Ouabain at Nanomolar Concentration Promotes Synthesis and Release of Angiotensin II from the Endothelium of the Tail Vascular Bed of Spontaneously Hypertensive Rats

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Abstract: The effects of 1 nM ouabain (OUA) on the contractile actions of phenylephrine (PHE, 0.001–100 μg) and functional activity of the sodium pump (NKA) in isolated-perfused tail vascular beds from WKY and SHR were investigated. In preparations from SHR, perfusion with OUA in the presence of endothelium (E+) increased the sensitivity (pED_{50}) of PHE (before: 2.14 ± 0.06 versus after: 2.47 ± 0.07; $P < 0.05$) without altering the maximal response (E_{max}). After endothelial damage, OUA reduced the E_{max} of PHE in SHR (before: 350 ± 29 versus after: 293 ± 25 mm Hg; $P < 0.05$). In SHR/E+, pretreatment with losartan (10 μM) or enalaprilat (1 μM) prevented the increased sensitivity to PHE induced by OUA. OUA increased NKA activity in SHR/E+ (before: 45 ± 6 versus after: 58 ± 5%, $P < 0.05$). Losartan (10 mg/Kg, *i.v.*) also abolished the increment in systolic and diastolic blood pressure induced by OUA (0.18 μg/Kg, *i.v.*) in anesthetized SHR. OUA did not alter the actions of PHE in either anesthetized WKY rats or vascular preparations. Results suggest that 1 nM OUA increased the vascular reactivity to PHE only in SHR/E+. This effect is mediated by OUA-induced activation of endothelial angiotensin converting enzyme that promotes the local formation of angiotensin II, which sensitizes the vascular smooth muscle to the actions of PHE.

Key Words: ouabain, SHR, ACE, angiotensin II, Na^+, K^+-ATPase, endothelium

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Ouabain, or a closely related isomer, is an endogenous compound secreted by some regions of the central nervous system[1,2] and the supra-renal cortex.[3,4] It is present in nanomolar concentration in the plasma of several animals such as rats[4] and humans.[5] It is thought to play a role in some forms of hypertension by inhibiting the Na^+-pump activity. The inhibition of the Na^+, K^+-ATPase activity in the vascular smooth muscle increases intracellular sodium[6,7] reducing the activity of the Na^+/Ca^{2+} exchanger and increasing intracellular calcium concentration. Thereafter, the calcium uptake by the sarcoplasmic reticulum increases.[6] When the vascular smooth muscle is stimulated the final result is an amplification of the calcium release and increase of the vascular tone.[7] In some vascular tissues, the α_2 and α_3 isoforms of Na^+, K^+-ATPase cluster with the Na^+/Ca^{2+} exchanger in the plasmalemmal micro domain adjacent to the sarcoplasmic reticulum.[7] This region, named plasmerosome, can regulate Ca^{2+} signaling and might explain how low concentrations of ouabain, which are selective for these isoforms, can affect the vascular tone.

There is evidence that the endothelium modulates the action of ouabain.[8–10] Previously we reported that nanomolar ouabain releases an endothelial potassium channel opener in isolated vascular preparations from normotensive animals that reduces the vasoconstrictor actions of phenylephrine.[10] In contrast, ouabain at micromolar concentrations releases a vasoconstricting factor in isolated aortae from spontaneously hypertensive rats (SHR).[8]

We previously demonstrated that nanomolar concentrations of ouabain increased the response to phenylephrine in the tail vascular bed from SHR.[11,12] The present study was undertaken to investigate the mechanism(s) of action by which nanomolar concentrations of ouabain sensitize the vasculature in SHR to the contractile actions of phenylephrine. The role of endothelial angiotensin converting enzyme (ACE) and Na^+, K^+-ATPase activity on the enhanced reactivity to phenylephrine produced by ouabain in the isolated tail vascular bed from SHR was also investigated.

MATERIALS AND METHODS

Animals

Three-month-old male WKY (n = 51) and SHR (n = 83) were used in this study. All experiments were conducted in compliance with the guidelines for biomedical research as stated by the Brazilian Societies of Experimental Biology. All rats had free access to water and were fed rat chow ad libitum.

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Blood Pressure, Heart Rate, and Body Weight Determinations

On the day before the experiments the rats were anesthetized with ether. The left femoral artery was cannulated with a polyethylene catheter (PE-50 with heparinized saline) that was exteriorized in the mid scapular region. On the day of the experiments, 24 hours later, body weight, blood pressure, and heart rate were measured in conscious animals.

Arterial blood pressure was measured by a pressure transducer (model 1050BP, UFI, Inc., Morro Bay, CA) and recorded using an interface and software for computer data acquisition (model MP100A, BIOPAC Systems, Inc., Santa Barbara, CA). Heart rate was determined from the intra-beat intervals.

"In Vitro" Experiments

Isolated Rat Tail Vascular Bed Preparation

Isolated rat tail vascular beds were used in this study as previously reported.[13] 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 24G ¾, Sorocaba, SP, BR) near the base of the tail. The vascular bed was flushed with Krebs-Henseleit buffer (KHB in mM) (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, BR). After a 30- to 45-minute equilibration period, the experimental protocol was initiated. Mean perfusion pressure (MPP) was measured by using a pressure transducer (TPS-2, InCor, São Paulo, SP, BR) and the data recorded using an interface and software for computer data acquisition (model MP100A, BIOPAC Systems, Inc., Santa Barbara, CA) 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.

