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A low concentration of ouabain (0.18 µg/kg) enhances hypertension in spontaneously hypertensive rats by inhibiting the Na^+ pump and activating the renin-angiotensin system

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Abstract

We investigated the effects of low ouabain concentrations on systolic (SAP) and diastolic (DAP) arterial pressures and on pressor reactivity in 3-month-old male spontaneously hypertensive rats (SHR). Arterial blood pressure (BP) and pressor reactivity to phenylephrine (PHE) were investigated before and after 0.18 µg/kg ouabain administration (N = 6). The influence of hexamethonium (N = 6), canrenone (N = 6), enalapril (N = 6), and losartan (N = 6) on ouabain actions was evaluated. Ouabain increased BP (SAP: 137 ± 5.1 to 150 ± 4.7; DAP: 93.7 ± 7.7 to 116 ± 3.5 mmHg; $P < 0.05$) but did not change PHE pressor reactivity. Hexamethonium reduced basal BP in control but not in ouabain-treated rats. However, hexamethonium + ouabain increased DAP sensitivity to PHE. Canrenone did not affect basal BP but blocked ouabain effects on SAP. However, after canrenone + ouabain administration, DAP pressor reactivity to PHE still increased. Enalapril and losartan reduced BP and abolished SAP and DAP responses to ouabain. Enalapril + ouabain reduced DAP reactivity to PHE, while losartan + ouabain reduced SAP and DAP reactivity to PHE. In conclusion, a small dose of ouabain administered to SHR increased BP without altering PHE pressor reactivity. Although the renin-angiotensin system (RAS), Na^+ pump and autonomic reflexes are involved in the effects of ouabain on PHE reactivity, central mechanisms might blunt the actions of ouabain on PHE pressor reactivity. The effect of ouabain on SAP seems to depend on the inhibition of both Na^+ pump and RAS, whereas the effect on DAP seems to depend only on RAS.

Key words: Ouabain; Hypertension; Na^+ pump; Renin-angiotensin system

Introduction

The existence of an endogenous steroidal ligand that inhibits Na^+ pump activity in humans and other mammals has been well established (1). This inhibitor was characterized as ouabain or a stereoisomer of ouabain (2,3). Endogenous ouabain increases in patients with essential hypertension and correlates with blood pressure (4,5). In addition, plasma ouabain levels increase in several hypertension models (6,7) and several studies have shown that acute or chronic administration of ouabain increases blood pressure in rodents (8-10). These results suggest a possible association between ouabain and the genesis or development and maintenance of arterial hypertension.

An explanation for this association is that ouabain binds to the α-subunit of the Na^+ pump, inhibiting its activity. Inhibition of this pump increases intracellular Na^+, which reduces the activity of the sarcolemmal Na^+/Ca^{2+} exchanger and thereby reduces Ca^{2+} extrusion. Consequently, the intracellular Ca^{2+} concentration increases and is taken up by the sarcoplasmic reticulum, which, upon activation, releases more calcium and increases the vascular smooth muscle tone (11). According to Vassallo et al. (12) and Rossoni et al. (9), acute treatment with ouabain enhances the vascular reactivity to vasopressor agents, and Barker et al. (13) reported that it also enhances the release of norepinephrine from the perivascular adrenergic nerve endings. Additionally, the hypertension induced by oua-

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bain has been associated with central mechanisms that increase sympathetic tone, subsequent to the activation of the cerebral renin-angiotensin (14,15) and endothelin systems (16). Thus, in addition to peripheral mechanisms, central mechanisms may contribute to the ouabain-induced elevation of arterial blood pressure.

Previous reports have shown that acute administration of very low doses of ouabain increases the blood pressure of spontaneously hypertensive rats (SHR) but not of normotensive rats (9,12). In the perfused tail vascular bed, 1 nM ouabain increases the pressor response to phenylephrine only in hypertensive rats by a mechanism involving an increase in the activity of endothelial angiotensin-converting enzyme (ACE) and the local synthesis of angiotensin II (17). However, the mechanism(s) by which acute administration of nanomolar concentrations of ouabain, similar to the normal plasma concentrations (2), exerts its effects *in vivo* on blood pressure and on pressor responses to vasoconstrictor agents is still under debate. It is not clear yet which is the contribution of central (15,16) or peripheral mechanisms (9,12,17), or of both simultaneously to this increased pressor response.

Since SHR have higher sensitivity to ouabain at nanomolar concentration (12), our aim was to investigate the effects of ouabain *in vivo* on arterial pressure and pressor reactivity to phenylephrine and to evaluate the role of autonomic reflexes, the renin-angiotensin system and Na^+ pump activity.

Material and Methods

Studies were performed in 3-month-old male SHR (N = 57). All experiments were conducted in compliance with the guidelines for biomedical research as stated by the Brazilian Societies of Experimental Biology. The experimental protocol was approved by the Ethics Committee of the Institute of Biomedical Sciences, EMESCAM (Protocol #003/2007). All rats had free access to water and rat chow.

