypertensive rats. As shown in Figure

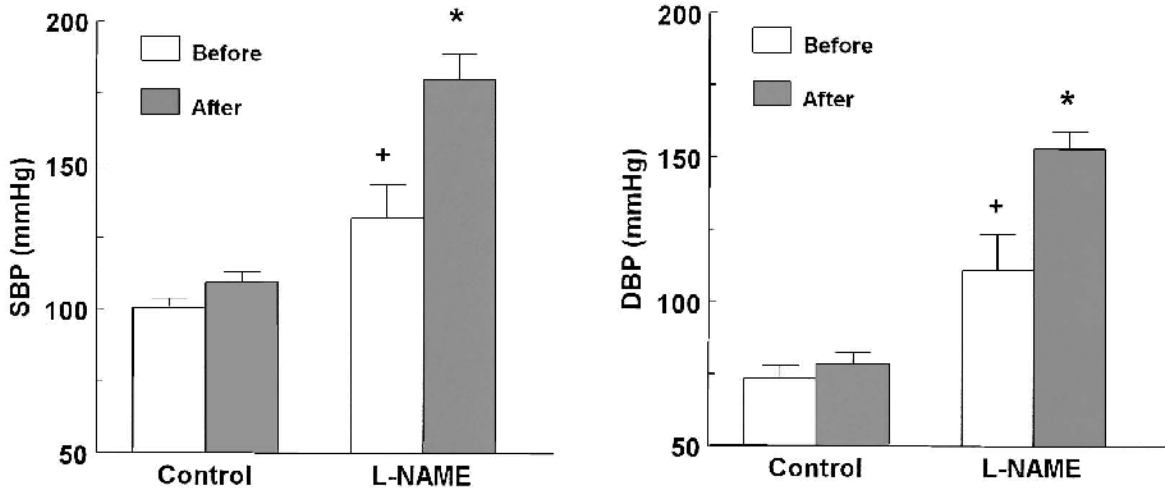

FIG. 1. Systolic (SBP) and diastolic (DBP) blood pressure before and 60 min after the administration of 0.18 μg/kg ouabain in control (N = 7) and in 7-day L-NAME–treated rats (N = 8). Results are expressed as mean ± SEM. +$p < 0.01$ L-NAME before versus control before, unpaired *t* test, and *$p < 0.01$ after versus before ouabain, paired *t* test.