The following protocols were used:

Effects of Ouabain on the Actions of Phenylephrine in the Presence and Absence of Endothelium

After a 30- to 45-minute stabilization period, increasing doses of phenylephrine (0.001–100 μg, as bolus injections of 100 μL) were administered into the perfusion medium in preparations from WKY (n = 15) and SHR rats (n = 13) with intact endothelium (E+) prior to and after a 60-minute equilibration period of perfusion of KHB containing 1 nM ouabain (E+/Oua 1 nM).

The same protocol, as described previously, was performed in preparations following endothelium (E-) damage by a bolus injection of 8 mg of CHAPS, {3-[(3-chloroamidopropyl) dimethylammonium]-1-propane-sulfonate} as previously described.[10] The absence of functional endothelium was confirmed by the inability of ACh (5 μg in 100 μL) to produce relaxation. Responses to phenylephrine were measured in the WKY (n = 7) and SHR groups (n = 13): at 30 minutes after endothelial damage (E-) and repeated following an equilibration period of 60 minutes with ouabain (1 nM) containing KHB (E-/Oua 1 nM).

Time-control dose-response curves to phenylephrine (0.001–100 μg, as bolus injections in 100 μL) were administered before and after a 60-minute equilibration period of infusion of ouabain-free KHB solution in preparations with and without endothelium from WKY (n = 7–9) and SHR rats (n = 7–9).

Effect of Renin-Angiotensin-System-Derived Products on the Effects of Ouabain on the Phenylephrine-Induced Pressure Response

The possibility that ouabain might stimulate the release of a renin-angiotensin-system-derived product was evaluated in preparations from SHR, by determining responses to phenylephrine before and 1 hour after incubation with ouabain (1 nM) plus an AT_1 receptor antagonist, losartan (10 μM, n = 7) or an angiotensin converting enzyme inhibitor (ACE), enalaprilat (1 μM, n = 8). The same protocol was performed in the absence and in the presence of KBS solution with losartan (10 μM, n = 7) or enalaprilat (1 μM) without ouabain for 60 minutes.

Functional Activity of the Na^+, K^+-ATPase

The functional activity of Na^+, K^+-ATPase in preparations from WKY (n = 6) and SHR rats (n = 5) was measured using K^+-induced relaxation as described by Webb and Bohr[14] and modified by Rossoni et al.[10] After a 30- to 45-minute equilibration period in normal KHB, preparations were perfused during 30 minutes with a K^+-free KHB. Afterward, the preparations were precontracted with phenylephrine (10^{-7} M) and once a plateau was attained, the concentration of KCl in the perfusate was increased in steps (1, 2, 4, and 6 mM), each one with 5 minutes' duration. This protocol was repeated 60 minutes after the initiation of perfusion with 1 nM of ouabain.

Time-control concentration-dependent curves to KCl (1, 2, 4, and 6 mM) were performed before and after 60 minutes of infusion of ouabain-free KBS solution in preparations from WKY (n = 7) and SHR rats (n = 7).

"In Vivo" Experiments

Effect of Ouabain on the Arterial Blood Pressure in Anesthetized Animals

Urethane (1.2 g/Kg, *i.p.*) was used as an anesthetic and supplemented when necessary. The jugular vein and the carotid artery were dissected and cannulated with a polyethylene catheter (PE-50 filled with heparinized saline) for drug admin-

istration and arterial blood pressure measurements, respectively. The arterial blood pressure was measured with a pressure transducer (model 1050BP, UFI, Inc., Morro Bay, CA) and recorded using an interface and software for computer data acquisition (model MP100A, BIOPAC Systems, Inc., Santa Barbara, CA). Heart rate was determined from the intra-beat intervals.

After a 30-minute stabilization period, systolic and diastolic blood pressures were measured before and 60 minutes after administration of 0.18 μg/Kg (approximately 0.3 nmol/Kg) ouabain, a dose that increases blood pressure in hypertensive but not in normotensive animals.[12,15,16] Additionally, the effects of ganglionic blockade with hexamethonium (5 mg/Kg, *i.v.*) or AT_1 receptor blockade with losartan (10 mg/Kg, *i.v.*) were investigated. Following anesthesia and stabilization, hexamethonium or losartan were administered. Thirty minutes later, a time when the depressor effects of hexamethonium or losartan had stabilized, ouabain (0.18 μg/Kg, *i.v.*) was administered. The systolic and diastolic blood pressures at 60 minutes after the ouabain administration were recorded.

Drugs and Reagents

Ouabain octahydrate, L-phenylephrine hydrochloride, hexamethonium hydrochloride, losartan, enalaprilat, acetylcholine chloride, CHAPS, and sodium pentobarbital were purchased from Sigma (St. Louis, MO); heparin was purchased from Roche (São Paulo, SP, BR).

The drugs were dissolved in bi-distilled water and all solutions were prepared freshly before use and protected from light.