Rats were anesthetized with urethane (1.2 g/kg, *ip*), and, after loss of the righting reflex, the carotid artery and jugular vein were dissected and cannulated with a polyethylene catheter (PE-50) and filled with heparinized saline (50 U/mL) for arterial blood pressure measurements and drug infusions, respectively. Arterial blood pressure was measured using a pressure transducer (Statham P23 AA, USA) connected to a preamplifier and to an acquisition system (model MP100A, BIOPAC System, Inc., USA). The protocols were performed with anesthetized rats due to the duration of the experiment and the necessity of stable arterial pressure maintenance.

Hemodynamic effects of ouabain

To determine whether nanomolar amounts of ouabain affected the arterial blood pressure and the pressor response to phenylephrine, ouabain (0.18 µg/kg, *iv*; in a volume of 17 µL/100 g rat weight) was administered. This dose of ouabain was expected to produce nanomolar levels of circulating ouabain (approximately 1.5 nM) similar to that found in the plasma of normotensive subjects (2), considering that the amount injected would be diluted in 20 mL of extracellular fluid per 100 g body weight. This dose produced an increase in blood pressure in hypertensive but not in normotensive animals, showing that hypertensive animals are more sensitive to ouabain (12).

Systolic (SAP) and diastolic (DAP) arterial pressures were monitored continuously. SAP was taken as an index of left ventricular function while DAP was taken as an index of peripheral resistance. After a 30-min stabilization period, the pressor reactivity protocol was performed by injecting increasing concentrations of phenylephrine in bolus (0.03 to 100 µg/kg) administered in small volumes (5 µL/100 g rat weight). After ouabain administration, all animals (N = 6) were followed for 1 h. SAP, DAP and heart rate (HR) were then measured again, and another dose-response curve to phenylephrine was constructed.

To ensure that the effects were not dependent on time, a time control experiment was performed. Saline (0.9%, *iv*) was administered to another group of rats under the same conditions. The stability of the protocol was confirmed by the maintenance of SAP and DAP (data not shown).

Ganglionic blockade

The ganglionic blocker hexamethonium (5 mg/kg) was used to investigate the modulation of the effects of ouabain by autonomic reflex control. To avoid a large drop of arterial pressure, only a partial ganglionic blockade was obtained with this concentration of hexamethonium. Due to the duration of the experiment two groups were used: one treated with hexamethonium and the other treated with hexamethonium plus ouabain. In the control group, after arterial pressure stabilization, a phenylephrine dose-response curve was constructed, and SAP, DAP and HR were measured. Next, hexamethonium was administered and after 30 min another concentration-response curve to phenylephrine was constructed. In the hexamethonium plus ouabain group, SAP, DAP and HR were measured after arterial pressure stabilization. Hexamethonium plus ouabain was then administered. After 60 min another phenylephrine dose-response curve was constructed, and SAP, DAP and HR were measured.

Na^+ pump blockade

To determine whether the effects of ouabain were caused by inhibition of the Na^+ pump activity, canrenone (1 mg/kg) a blocker of ouabain effects, was used because of its antagonistic properties at the digitalis receptor site (18,19). This small concentration of canrenone was used to avoid the changes induced by larger concentrations in myocardial contractility, vascular tone or arterial blood pressure, as previously reported (18).

Also, two groups were used, one treated with canrenone and the other treated with canrenone plus ouabain. The same protocol as described above for hexamethonium was performed using canrenone.

ACE and AT_1 blockade

In the last two protocols, the participation of the renin-angiotensin system was investigated by using the ACE inhibitor enalapril maleate (5 mg/kg), and losartan (10 mg/kg), which is an angiotensin type 1 (AT_1) receptor antagonist.

Due to the duration of the experiment, these protocols were performed with different groups of rats, one for losartan or enalapril maleate and the other for losartan or enalapril maleate plus ouabain. The same protocol as used for carenone was then performed using enalapril maleate or losartan.

Drugs used

Ouabain octahydrate, l-phenylephrine hydrochloride, hexamethonium hydrochloride, canrenone, losartan, enalapril maleate, and urethane were purchased from Sigma (USA), and heparin was purchased from Roche Pharmaceuticals (Brazil).

Statistical analysis

Data are reported as means ± SEM. Non-linear regression analysis for the determination of the maximum change in pressure (Emax) and the logarithm of the dose producing one-half of Emax (-log EC50 or pD_2) were employed to obtain fitting curves using the GraphPad Prism Software (USA). To compare the effect of different drugs on the response to phenylephrine, some results are reported as "differences of areas under the concentration-response curves" (dAUC) for control and experimental situations. The AUC was calculated from each concentration-response curve, and the differences are reported as a percentage of AUC for the corresponding control situation. Data were analyzed statistically by the Student *t*-test and ANOVA. When ANOVA showed a significant effect of treatment, the Tukey *post hoc* test was used to compare means. $P < 0.05$ was considered to be significant.

Results

Table 1 shows that 1 h after the administration of ouabain (*iv*), baseline SAP and DAP significantly increased. However, ouabain did not change HR. The systolic and diastolic pressure reactivity to phenylephrine did not change after ouabain administration, as shown in Figure 1. Tables 2 and 3 also show that Emax and pD_2 did not change.