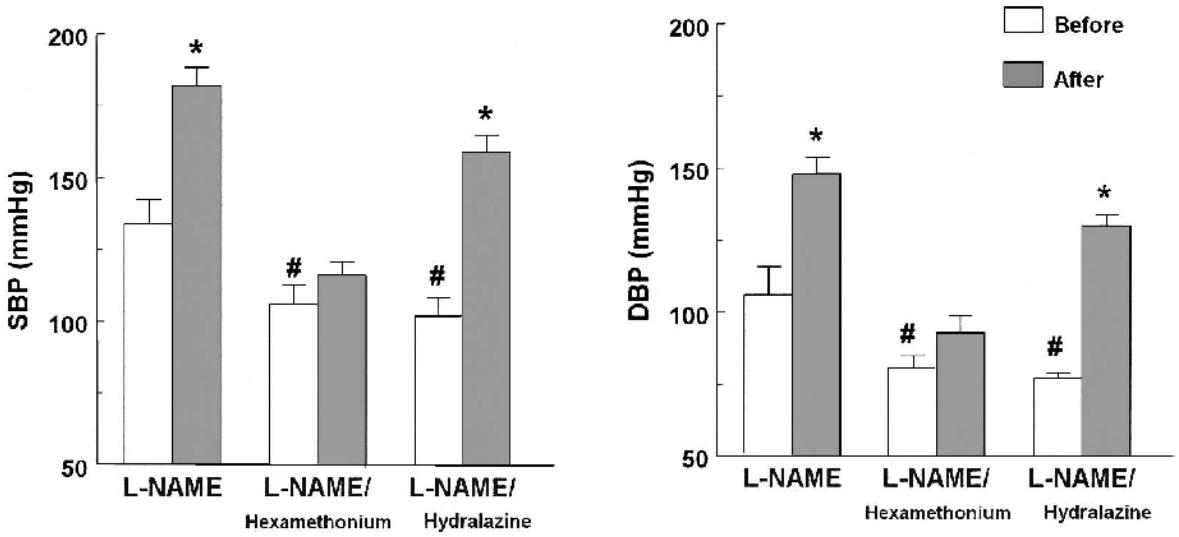

FIG. 2. Effect of ganglionic blockade with hexamethonium or reduction in the blood pressure induced by hydralazine on the pressor actions of ouabain in anesthetized L-NAME–treated rats. Systolic (SBP) and diastolic (DBP) blood pressure before and 60 min after the administration of 0.18 μg/kg ouabain in control L-NAME–treated rats (N = 8), ganglionic-blocked L-NAME–treated rats (N = 6), and reduced-blood-pressure L-NAME–treated rats (N = 5). Results are expressed as mean ± SEM. #$p < 0.01$ L-NAME plus hexamethonium or hydralazine before versus L-NAME before, unpaired *t* test, and *$p < 0.01$ after versus before ouabain, paired *t* test.

3A, phenylephrine dose-dependently increased MPP in the absence and presence of ouabain. In the presence of ouabain, the response to phenylephrine was reduced. It is important to emphasize that perfusion for 60 min with ouabain-free KHB solution (time control) did not change the baseline perfusion pressure or the pressor response to phenylephrine (results not shown).

Effect of endothelial damage on the effects of ouabain on the phenylephrine-induced pressure response

CHAPS-induced endothelial damage in the preparations from L-NAME–treated rats did not alter the phenylephrine pressor response (Fig. 3B). In contrast to preparations with an intact endothelium, 10 n*M* ouabain produced an increase in the pressor response to phenylephrine (Fig. 3B). Baseline perfusion pressure did not change after endothelial damage in the L-NAME group, maintaining a similar level after ouabain treatment (results not shown).

Before endothelial damage, the relaxation produced by ACh in the L-NAME group (20.3 ± 4.1% relaxation) was smaller when compared with control rats (79.2 ± 8.1% relaxation), but after endothelial damage, ACh produced a small but significant constrictor effect (before: 20.3 ± 4.1% relaxation vs. after: 2.8 ± 3.5% constriction, $p < 0.01$, paired *t* test). The relaxation produced by sodium nitroprusside was unchanged (before: 81.2 ± 2.0% relaxation vs. after endothelial damage: 87.3 ± 4.6% relaxation, $p > 0.05$, paired *t* test).

Effect of K^+-channel blockade on the effects of ouabain on the phenylephrine-induced pressure response

No changes in baseline perfusion pressure were observed in the L-NAME–treated group during the perfusion of the preparations with TEA, even after ouabain treatment (results not shown). Surprisingly, after continuous infusion of TEA, the phenylephrine pressor response was reduced in all doses studied. However, 60 min after ouabain (10 n*M*) plus TEA, an increase of phenylephrine pressure response was obtained (Fig. 4).

In the L-NAME–treated group, TEA inhibited the actions of ACh. Here, ACh produced vasoconstriction instead of vasodilatation (before: 17.2 ± 3.8% relaxation vs. after TEA: 9.1 ± 5.0% contraction, $p < 0.01$, paired *t* test).

Functional activity of Na^+, K^+-ATPase

As shown in Figure 5A, the functional activity of Na^+, K^+-ATPase was reduced after L-NAME treatment. The curve for relaxation induced by increasing concentrations of KCl was displaced upward as compared with controls, suggesting a decrease in the functional activity of the Na^+, K^+-ATPase caused by L-NAME.

In the control group, 10 n*M* ouabain reduced the relaxation induced by potassium (Fig. 5B). In contrast, 10 n*M* ouabain did not alter the effects of KCl in preparations obtained from L-NAME–treated rats (Fig. 5C).