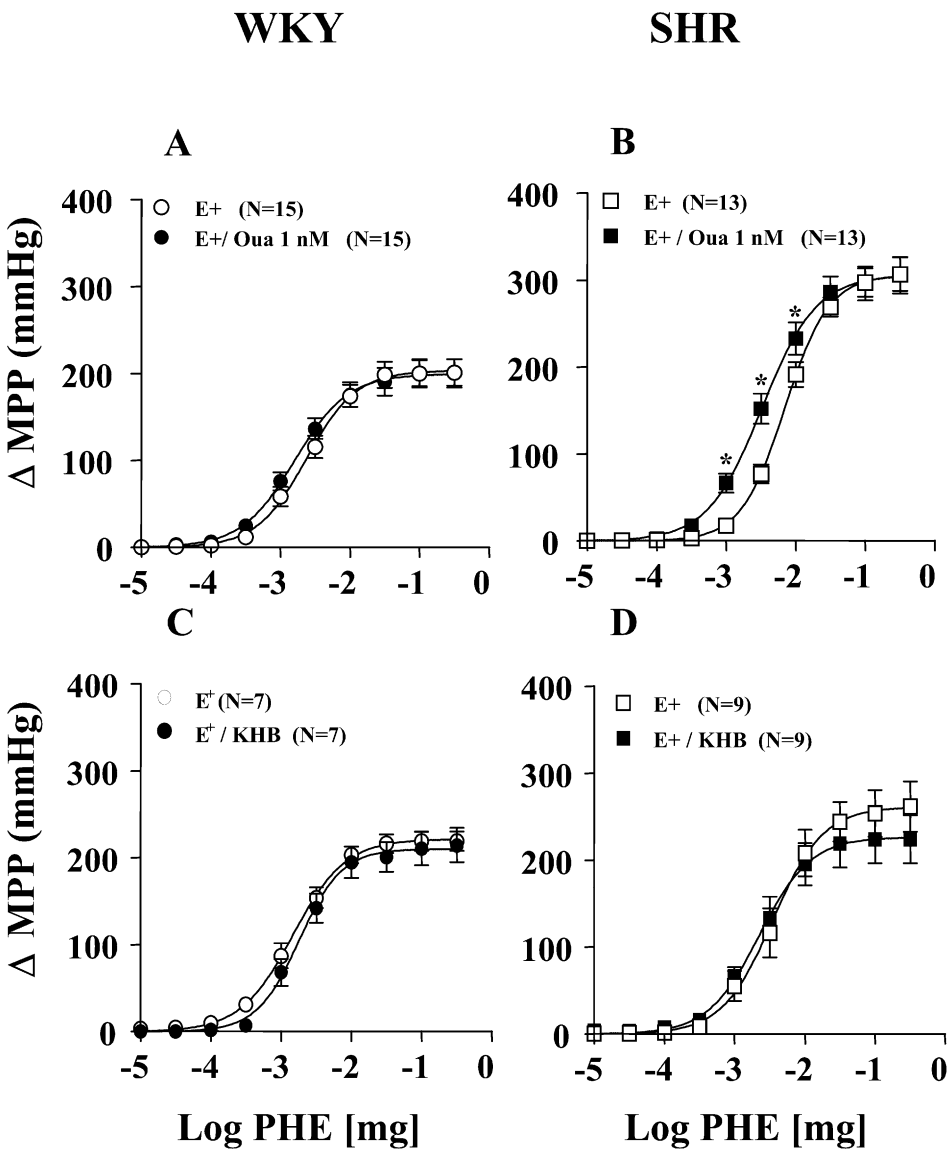

FIGURE 1. Changes of the mean perfusion pressure (MPP) produced by phenylephrine (PHE) in tail vascular bed from SHR (left panels) and WKY (right panels). Dose-response curves were performed in preparations with intact endothelium: before (E+) and 60 minutes after 1 nM ouabain (E+/OUA) (panels A and B). Panels C and D show the time-control dose-response curves to phenylephrine before (E+) and after 1 hour of infusion with Krebs-Henseleit buffer (E+/KHB). Number of preparations indicated in parentheses. Results are expressed as means ± SEM. * $P < 0.01$, after versus before OUA, 2-way ANOVA, repeated measures.

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).

For each concentration-response curve for phenylephrine, the maximum effect (E_{max}) and the bolus dose (μg) that produced one-half E_{max} (log ED_{50}) were estimated using nonlinear regression analyses (GraphPad Prism Software, San Diego, CA). The sensitivity of the agonists is expressed as pED_{50} ($-\log EC_{50}$).

Results are presented as means ± SEM. Data were analyzed using a *t*-test and ANOVA. When the ANOVA showed a significant treatment effect, a Tukey's *post-hoc* test was used to compare means. $P < 0.05$ was considered significant.

RESULTS

Three-month-old male awake SHR had a higher mean arterial pressure (SHR: 179 ± 1 versus WKY: 106 ± 2 mm Hg, $P < 0.001$) and a lower body weight when compared with WKY rats (SHR: 227 ± 4 versus WKY: 289 ± 5 g, $P < 0.05$). However, no change in heart rate was observed (SHR: 363 ± 4 versus WKY: 347 ± 5 bpm, $P > 0.05$).