To investigate whether the reflex activity involving the autonomic nervous system could mask the effects of ouabain, a ganglionic blocker was used. As expected, the administration of hexamethonium reduced baseline SAP and DAP, but these parameters increased after ouabain treatment (Table 1).

The effects on pressor reactivity were investigated using phenylephrine, a vascular smooth muscle α1-adrenoceptor agonist. Under control conditions, the concentration-response curves to phenylephrine after hexamethonium showed similar values of pD_2 and Emax for both SAP and DAP. After ouabain administration, the systolic pressure reactivity to phenylephrine did not change, but the diastolic sensitivity to phenylephrine increased (Figure 2, Tables 2 and 3). We observed an upward displacement of the concentration-response curve of DAP to phenylephrine after ouabain administration. Two-way randomized ANOVA, performed in order to evaluate the behavior of the concentration-response DAP curves, significance was found. Then, to better understand this effect, we generated dAUCs and observed a large increase in diastolic pressure reactivity to phenylephrine after treatment with hexamethonium plus ouabain (Figure 2C).

Canrenone, an Na^+ pump blocker, was used to evaluate the influence of the Na^+ pump on the actions of ouabain. Canrenone administration did not alter baseline SAP or DAP. One hour after ouabain administration, the SAP of rats previously treated with canrenone did not change,

Table 1. Baseline systolic and diastolic arterial pressure (SAP and DAP) and heart rate (HR) of all groups.

Groups	SAP (mmHg)	DAP (mmHg)	HR (bpm)
Ct (N = 6)	137 ± 5.1	93.7 ± 7.7	394 ± 10
Oua (N = 6)	150 ± 4.7*	116.0 ± 3.5*	381 ± 17
Ct (N = 6)	149 ± 5.2	103 ± 5.9	359 ± 11
Hexa (N = 6)	118 ± 4.9*	79 ± 7.4*	336 ± 12
Hexa + Oua (N = 6)	145 ± 4.4$^+$	103 ± 7.8$^+$	348 ± 10
Ct (N = 6)	140 ± 4.2	94.8 ± 4.4	341 ± 14
Can (N = 6)	146 ± 9.8	96.8 ± 7.3	362 ± 6
Can + Oua (N = 6)	155 ± 2.7	114.0 ± 4.2*$^+$	362 ± 8
Ct (N = 6)	137 ± 3.9	77.0 ± 5.0	330 ± 9
Ena (N = 6)	115 ± 5.3*	50.0 ± 1.9*	326 ± 10
Ena + Oua (N = 6)	122 ± 6.4	49.5 ± 3.4*	330 ± 13
Ct (N = 6)	128 ± 4.2	72.5 ± 4	332 ± 13
Los (N = 6)	113 ± 2.9*	57.1 ± 5*	337 ± 18
Los + Oua (N = 6)	110 ± 3.7*	53.6 ± 4.9*	334 ± 13

Ct = measurements made under control conditions; Oua = measurements made 1 h after ouabain administration; Hexa = hexamethonium; Can = canrenone; Ena = enalapril maleate; Los = losartan. Data are reported as means ± SEM. *$P < 0.05$ *vs* its control value. $^+P < 0.05$ Hexa + Oua *vs* Hexa; Can + Oua *vs* Can (one-way randomized ANOVA).

but the DAP increased (Table 1). In ouabain-untreated rats, canrenone did not alter the pD_2 and Emax of the SAP and DAP concentration-response curves to phenylephrine. Ouabain administration also did not change the pD_2 and Emax of the SAP concentration-response curves to phenylephrine (Tables 2 and 3). However, we observed

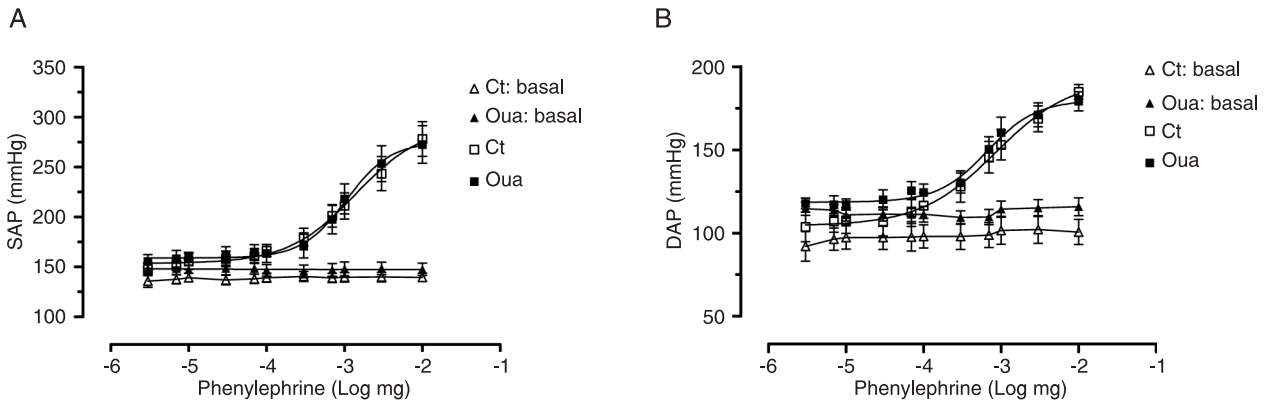

Figure 1. Changes in systolic (A) and diastolic (B) arterial pressure (SAP and DAP), and basal pressure in the dose-response curve for phenylephrine before (Ct; N = 6) and 1 h after ouabain (Oua; N = 6) administration. Data are reported as means ± SEM (Student *t*-test).