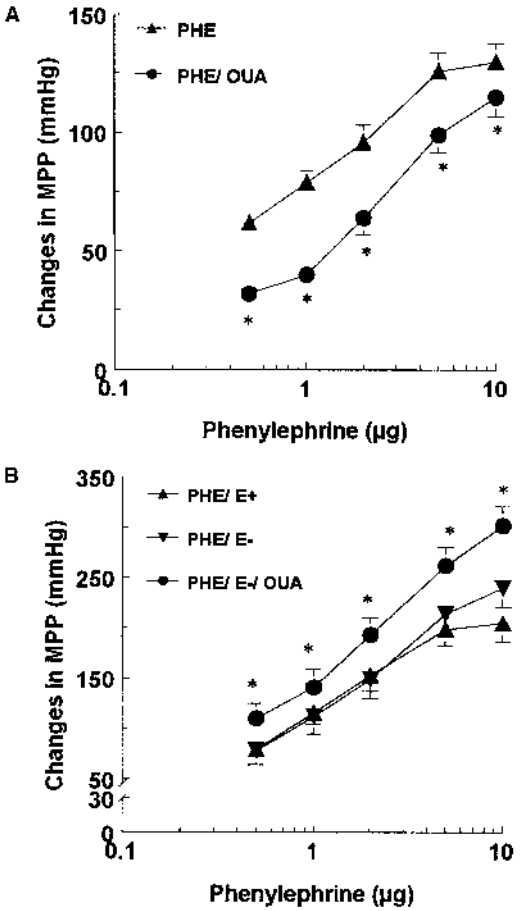

FIG. 3. Changes of the mean perfusion pressure (MPP) produced by phenylephrine (PHE) in tail vascular bed from L-NAME–treated rats (N = 7–8). Dose–response curves were made in preparations with intact endothelium (E+): (**A**) before (PHE) and after 10 n*M* ouabain (PHE/OUA) and (**B**) with intact endothelium (PHE/E+), endothelium damaged with CHAPS (PHE/E–), and endothelial damage plus 10 n*M* ouabain (PHE/E–/OUA). Results are expressed as mean ± SEM. *p < 0.01 PHE/OUA versus PHE, two-way analysis of variance, repeated measures.

DISCUSSION

L-NAME–induced hypertension is thought to be due to its inhibition of the synthesis of nitric oxide and the removal of its tonic vasodilatory influence (23). This is accompanied by an increase in sympathetic tone (24), an increase in the activity of the renin-angiotensin system (23), and an increase the cyclooxygenase pathway–derived vasoconstrictors (26), all of which can contribute to L-NAME–induced hypertension. Our results are consistent with an inhibitory action of L-NAME on NOS. The inhibition of NO synthesis and its subsequent release from the endothelium in rats treated with L-NAME for 7 days results in hypertension and is accompanied by the loss of endothelium-dependent relaxation produced by ACh. Importantly, our results show that the hypertensive actions of ouabain as well as its ability to modulate the actions of phenylephrine are changed in L-NAME–treated rats.

The results presented here show that in anesthetized L-NAME–hypertensive rats, a subpressor dose of ouabain in normotensive rats (15,28) increased arterial blood pressure in vivo. In vitro, a low concentration of ouabain reduced vascular reactivity to phenylephrine in isolated tail vascular preparations from L-NAME–hypertensive rats, an effect that was reversed when endothelium was damaged or K^+ channels were blocked. The latter two observations are consistent with the notion that ouabain can cause the release of an endothelium-derived vasodilator that acts as a K^+-channel opener. Additionally, the functional activity of Na^+, K^+-ATPase was shown to be reduced in isolated tail vascular beds.

It is well established that Na^+, K^+-ATPase, or its functional counterpart, the Na^+ pump, participate in the modulation of vascular smooth muscle contractility and tone (31). Activation of Na^+, K^+-ATPase in the vascular smooth muscle causes hyperpolarization and relaxation, and its inhibition causes depolarization and contraction (13,31). Changes in the functional state of the vascular Na^+ pump or its regulation may either contribute to the