Effects of Ouabain on the Actions of Phenylephrine in the Presence and Absence of Endothelium

In endothelium-intact tail vascular bed preparations from WKY and SHR, perfusion of 1 nM ouabain for 60 minutes did not change baseline mean perfusion pressure (in mm Hg, WKY: before: 75 ± 3 versus after ouabain: 80 ± 4, $P >$ 0.05; SHR: before: 95 ± 4 versus after ouabain: 84 ± 2, $P >$

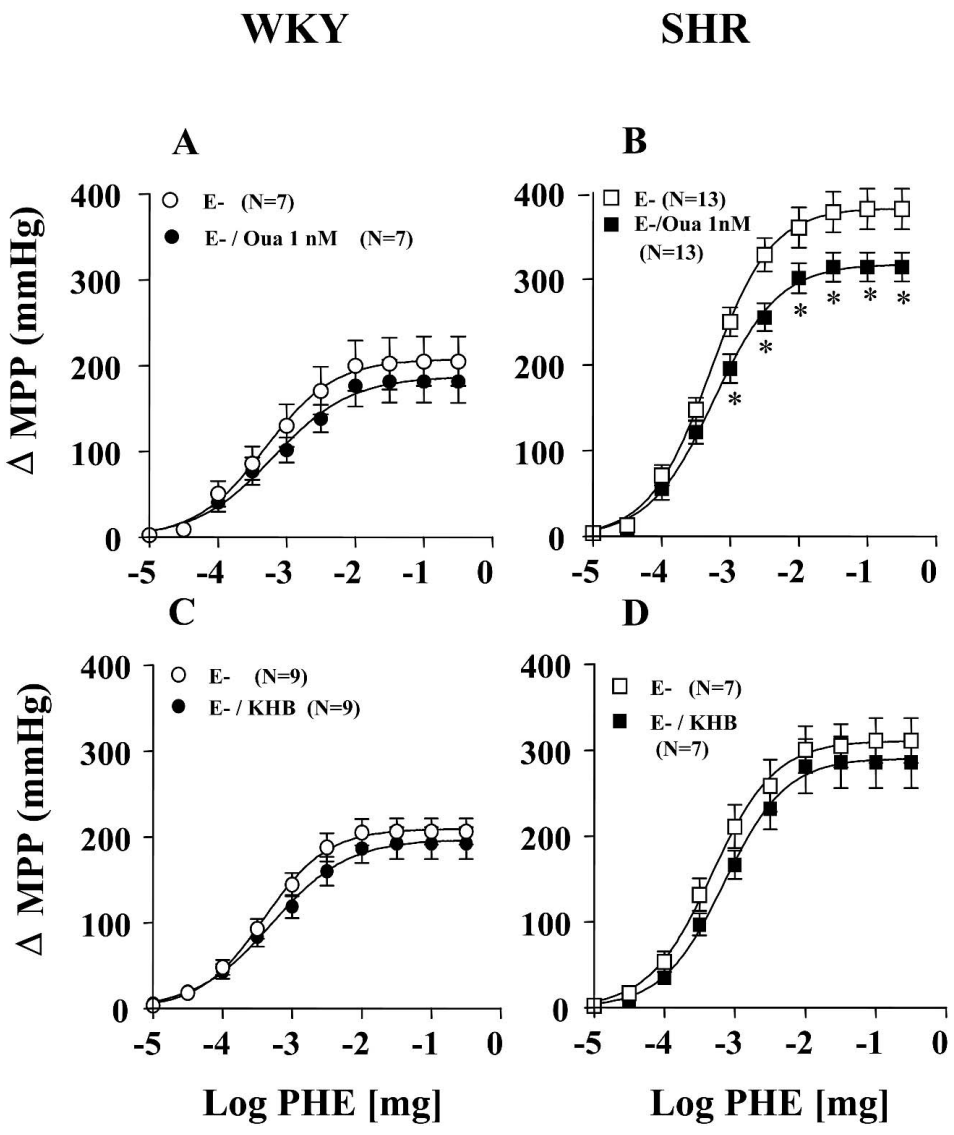

FIGURE 2. Changes of the mean perfusion pressure (MPP) produced by phenylephrine (PHE) in tail vascular bed from SHR (left panels) and WKY (right panels). Dose-response curves were performed in preparations with damaged endothelium before (E-) and after 1 hour perfusion of 1 nM ouabain (E-/OUA) (panels A and B). Panels C and D show the time-control dose-response curves to phenylephrine before (E-) and after 1 hour of infusion with Krebs-Henseleit buffer (E-/KHB). Number of preparations indicated in parentheses. Results are expressed as means ± SEM. *$P <$ 0.01, after versus before OUA, 2-way ANOVA, repeated measures.

0.05). The perfusion with ouabain did not change the response to phenylephrine in the tail vascular bed from WKY (Fig. 1A). However, in preparations from SHR ouabain increased pED_{50} to phenylephrine (2.14 ± 0.06 before versus 2.47 ± 0.07 after ouabain, $P < 0.01$) without changing E_{max} (303 ± 18.7 before versus 302 ± 20.3 mm Hg after ouabain, $P > 0.05$) (Fig. 1B). It is important to emphasize that perfusion for 60 minutes with ouabain-free KHB solution (time control) did not change the baseline perfusion pressure or the pressor response to phenylephrine in WKY or SHR vascular bed (Fig. 1 C and D).

In the absence of endothelium, 1 nM ouabain did not alter E_{max} or pED_{50} values for phenylephrine in tail vascular bed preparations from WKY rats (Fig. 2A). This result was similar to that obtained in preparation with intact endothelium (compare with Fig. 1A). On the other hand, in endothelium-denuded tail vascular bed from SHR 1 nM ouabain produced a reduction in the E_{max} to phenylephrine (381 ± 23.5 before versus 314 ± 16.9 mm Hg after ouabain; $P < 0.01$), without change in pED_{50} values (3.30 ± 0.06 before versus 3.25 ± 0.09 after ouabain, $P > 0.05$) (Fig. 2B). This result was opposite to that obtained in preparations from SHR with an intact endothelium (compare with Fig. 1B). The results of control experiments showed that perfusion for 60 minutes with ouabain-free KHB did not change the baseline perfusion pressure or the pressor responses to phenylephrine in tail vascular beds without endothelium from either WKY or SHR (Fig. 2 C and D).