Table 2. Maximal response (Emax) and sensitivity (pD_2) of systolic arterial pressure concentration-response curves to phenylephrine of all groups.

Groups	Emax (mmHg)	pD_2
Ct (N = 6)	278 ± 17.3	2.20 ± 0.7
Oua (N = 6)	273 ± 18.9	2.95 ± 0.05
Ct (N = 6)	276 ± 18.0	2.80 ± 0.09
Hexa (N = 6)	285 ± 12.7	2.89 ± 0.07
Hexa + Oua (N = 6)	294 ± 10.8	2.97 ± 0.14
Ct (N = 6)	305 ± 7	2.7 ± 0.06
Can (N = 6)	301 ± 8.2	2.9 ± 0.05
Can + Oua (N = 6)	269 ± 19	2.8 ± 0.04
Ct (N = 6)	285 ± 2.3	2.78 ± 0.08
Ena (N = 6)	269 ± 5.4*	2.86 ± 0.2
Ena + Oua (N = 6)	269 ± 3.8*	2.19 ± 0.21^{+}
Ct (N = 6)	281 ± 8.1	2.7 ± 0.15
Los (N = 6)	275 ± 7.7	2.5 ± 0.10
Los + Oua (N = 6)	246 ± 6.9*$^{+}$	2.7 ± 0.06

Ct = measurements made under control conditions; Oua = measurements made 1 h after ouabain administration; Hexa = hexamethonium; Can = canrenone; Ena = enalapril maleate; Los = losartan. Data are reported as means ± SEM. *P < 0.05 *vs* its control value. $^{+}$P < 0.05 Los + Oua *vs* Los; Ena + Oua *vs* Ena (one-way randomized ANOVA).

Table 3. Maximal response (Emax) and sensitivity (pD_2) of diastolic arterial pressure concentration-response curves to phenylephrine in all groups.

Groups	Emax (mmHg)	pD_2
Ct (N = 6)	185 ± 4.4	2.78 ± 0.42
Oua (N = 6)	179 ± 5.8	3.09 ± 0.13
Ct (N = 6)	179 ± 12.8	2.95 ± 0.11
Hexa (N = 6)	185 ± 8.7	3.06 ± 0.13
Hexa + Oua (N = 6)	192 ± 6.0	3.34 ± 0.12*
Ct (N = 6)	184 ± 7.3	3.0 ± 0.28
Can (N = 6)	197 ± 4.8	3.1 ± 0.06
Can + Oua (N = 6)	186 ± 6	3.0 ± 0.07
Ct (N = 6)	176 ± 5.5	2.20 ± 0.70
Ena (N = 6)	155 ± 9.4	2.45 ± 0.57
Ena + Oua (N = 6)	157 ± 7.1	2.51 ± 0.15
Ct (N = 6)	173 ± 7.5	2.6 ± 0.52
Los (N = 6)	162 ± 5.7	2.9 ± 0.09
Los + Oua (N = 6)	159 ± 9.9	2.8 ± 0.11

Ct = measurements made under control conditions; Oua = measurements made 1 h after ouabain administration; Hexa = hexamethonium; Can = canrenone; Ena = enalapril maleate; Los = losartan. Data are reported as means ± SEM. *P < 0.05 *vs* its control value (one-way randomized ANOVA).

a marked difference in the DAP concentration-response curves between groups when the first six phenylephrine concentrations were used (Figure 3B). We then performed a two-way randomized ANOVA to evaluate the behavior of the whole concentration-response DAP curves, which revealed significance. The effects of the first six phenylephrine concentrations were then appreciated by comparing the three groups (control, canrenone and canrenone plus ouabain) using dAUC. It was observed that stimulation with low concentrations of phenylephrine induced increased diastolic pressure reactivity after treatment with canrenone plus ouabain (Figure 3C).

Enalapril and losartan were used to investigate the effect of the renin-angiotensin system on the actions of ouabain on blood pressure and pressure reactivity to phenylephrine. Administration of both drugs reduced the baseline SAP and DAP. Ouabain administration to rats previously treated with enalapril or losartan did not change the baseline SAP and DAP, suggesting that AT_1 blockade or ACE inhibition abolished the increase in SAP and DAP produced by ouabain (Table 1). Losartan administration did not change pressure reactivity to phenylephrine for SAP and DAP. However, after ouabain administration, losartan produced a downward displacement of the whole concentration-response curve of SAP and DAP to phenylephrine. For SAP, Emax was reduced without changing pD_2 (Figure 4A, Table 2) and for DAP, these parameters did not change (Figure 4B, Table 3). dAUCs were used to analyze the whole effects of losartan and losartan plus ouabain, suggesting a reduction of the systolic and diastolic pressure reactivity to phenylephrine after treatment with the combination of the two drugs (Figure 4C,D).