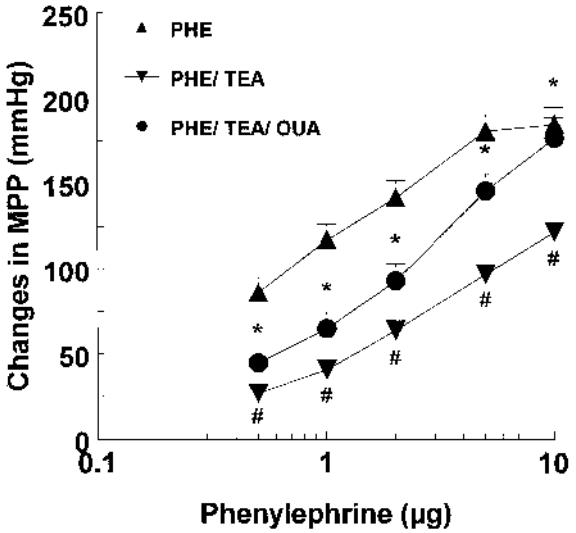

FIG. 4. Changes of the mean perfusion pressure (MPP) produced by phenylephrine (PHE) in tail vascular bed from L-NAME–treated rats (N = 7) before (PHE) and after treatment with 20 m*M* tetraethylammonium (PHE/TEA) and after perfusion of TEA plus 10 n*M* ouabain (PHE/TEA/OUA). Results are expressed as mean ± SEM. #p < 0.01 PHE/TEA versus PHE and *p < 0.01 PHE/TEA/OUA versus PHE/TEA, two-way analysis of variance, repeated measures.