Effect of Renin-Angiotensin-System-Derived Products on the Effects of Ouabain on the Phenylephrine-Induced Pressure Response

No changes in baseline perfusion pressure or in phenylephrine pressor response were observed in preparations from SHR group during the perfusion with enalaprilat (1 μM) or losartan (10 μM), even after ouabain treatment (Figs. 3C and D). The infusion with ouabain (1 nM) plus enalaprilat (Fig. 3A) or losartan (Fig. 3B) for 60 minutes did not change pED_{50} or E_{max} for phenylephrine suggesting that AT_1 blockade or

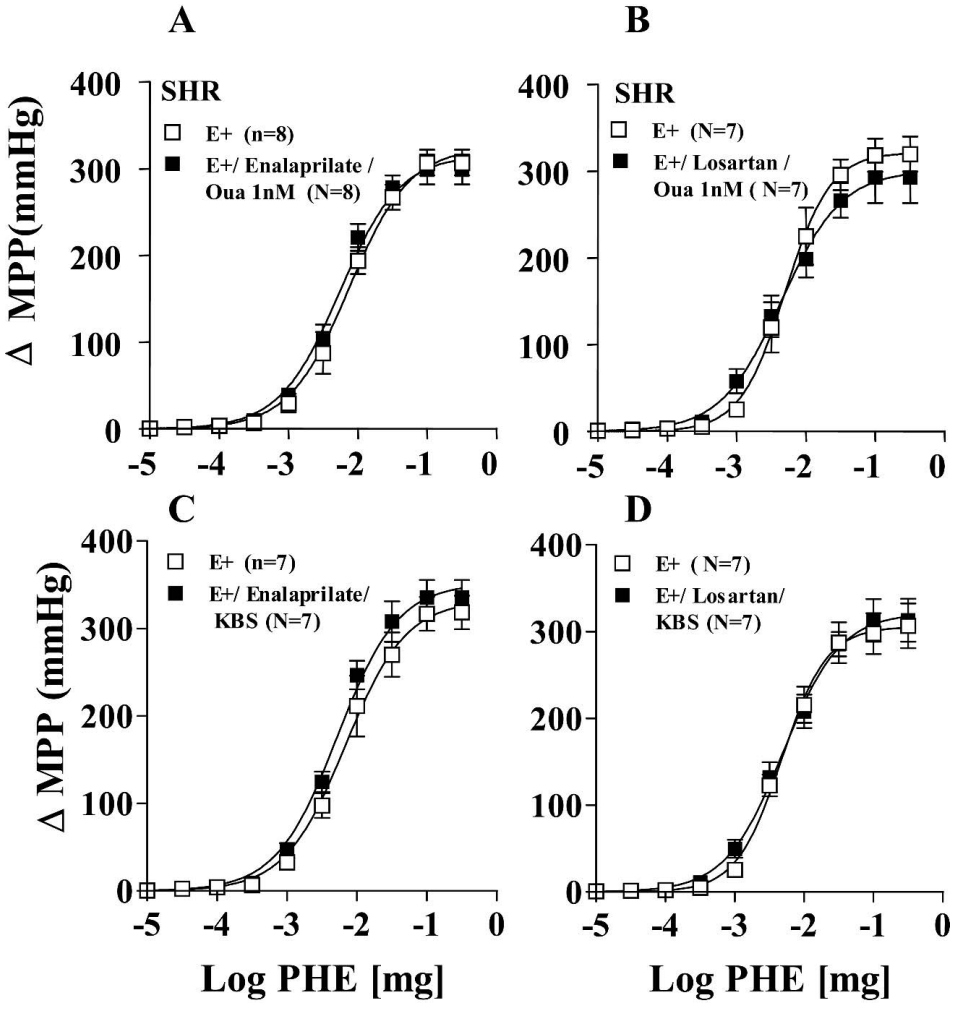

FIGURE 3. Changes of mean perfusion pressure (MPP) produced by phenylephrine (PHE) in tail vascular bed from SHR. Dose-response curves were performed in preparations 30 minutes before enalaprilat (Ena: 1 μM-Panel A) or losartan (Los: 10 μM-Panel B) infusion (E+) and after enalaprilat or losartan plus 1 nM ouabain infusion (E+/Ena or Los/OUA). Panels C and D show the dose-response curves to phenylephrine performed 30 minutes before enalaprilat (Ena-Panel C) or losartan (Los-Panel D) infusion (E+) and after enalaprilat or losartan plus Krebs-Henseleit buffer infusion (E+/Ena or Los/KBS). The number of preparations is indicated in parentheses. Results are expressed as means ± SEM. 2-way ANOVA, repeated measures.

ACE inhibition abolished the sensitization induced by ouabain on the phenylephrine pressor response.