When evaluating SAP, we observed that enalapril reduced Emax under non-treated conditions but did not change the pD_2 to phenylephrine. Following ouabain administration, pD_2 was also reduced when compared to the non-treated condition while Emax was more reduced (Figure 5A, Table 2). For DAP, these parameters, Emax and pD_2, did not show significant changes (Figure 5B, Table 3). However, when observing the behavior of the curves, we noticed a downward displacement in the concentration-response curve for DAP to phenylephrine after ouabain administration. With dAUC generation, we could observe a reduction of DAP reactivity to phenylephrine after treatment with enalapril plus ouabain, which was similar to the behavior of the losartan plus ouabain group (Figure 5C,D), but not for SAP.

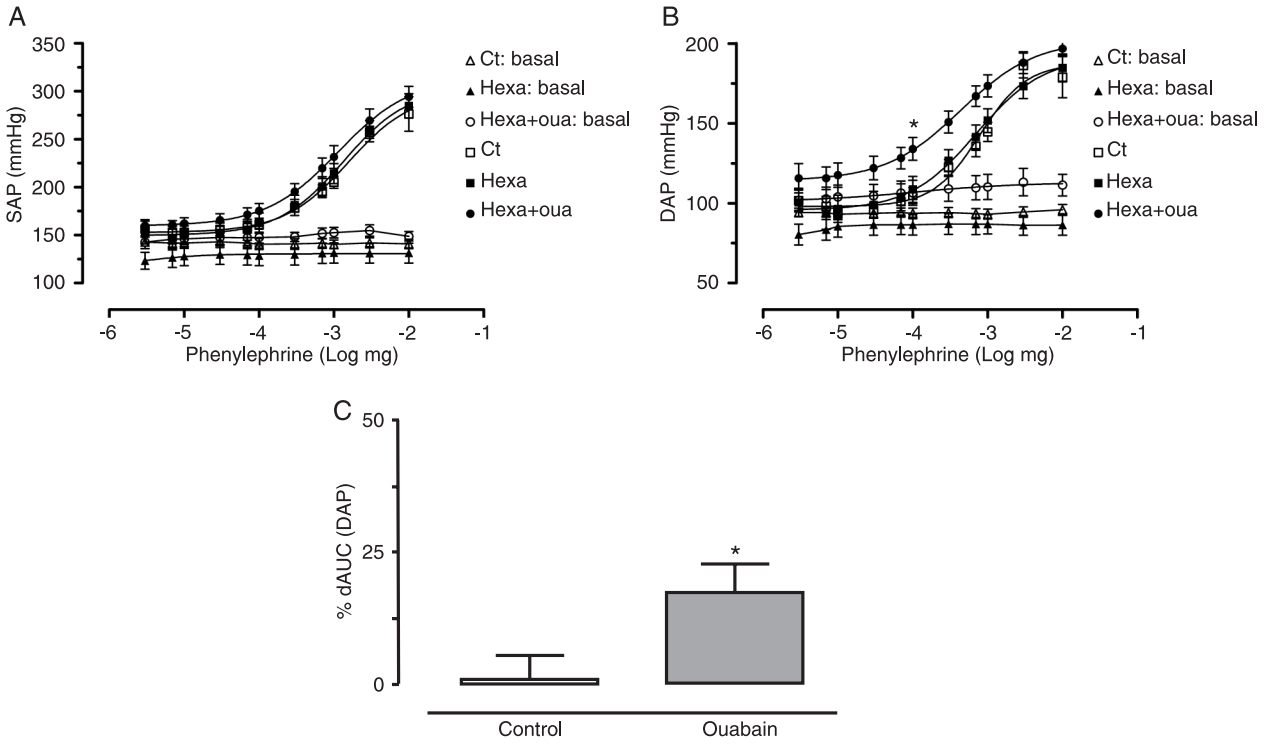

Figure 2. Dose-response curve of systolic (A) and diastolic (B) arterial pressure (SAP and DAP) and basal pressure to phenylephrine before (Ct; N = 6) and after hexamethonium (Hexa; N = 6) and after hexamethonium plus ouabain (Hexa + Oua; N = 6) administration. *P < 0.05 for Hexa + Oua *vs* Ct (one-way randomized ANOVA). *Panel C* shows differences in area under the concentration-response curve (dAUC) of DAP to phenylephrine before and after hexamethonium in control and ouabain-treated rats. Data are reported as means ± SEM. *P < 0.05 *vs* Control (Student *t*-test).