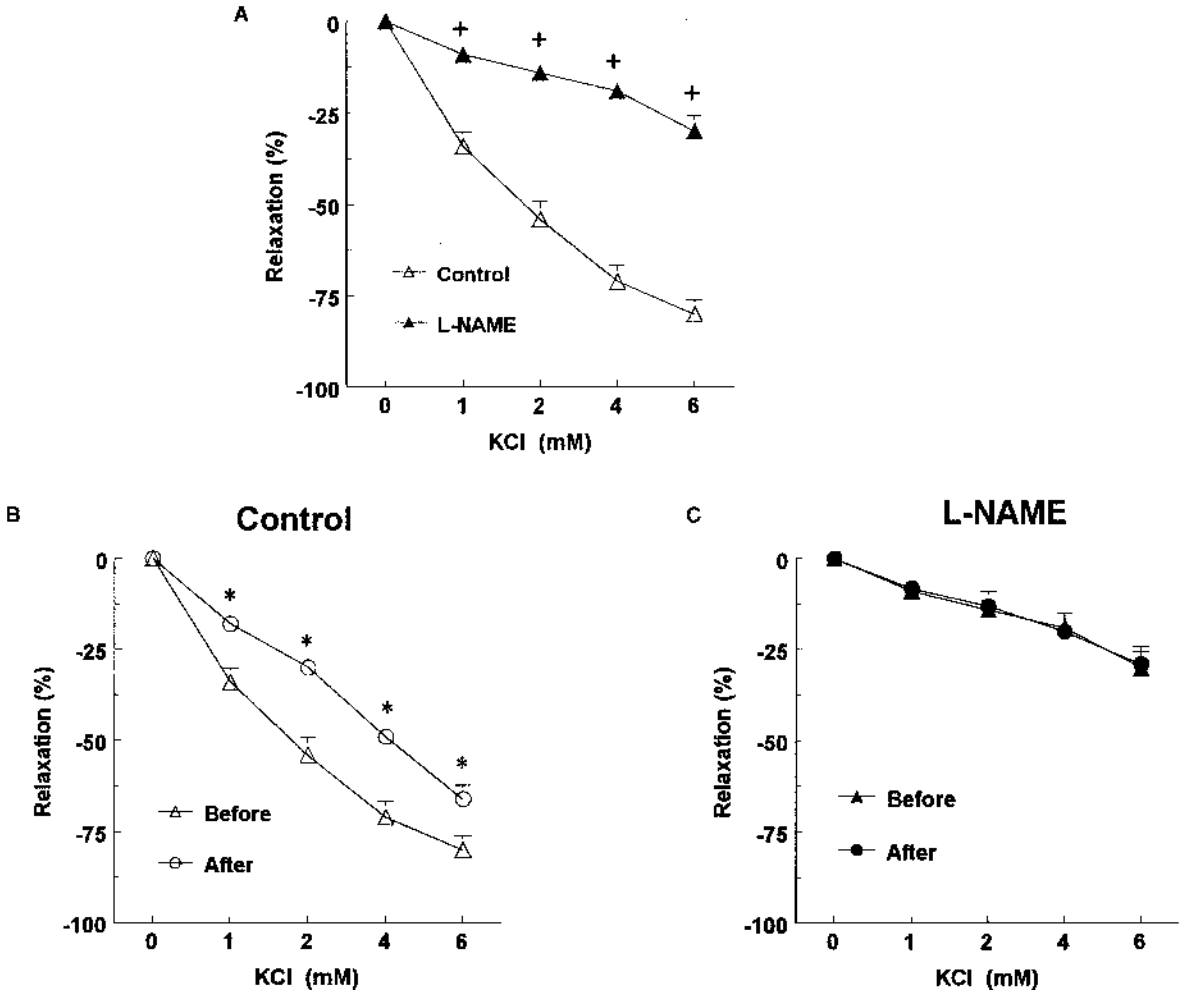

FIG. 5. Reversal of PHE-induced contractions in KCl-free KHB produced by the addition of increasing concentrations of KCl in the absence and presence of 10 n*M* ouabain. (**A**) Curves in control (N = 6) and L-NAME–treated rats (N = 6) before treatment with ouabain; (**B**) curves in control rats and (**C**) in L-NAME–treated rats before and in the presence of 10 n*M* ouabain. Results are expressed as mean ± SEM. SEM is not shown when smaller than the symbols. +p < 0.01 L-NAME versus control rats, two-way analysis of variance, completely randomized, and *p < 0.01 after versus before ouabain treatment, two-way analysis of variance, repeated measures.

development of hypertension or be a compensatory mechanism against the elevated pressure (13,31,32). The reduction in the functional activity of Na^+, K^+-ATPase probably contributes to the hypertension produced by L-NAME.

These characteristics, an endothelial dysfunction plus an increased vascular reactivity to pressor agents, are present in different models of hypertension. This includes those models in which plasma levels of ouabain are increased such as those resulting from volume-dependent hypertension (33,34).

Ouabain, one of the endogenous inhibitors of Na^+, K^+-ATPase, is present in nanomolar concentration in the plasma and several tissues (6–8,33,34). Previous reports showed that the chronic administration of ouabain increases arterial blood pressure in normotensive animals (9–11). We recently showed that ouabain at concentrations in the range of those found in the plasma of hypertensive patients (8,12,13) inhibits the functional activity of the Na^+, K^+-ATPase (18,35) and may sensitize the vascular smooth muscle to the actions of vasopressor substances (15,16,18,28,35). In agreement with others (36,37), we also demonstrated that in normotensive anesthetized rats, ouabain (18 μg/kg, ~30 nmol/kg) produced a pressor response by acting on sympathetic nerve endings as well as on vascular smooth muscle under conditions in which the pressor responses to phenylephrine were not altered (38). These effects might be relevant for the genesis and maintenance of hypertension. However, the mechanism that links ouabain and hypertension or by

which ouabain might act to induce hypertension is not completely understood. Other reports suggested that low doses of ouabain (~0.3 nmol/kg), which do not affect normotensive animals, might increase pressor and vascular reactivity in SHR and in volume-dependent hypertensive rats (15,28).

To investigate whether ouabain also increases arterial blood pressure in L-NAME–induced hypertension, a low dose of ouabain was used in anesthetized L-NAME–treated rats. In anesthetized control rats, 0.18 μg/kg ouabain (~0.3 nmol/kg) had no effects on arterial blood pressure, reproducing previous results (15,28). However, similar to our results from other studies using SHR (15), DOCA-Salt, and 1K1C (28) hypertensive rats, this dose of ouabain increased systolic and diastolic blood pressures in L-NAME–treated rats. Indeed, the present results show that pretreatment of anesthetized L-NAME rats with hexamethonium, which reduced arterial pressure in these animals, blocked the increase in systolic and diastolic blood pressures produced by the acute administration of 0.18 μg/kg ouabain. In a previous study (38), we showed that higher doses of ouabain, 6 and 18 μg/kg, produce dose-related increases in arterial pressure in reflex-blocked normotensive rats. Together, these observations suggest that the low dose of ouabain used in the current study has selective actions at preganglionic neurons and/or the central nervous system to increase sympathetic tone. The small elevation in arterial pressure produced by 0.18 μg/kg ouabain following hexamethonium, although not significant for the group size used, might be due to additional actions of ouabain on perivascular sympathetic nerves and/or the vasculature as we have found for higher doses of ouabain in hexamethonium-blocked normotensive rats (38). To exclude the possibility that prevention of the pressor effects of low-dose ouabain administration by hexamethonium was not due to its effect in reducing arterial pressure in L-NAME–treated rats, a control study with hydralazine was performed in these animals. The pretreatment with hydralazine reduced arterial pressure to similar levels to those observed for hexamethonium pretreatment in L-NAME–treated rats, and in this condition, ouabain was also able to increased systolic and diastolic blood pressures. These results suggested that the reduction in arterial pressure, per se, did not prevent the hypertensive actions of this dose of ouabain in L-NAME–induced hypertension.