Functional Activity of the Na^+, K^+-ATPase

As shown in the Figure 4A, 1 nM ouabain did not alter the relaxation induced by increasing concentrations of KCl in preparations from WKY rats. In contrast, in preparations obtained from SHR rats, 1 nM ouabain increased the relaxation induced by KCl (Fig. 4B). It is important to emphasize that perfusion for 60 minutes with ouabain-free KHB solution (time control) did not change the relaxation induced by KCl in tail vascular bed from WKY or SHR rats (Figs. 4C and D).

Effect of Ouabain on the Arterial Blood Pressure in Anesthetized Animals

Anesthesia with urethane reduced systolic and diastolic blood pressures in both groups, similar to previous reports.[12,15,16] In WKY, neither systolic nor diastolic arterial pressures changed 60 minutes after administration of ouabain (0.18 μg/kg, *i.v.*) (Fig. 5). In contrast, this treatment significantly increased both systolic and diastolic blood pressures in SHR (Fig. 5). In both groups, ouabain did not change the heart rate (results not shown). Ganglionic blockade with hexamethonium did not change the effects on blood pressure induced by ouabain in SHR (Fig. 6); however, after losartan treatment this effect was completely blocked (Fig. 6).

DISCUSSION

Results presented here show that 1 nM of ouabain increases the sensitivity to phenylephrine in the tail vascular bed from SHR by a mechanism involving an increase in the activity of endothelial ACE and the local synthesis of angiotensin II. This observation with respect to putative mediators involved in elevations of vascular tone produced by ouabain is novel and

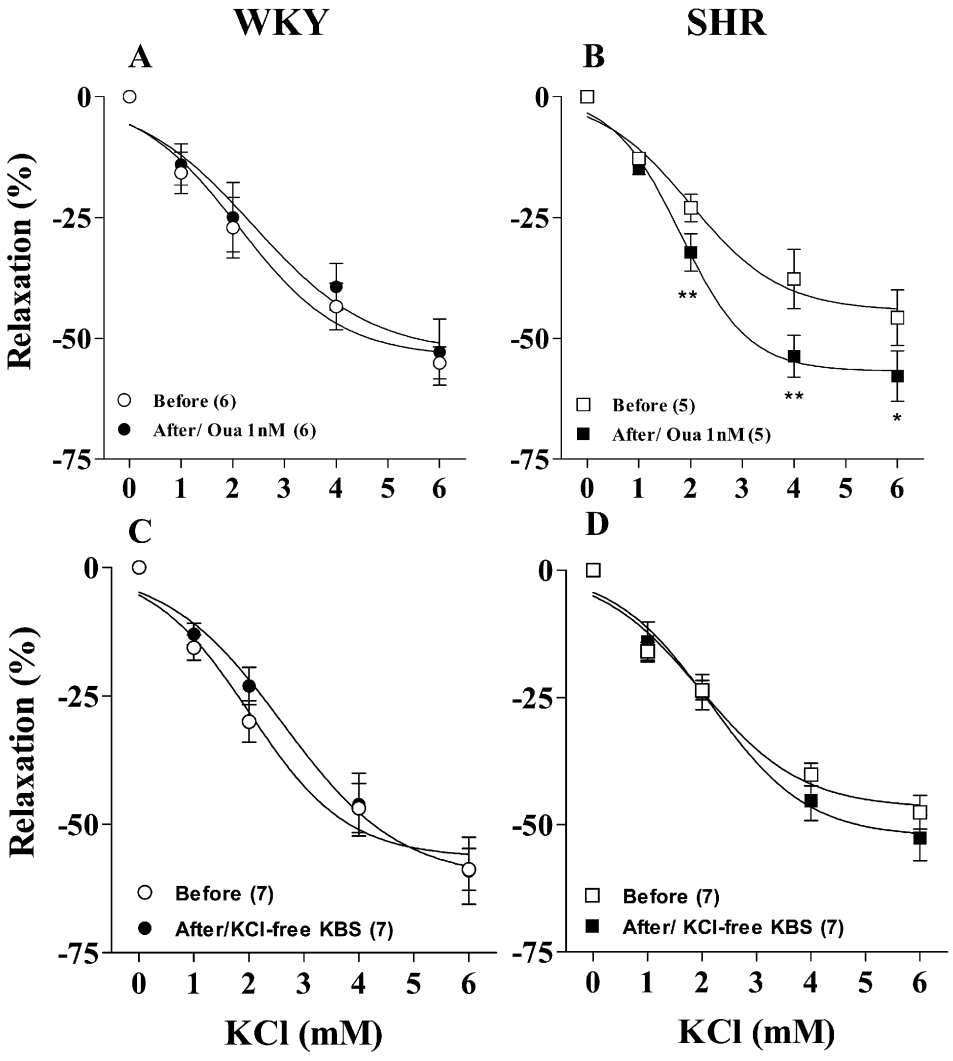

FIGURE 4. Reversal of PHE-induced contractions in KCl-free KHB produced by the addition of increasing concentrations of KCl in the absence and presence of 1 nM ouabain. (Panel A) Curves in WKY and (Panel B) curves in SHR before and after 60 minutes after 1 nM ouabain infusion. (Panel C) Curves in WKY and (Panel D) curves in SHR before and 60 minutes after KCl-free Krebs-Henseleit buffer infusion. The number of preparations is indicated in parentheses. Results are expressed as means ± SEM. * $P < 0.01$ after versus before OUA, 2-way ANOVA, repeated measures.