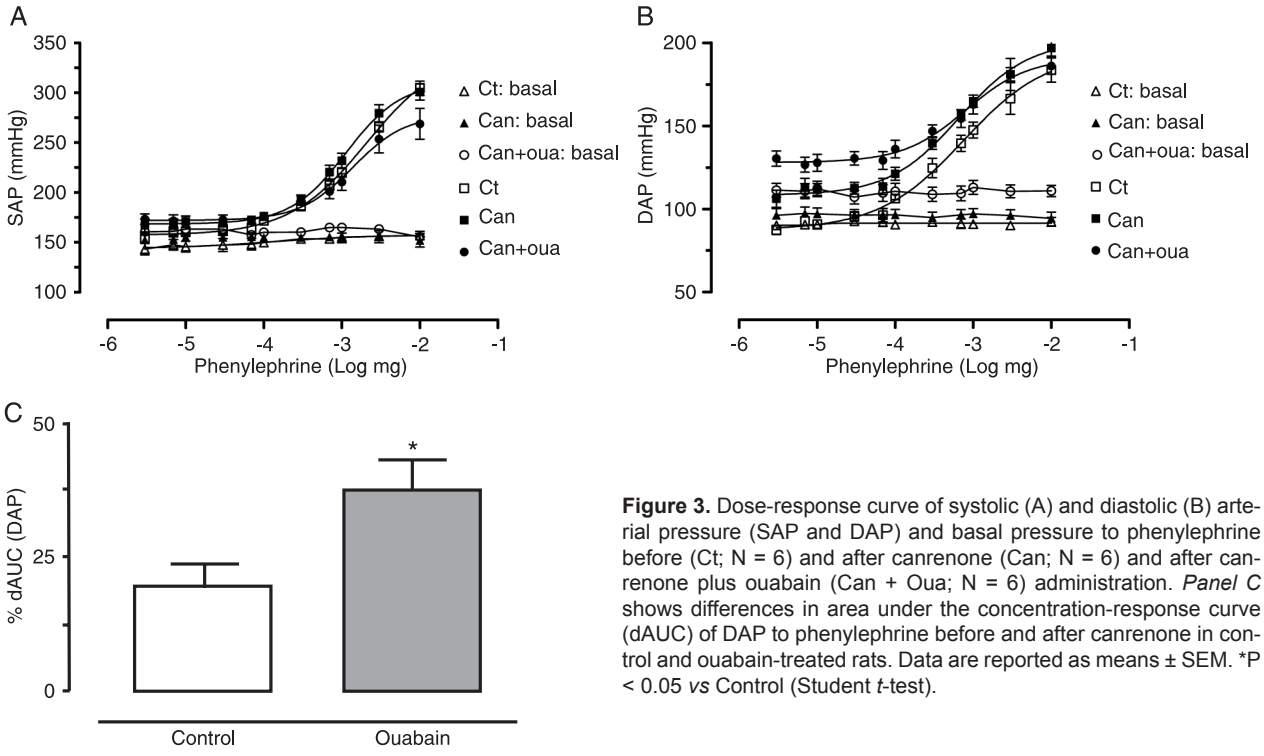

Figure 3. Dose-response curve of systolic (A) and diastolic (B) arterial pressure (SAP and DAP) and basal pressure to phenylephrine before (Ct; N = 6) and after canrenone (Can; N = 6) and after canrenone plus ouabain (Can + Oua; N = 6) administration. *Panel C* shows differences in area under the concentration-response curve (dAUC) of DAP to phenylephrine before and after canrenone in control and ouabain-treated rats. Data are reported as means ± SEM. *P < 0.05 *vs* Control (Student *t*-test).