There are several reports that suggest ouabain can have a direct action on the vasculature to increase vascular resistance or to sensitize vascular beds to pressor responses produced by norepinephrine, angiotensin II, and phenylephrine (15,16,18–20,28,37,39,40). Low nanomolar concentrations of ouabain were shown to augment caffeine contractions in rat isolated mesenteric arteries (41) and cause an increase in the concentration of cytosolic free calcium (17). We have shown that 10 n*M* ouabain did not alter baseline perfusion pressure but increased the sensitivity to phenylephrine-evoked pressor responses in a perfused rat tail vascular bed preparation from normotensive rats (18,28,35). Therefore, experiments were performed to see whether a similar mechanism might occur in L-NAME–hypertensive rats. We found that the actions of phenylephrine in the tail vascular bed preparations from L-NAME–treated rats were reduced after treatment with 10 n*M* ouabain. This was an unexpected observation and opposite to what we previously observed in tail vascular beds from normotensive and other models for hypertension in rats (15,28). It is proposed that this observation, in part, might be due to an endothelial modulation of the actions of ouabain.

Several reports show that the endothelium modulates the effects induced by ouabain. Endothelium from normotensive animals releases a factor in the presence of ouabain, which counteracts the vasoconstrictor and the sensitizing action of this cardiotonic compound (18–20,42). Wolfson and Poston (43) reported that ouabain, directly or indirectly, might alter the synthesis and/or the release of a relaxing factor. Therefore, we studied in L-NAME–treated rats the participation of the endothelium to explain the reduction of the phenylephrine pressor response induced by ouabain. When experiments were performed using vessels from L-NAME rats with damaged endothelium, ouabain increased the pressor response evoked by phenylephrine. These data suggested an endothelial modulation of ouabain actions in L-NAME–treated rats. This and others results support the notion that the endothelium might modulate the actions of ouabain (18–20,42). However, the nature of factor released by the endothelium from the L-NAME–treated group is not known.

Ouabain has been shown to release nitric oxide from endothelial cells and to induce NOS expression and activity in vascular smooth muscle cell (21,22). Also, recently we demonstrated in rats made hypertensive by chronic ouabain treatment that there is an increase in the nitric oxide modulation of contractile responses produced by phenylephrine that is accompanied with an increase in the expression of the endothelial NOS protein (11). In the current study, because nitric oxide production was already blocked by L-NAME treatment, the ability of ouabain to produce the release of another vasodilator should be considered. The cyclooxygenase pathway was discarded in this study because there are reports (19,20,42) showing that the blockade of this pathway by indomethacin does not potentiate the effects of ouabain.