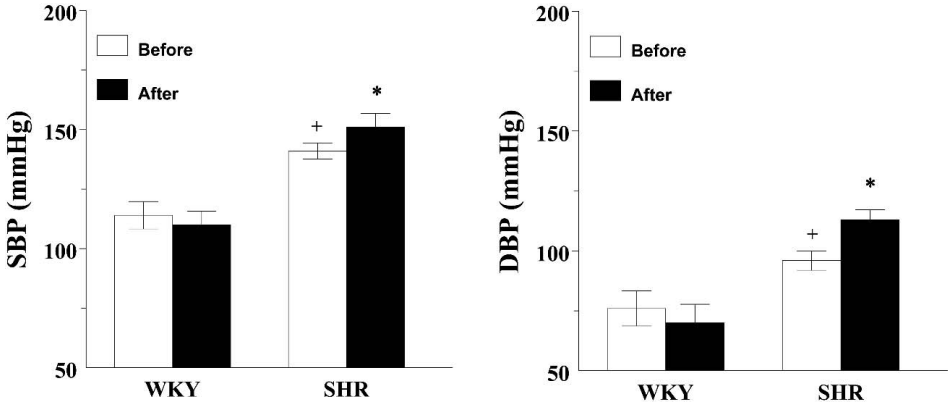

FIGURE 5. Systolic (SBP) and diastolic (DBP) blood pressure before and 60 minutes after the administration of 0.18 μg/kg ouabain in WKY (N = 8) and in SHR (N = 8) anesthetized rats. Results are expressed as means ± SEM. +$P < 0.01$ SHR versus WKY before ouabain, unpaired *t*- test and * $P < 0.01$ after versus before ouabain, paired *t*-test.

here-to-fore unreported. In addition, 1 nM ouabain activated instead of inhibited, Na^+, K^+-ATPase activity in the vascular smooth muscle, an observation similar to that described by Gao et al[17] in cardiac myocytes.

Previous reports show that 10 nM ouabain increases the pressor responses to phenylephrine in tail vascular bed from both normotensive and spontaneously hypertensive and volume-dependent hypertensive rats.[10–12,15] These actions are also seen in anesthetized rats and are still present in hypertensive models even when normotensive animals show no response.[12,15] However, the normal plasma concentrations of ouabain are lower than 10 nM[18] and the purpose of this experiment was to test possible effects at a concentration of ouabain near the physiological norm.

Ouabain in the quantity of 1 nM increased the sensitivity to phenylephrine only in tail vascular bed from SHR. This result reinforces our previous observations[12] that tail vascular beds from SHR are more responsive to the effects induced by nanomolar concentrations of ouabain.

It has been previously demonstrated that the endothelium modulates the actions of higher doses of acutely administered ouabain in vascular preparations from normotensive and hypertensive rats.[8,10] For this reason, we investigated the possible role of the endothelium on the actions of 1 nM ouabain in preparations from WKY and SHR. Endothelial damage increased maximal response and sensitivity to phenylephrine before ouabain administration, as expected. In de-endothelized preparations ouabain did not alter the pressor responses to phenylephrine in preparations from WKY rats but reduced maximal response to this α_1-adrenoceptor agonist in preparations from SHR.

Endothelium-derived factors might modulate positively or negatively the vascular actions of ouabain. In normotensive Wistar rats and in L-NAME hypertensive rats the acute administration of ouabain induces the release of EDHF.[10,16] In rats made hypertensive by a chronic ouabain treatment an enhanced endothelial release of nitric oxide and EDHF was reported[15]; meanwhile in SHR it releases an unknown vasoconstrictor factor.[8] There are reports showing that the effects induced by acute treatment with ouabain in aorta from SHR did not involve products from the arachidonic-acid pathway or free radicals release.[8] One pathway that may play a role on

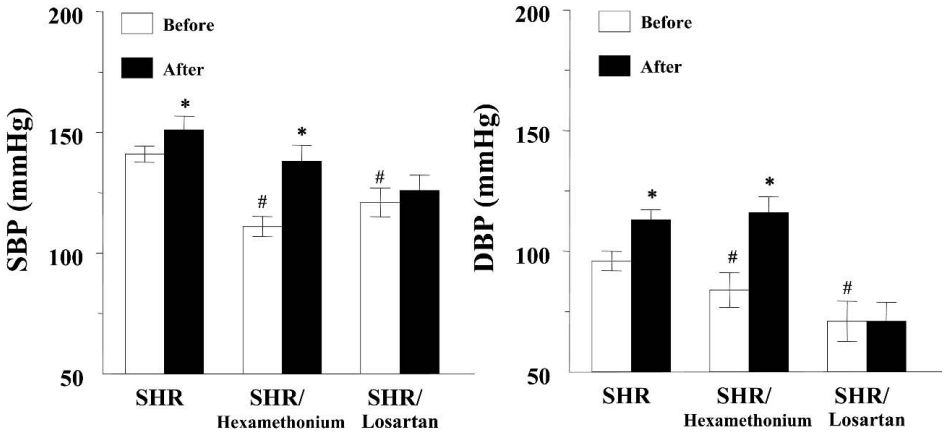

FIGURE 6. Effect of ganglionic blockade with hexamethonium (5 mg/Kg, *i.v.*) or AT_1 blockade with losartan (10 mg/Kg, *i.v.*) on the pressor actions of ouabain in anesthetized SHR. Systolic (SBP) and diastolic (DBP) blood pressure before and 60 minutes after the administration of 0.18 μg/kg ouabain in control SHR (N = 8), ganglionic blocked SHR (N = 8), and AT_1 blocked SHR (N = 8). Results are expressed as means ± SEM. # $P < 0.01$ SHR plus hexamethonium or losartan before versus SHR before, unpaired *t*-test and * $P < 0.01$ after versus before ouabain, paired *t*-test.