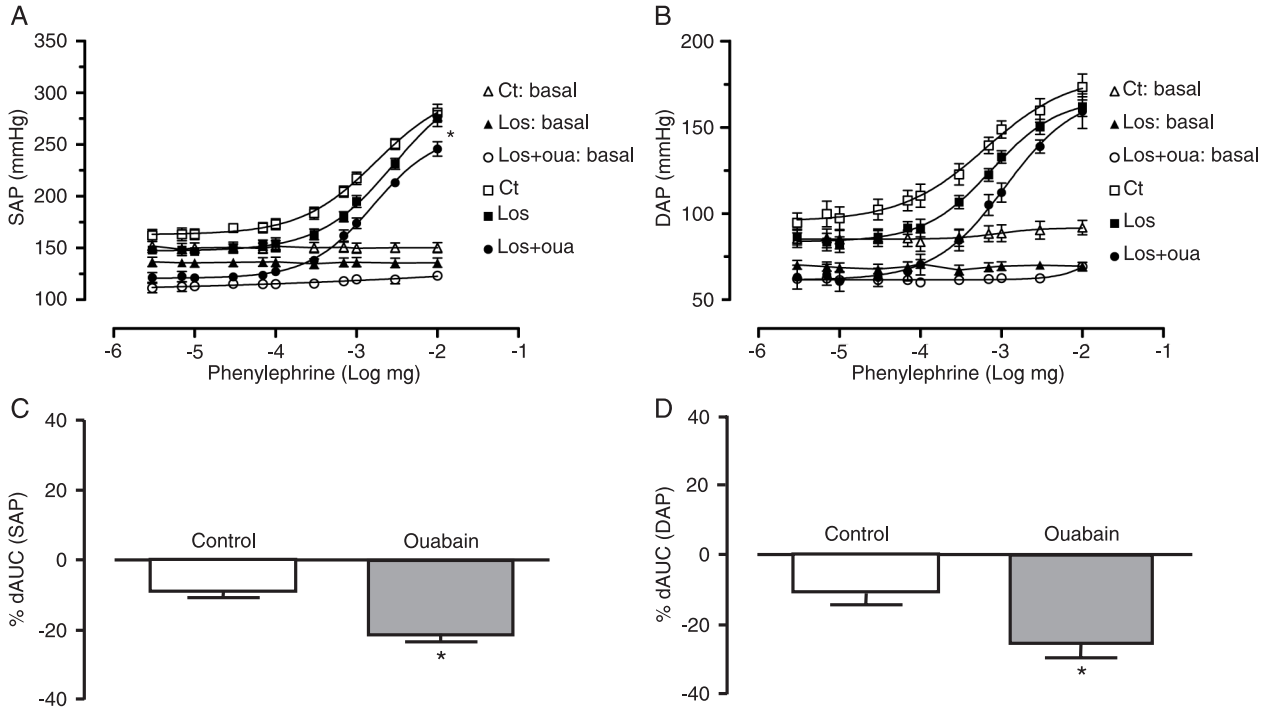

Figure 4. Dose-response curve of systolic (A) and diastolic (B) arterial pressure (SAP and DAP) and basal pressure to phenylephrine before (Ct; N = 6) and after losartan (Los; N = 6) and after losartan plus ouabain (Los + Oua; N = 6) administration. *P < 0.05 Los + Oua *vs* Ct and *vs* Los (one-way randomized ANOVA). *Panels C* and *D* show differences in area under the concentration-response curve (dAUC) of SAP (C) and DAP (D) to phenylephrine before and after losartan in control and ouabain-treated rats. Data are reported as means ± SEM. *P < 0.05 *vs* Control (Student *t*-test).

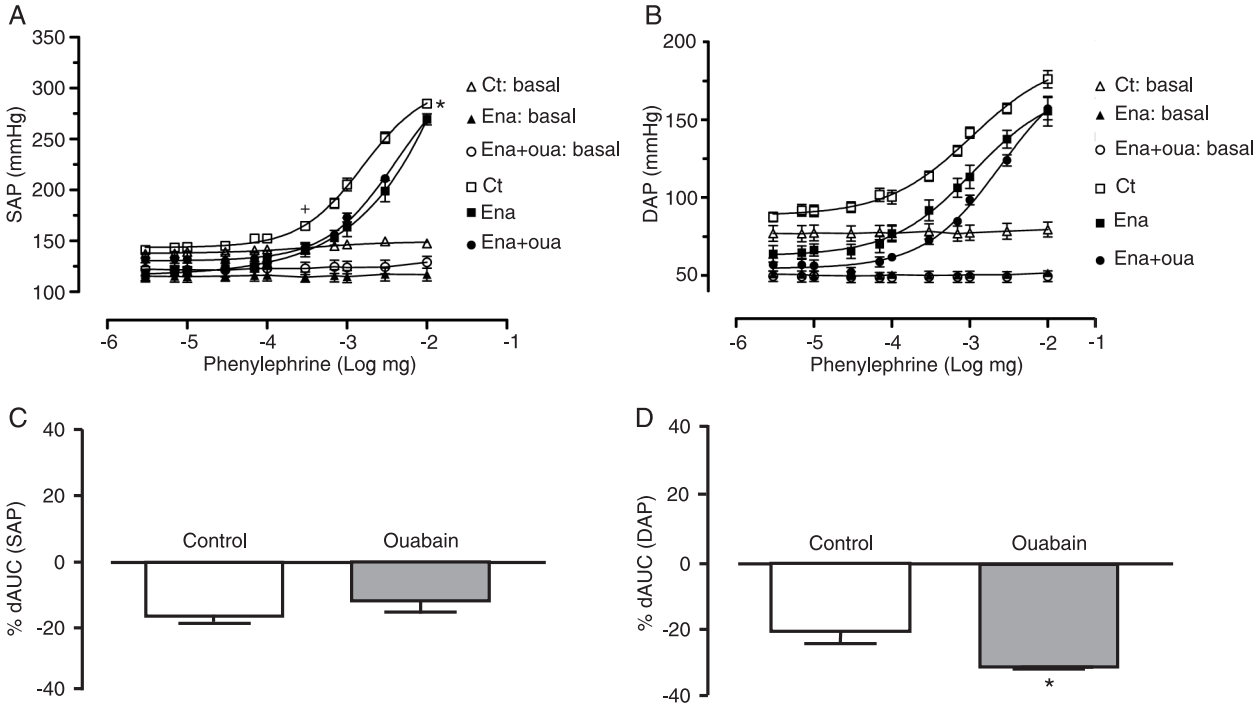

Figure 5. Dose-response curve of systolic (A) and diastolic (B) arterial pressure (SAP and DAP) and basal pressure to phenylephrine before (Ct; N = 6) and after enalapril maleate (Ena; N = 6) and after enalapril maleate plus ouabain (Ena + Oua; N = 6) administration. *P < 0.05 Ct *vs* Ena and *vs* Ena + Oua, $^{+}$P < 0.05 Ena + Oua *vs* Ena (one-way randomized ANOVA). *Panels C* and *D* show differences in area under the concentration-response curve (dAUC) of SAP (C) and DAP (D) to phenylephrine before and after enalapril maleate in control and ouabain-treated rats. Data are reported as means ± SEM. *P < 0.05 *vs* Control (Student *t*-test).