Endothelium-derived hyperpolarizing factor (EDHF) is a putative mediator that could counteract the actions of ouabain on the vasculature. EDHF is thought to act by opening K^+ channels and/or by stimulating smooth muscle Na^+, K^+-ATPase (44). Previously, using tail vascular beds from normotensive rats treated with TEA, we showed an increase in the sensitivity to phenylephrine induced by ouabain, reinforcing the suggestion that ouabain might stimulate the release of a K^+-channel opener, one putative EDHF factor (18). Recently, it was also demonstrated that after chronic treatment with L-NAME, there is an increase of EDHF production by the endothelium, which might be a compensatory mechanism for NOS blockade (25,27). Therefore, we used TEA to evaluate the possibility of ouabain inducing the release of a K^+-channel opener from the endothelium of L-NAME–treated rats. In preparations from L-NAME–treated rats with an intact endothelium, acetylcholine produced a vasoconstriction in the presence of TEA. Surprisingly, 20 m*M* TEA reduced the effects of phenylephrine on the vascular bed from L-NAME–treated rats. This action of TEA in tail vascular beds from L-NAME–treated rats was not observed in preparations from normotensive rats (18) and was completely different from that obtained when the endothelium was damaged. It is possible to speculate that in the presence of TEA, the endothelium from L-NAME–treated rats may produce on endothelial relaxing factor, but TEA also promoted a reduction in phenylephrine-induced contraction in tail vascular bed without endothelium (results not shown). Also, another possibility to consider is that TEA may have an α_1-adrenoceptor–blocking property. However, this hypothesis was discarded since TEA also reduced the serotonin-induced contraction in tail vascular bed from L-NAME–hypertensive rats (results not shown). The mechanism by which TEA interferes with phenylephrine in preparations from L-NAME–treated animals is not known, but these results suggested that TEA effects are present only in the hypertensive animals, and this effect is not dependent on the release of an endothelial vasodilator factor or on the α_1-adrenoceptor–blocking property promoted by TEA. On the other hand, TEA plus ouabain treatment enhanced the pressor response to phenylephrine, similar to what happened after endothelial damage. This finding suggested that ouabain in the L-NAME hypertensive rats might induce the release of a K^+-channel opener as we have previously demonstrated in normotensive rats (18).

The mechanism to explain the effects of ouabain is the inhibition of the Na^+ pump, which leads to an increase in the concentration of intracellular Na^+ and reduces the activity of the membrane Na^+/Ca^{2+} exchanger, reducing Ca^{2+} extrusion (12,13,41). Consequently, the concentration of intracellular Ca^{2+} increases (13,14). In vascular smooth muscle, the α_2 and α_3 isoforms of the Na^+, K^+-ATPase, which have high, nanomolar affinities for ouabain, have been shown to be preferentially localized in the sarcolemma near the sarcoplasmic reticulum, the plasmerosome (14), and not evenly distributed across the cell membrane as is the ouabain low-affinity α_1 isoform. Selective inhibition of the Na^+ pump by nanomolar concentrations of ouabain at the α_2 and α_3 sites permits a localized increase in Ca^{2+} that is quickly sequestered by the reticulum. It is proposed that because it is rapidly sequestered, this increase in Ca^{2+} does not alter resting vascular tone but instead results in an increased release of Ca^{2+} upon depolarization that leads to an increased response of the vasculature to vasoconstrictor agents (41).

To study the putative participation of the inhibition of Na^+, K^+-ATPase by ouabain, the functional activity of Na^+, K^+-ATPase was evaluated. As noted above, the results showed that in L-NAME–induced hypertension, the activity of Na^+, K^+-ATPase was reduced compared with normotensive animals. Similar results were observed in hearts from L-NAME–hypertensive rats (45). Because nitric oxide is an endothelial factor that activates Na^+, K^+-ATPase (31,46), the decrease in NO with L-NAME treatment might explain the reduced functional activity of Na^+, K^+-ATPase. This enzyme is an important factor to maintain a reduced vascular tone, and, being inhibited, it could act as a coadjutant for the elevation of arterial blood pressure. Similar to other results previously obtained in our laboratory (18,35), this study showed that treatment with ouabain (10 n*M*) for 60 min inhibited Na^+, K^+-ATPase activity. However, in L-NAME–treated rats, 10 n*M* ouabain did not produce any additional reduction in the functional activity of the pump. Because the effect of ouabain at nanomolar concentrations is most likely dependent on the inhibition of the α_2 and α_3 isoforms of Na^+, K^+-ATPase, the lack of additional inhibition of the functional activity of the pump in preparations from L-NAME–treated rats suggests a selective decrease in the activities of these isoforms. A similar behavior is also present in tail vascular bed from diabetic rats (35). In these rats, the sensitization to vasopressor agents induced by ouabain is lost as consequence of the reduction of the functional activity of Na^+, K^+-ATPase (35).