ouabain action is the local renin-angiotensin system. There are evidences that 10 nM ouabain interact with angiotensin II to stimulate aldosterone production in bovine adrenal glomerulosa cells.[19] At the same time angiotensin II, via AT_2 receptors, might induce its own release from these cells.[20] Besides these peripheral actions other reports presented links between ouabain and angiotensin II in some areas of the central nervous system in SHR and in a chronic ouabain-treated model.[1,21] The central mechanism already described suggests that ouabain stimulates ACE leading to the production of angiotensin II that further stimulates the sympathetic tone and ultimately increases arterial blood pressure. Considering that ouabain stimulates ACE centrally a question arises. Could ouabain stimulate local ACE at a vascular level? If this is true angiotensin II could be the unknown endothelial vasoconstrictor factor previously suggested.

The perfusion of 1 nM ouabain plus an ACE inhibitor, enalaprilat, or an AT_1-angiotensin receptor antagonist, losartan, did not change the vasoconstrictor response induced by phenylephrine suggesting that the sensitization induced by ouabain on the phenylephrine pressor response is mediated by the local vascular renin-angiotensin system. The fact that ouabain effect was also abolished by the endothelium damage suggests that the renin-angiotensin system activated by ouabain is localized in the endothelium. This is the first report to demonstrate that ouabain stimulates the production of endothelial angiotensin II, which act as a positive endothelial modulator. This property is similar to what was previously reported in the central areas of SHR, where ouabain stimulates angiotensin II production.[1]

The mechanism by which angiotensin II increases the sensitivity to alpha-adrenergic stimulation is probably related to changes on the vascular smooth muscle function. As previously reported by Henrion et al[22], subthreshold concentration of angiotensin II increases the vasoconstriction induced by noradrenaline in rabbit facial artery segments by a PKC-dependent mechanism. This mechanism could explain the increase in the blood pressure induced by acute administration of ouabain in SHR by an enhanced vascular resistance.[12]

To evaluate if these effects on blood pressure are also dependent on the renin-angiotensin system, "in vivo" experiments were performed. In anesthetized WKY rats 0.18 μg/kg ouabain (~0.3 nmol/Kg) had no effects on arterial blood pressure, reproducing previous results.[12,15,16] In addition, similar to our previous results using SHR,[12] and other models of hypertension,[15,16] this dose of ouabain increased systolic and diastolic blood pressures in SHR.

Although the ouabain effect on arterial blood pressure was not changed by the ganglionic blockade with hexamethonium, it was completely blocked by pretreatment with an AT_1-angiotensin receptor antagonist, losartan. The present results were completely different to those obtained with L-NAME-treated rats where the pretreatment with hexamethonium blocked the increase in systolic and diastolic blood pressures produced by the acute administration of 0.18 μg/ kg ouabain.[16] All together, these observations suggest that the acute low dose of ouabain used in the present study did not have actions at preganglionic neurons and/or at the central nervous system. Moreover, these results reinforce our hypothesis that in SHR ouabain activates the local renin-angiotensin system, which induces an increase in the vascular resistance and blood pressure.

Previous reports suggested that ouabain inhibited ACE activity in endothelial cells from bovine pulmonary artery[23] instead of activating it, as suggested by the findings presented here. The proposed mechanism was that intracellular sodium increment would inhibit ACE activity as a result of the inhibition of the Na^+, K^+-ATPase activity. However, these authors used an ouabain concentration (10–20 nM) higher than ours, which is known to inhibit the Na^+, K^+-ATPase activity but does not increase the bulk intracellular sodium.[24] In contrast to that, low concentration of digitalis compounds are reported to activate the Na^+, K^+-ATPase activity instead of inhibiting it.[17] We then investigated the effects of 1 nM ouabain on the Na^+, K^+-ATPase functional activity. Ouabain (1 nM) did not alter the relaxation induced by increasing concentrations of KCl in the WKY group, but increased the relaxation in preparations obtained from SHR.

Even accepting that intracellular sodium modulates ACE activity the increased Na^+, K^+-ATPase activity would reduce intracellular sodium activating ACE. Although knowing that low concentrations of ouabain activates the Na^+, K^+-ATPase activity we should stress that the angiotensin II, which is formed by local ACE, is also capable to activate the Na^+, K^+-ATPase activity.[25,26] This activation of Na^+, K^+-ATPase induced by ouabain would explain why in tails from SHR with endothelium an increased maximal response was not observed, but only an increased sensitivity. Moreover, this effect can help us to explain the reduction in the maximal response induced by ouabain in preparations without endothelium, since increased Na^+, K^+-ATPase activity reduces intracellular Ca^{2+} levels and contraction.

In conclusion, our results suggest a new mechanism for the peripheral action of low concentrations of ouabain that might increase the sensitivity of the vascular smooth muscle from spontaneously hypertensive rats to phenylephrine stimulating the endothelial ACE activity and increasing the angiotensin II release.

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