The reduction in a functional Na^+, K^+-ATPase activity and the release of a vasodilator factor might explain why ouabain did not enhance the vascular reactivity to phenylephrine in the preparations from L-NAME–treated animals with an intact endothelium. The loss of its action on

the Na^+ pump and the release of an endothelial factor with opposing actions on vascular tone could mask an action of ouabain to enhance pressor responses to vasoconstrictors. However, an action of ouabain solely on the Na^+ pump does not appear to account fully for our observations made on the ability of ouabain to enhance the activity of phenylephrine in preparations from L-NAME animals following removal of the endothelium. If we assume that the vascular smooth muscle relaxation in response to the addition of extracellular K^+ is due to an activation of the Na^+, K^+-ATPase localized on the sarcolemma of vascular myocytes, then it is difficult to explain how ouabain enhanced the actions of phenylephrine in the absence of the endothelium under conditions in which ouabain did not appear to produce a further inhibition of the pump than did treatment with L-NAME. At present, we can only speculate the possible basis for this observation. One possibility is that the method we used to evaluate the functional activity of the pump is not sensitive enough to detect a further inhibition by ouabain. Consequently, a small inhibition not detected by our assay might have been sufficient to increase Ca^{2+} in the plasmerosome region (14) and thus enhance the actions of phenylephrine. Secondly, because endothelial removal permitted the demonstration of an enhancing action of ouabain, it is possible that with intact endothelium and as a consequence of L-NAME treatment, ouabain caused the release of an endothelial hyperpolarizing factor that produced a stimulation of the pump and masked or reversed any inhibitory actions of ouabain. Also, as the recent work of by Manunta et al. (47) has shown, it is possible that pressor effects of ouabain can be caused by additional actions at a site(s) other than the Na^+ pump.

The results of our studies with TEA and the functional activity of the Na^+ pump in L-NAME–treated animals strongly suggests that endothelial modulation of the actions of ouabain appears to be greater in L-NAME–treated rats than in control animals. Further experimentation is required to determine the mechanism(s) by which this occurs. Regardless of the mechanism(s), our results suggest that direct vascular actions of nanomolar concentrations of ouabain in this model of hypertension do not appear to be the primary means by which a low dose of ouabain that is subpressor in anesthetized normotensive rats produces a pressor response in anesthetized L-NAME–treated rats.

As discussed above, the in vivo pressor actions of ouabain in L-NAME–treated rats used in the current study appear to be due mainly to actions of ouabain at the preganglionic level or higher, suggesting that ouabain enhanced sympathetic outflow in these animals, as has been suggested by others (50). In part, the hypertensive actions of ouabain in L-NAME–treated rats might be due to the diverse actions of ouabain on baroreflex activity in normotensive and L-NAME–hypertensive rats (48,49). In the normotensive anesthetized rat, ouabain enhances the activity of the baroreflex (48). Thus, actions of low doses of ouabain to sensitize the vasculature to pressor agents might be masked by an enhanced baroreflex that reduces sympathetic outflow. On the other hand, in L-NAME–hypertensive rats, ouabain produces an L-arginine–sensitive inhibition of baroreflex activity (49). Thus, it is possible that the actions of both ouabain and L-NAME to increase sympathetic outflow are not masked by baroreflex activity. At an apparent variance with this interpretation are our observations that show a decrease in the vasoconstrictor actions of phenylephrine in isolated tail vascular bed preparations from L-NAME–treated rats. Although the reactivity to phenylephrine was reduced, a dose-related vasoconstrictor response was observed. We speculate that in vivo, the increase in sympathetic outflow produced by ouabain in the L-NAME–treated rats was sufficient to produce an increase in arterial pressure even in the face of a reduced responsiveness to the actions of α-adrenoceptor agonists.

In conclusion, we demonstrated that L-NAME–induced hypertension is also associated with a reduced functional activity of Na^+, K^+-ATPase in the tail vascular bed. In this model of hypertension, a low dose of ouabain (0.18 μg/kg) increased arterial blood pressure in vivo. This effect cannot be explained by a direct vascular action but can be explained by an increase of the sympathetic tone. In the tail vascular bed, ouabain did not produce additional inhibition of the activity of Na^+, K^+-ATPase and, at the same time, reduced the vascular reactivity to phenylephrine. An endothelium-derived K^+-channel opener whose release is induced by ouabain may cause the last effect.

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