Discussion

The results presented here suggest that the acute administration of a small concentration of ouabain enhances hypertension in SHR, increasing systolic and diastolic arterial pressure without altering the pressor reactivity to phenylephrine. This increase in blood pressure seems to depend on ouabain inhibiting the Na^+ pump, which increases systolic pressure, and activating the local renin-angiotensin system, which increases diastolic pressure. The lack of the effect of ouabain on phenylephrine pressor reactivity seems to depend on central mechanisms that might blunt these changes in pressor reactivity. It is important to emphasize that anesthesia with urethane reduces systolic and diastolic blood pressure in SHR, but when compared with normotensive animals, the blood pressure levels of these animals were still elevated, as previously reported by our group (9,12,17).

Previous reports have shown that nanomolar concentrations resulting from acute ouabain administration increased the blood pressure of SHR, of rats with renovascular hypertension and rats with L-NAME-induced hypertension, whereas no change in blood pressure was observed in normotensive rats (9,12). This occurs also with chronic ouabain treatment, as observed by Xavier et al. (20). The cited study demonstrated that ouabain treatment for 5 weeks increased the blood pressure of SHR but not of normotensive rats (20). Our results show that acute administration of a small concentration of ouabain enhances SHR hypertension. The increase in baseline systolic pressure might result from the classic effect of ouabain promoting a positive inotropic effect by inhibiting the Na^+ pump (11,21) and/or increasing the sympathetic tone (14). The increase in baseline diastolic pressure suggests that ouabain increases pressor reactivity to vasoconstrictor agents and, consequently, the peripheral vascular resistance, as previously demonstrated (12,17,22).

Since normal plasma concentrations of ouabain are in the nanomolar range (2,23), the purpose of the present study was to investigate the mechanism(s) of ouabain action on arterial pressure using a dose that might generate a blood concentration near the physiological range by dilution in 20 mL of extracellular fluid per 100 g body weight.

In the present study, ouabain increased the systolic and diastolic arterial pressures. Therefore, we investigated some possibilities to explain these effects. Given that ouabain might induce hypertension by central mechanisms, we first investigated the possible influence of autonomic reflex

modulation via ganglionic blockade with hexamethonium. The central mechanism is associated with an increased sympathetic tone and resetting of the baroreceptor reflex (14). Considering that sympathetic hyperactivity and hypertension induced by ouabain are prevented by AT_1 and endothelin-1 (ET_1) receptor blockade (16,24), the central renin-angiotensin and endothelin systems are suggested to be the sympathoexcitatory components involved in the hypertensinogenic effect of ouabain. At the same time, previous reports have shown that in reflex-blocked rats hexamethonium prevents the hypertension induced by chronic ouabain treatment (25). However, our findings showed that the ouabain effect of increasing the baseline arterial pressure was not altered after ganglionic blockade with hexamethonium, suggesting a peripheral effect. The present findings agree with results reported by Barker et al. (13) who demonstrated that acute administration of small concentrations of ouabain increases the blood pressure of normotensive rats, and this effect is not modulated by autonomic reflexes but rather depends on ouabain actions on sympathetic nerve endings and on vascular smooth muscle. These observations suggest that under ganglionic blockade the increase of baseline systolic and diastolic arterial pressures evoked by ouabain is not mediated by central nervous system mechanisms.

However, when we tested the pressor reactivity to phenylephrine, we observed that co-treatment with hexamethonium plus ouabain increased the diastolic pressure sensitivity but not the systolic pressure reactivity to phenylephrine. These findings suggest that in conditions in which the autonomic reflexes are functional, central mechanisms might modulate ouabain actions during phenylephrine administration.

Previous reports have shown a putative association between ouabain and the genesis or development and maintenance of arterial hypertension (8-10). The possible explanation for this association is that ouabain binds to the α-subunit of the Na^+ pump, inhibiting its activity (11). However, the underlying mechanisms by which ouabain elevates blood pressure have not been completely described. A recent study showed that chronic ouabain administration induces up-regulation of the α2 Na^+ pump and Na^+/Ca^{2+} exchanger type 1 expression in arterial smooth muscle, probably trying to compensate for the ouabain-induced reduction in sodium pump activity (26). This increased Ca^{2+} signaling may explain the augmented myogenic responses and enhanced phenylephrine-induced vasoconstriction in arteries from ouabain-induced hypertensive rats, as well as the high blood pressure (26).

Thus, given that ouabain has the ability to inhibit the Na^+ pump in several tissues, including vascular smooth muscle (22), this could explain its pressor effects on baseline diastolic pressure. We then investigated whether the ouabain pressor effects are related to the inhibitory actions on the Na^+ pump. Previous reports have suggested that canrenone interacts with the digitalis receptor site of the Na^+ pump and antagonizes the binding of ouabain (19,27). Indeed, acute administration of canrenone blocks the positive inotropic effect of ouabain in isolated perfused hearts (18). To clarify this issue, we first tested the effects of canrenone or canrenone plus ouabain on arterial pressure. The present findings show that canrenone abolished the pressor effect of ouabain on the systolic pressure, suggesting that this effect is associated with inhibition of the Na^+ pump.

In contrast, canrenone did not abolish the pressor effect of ouabain on diastolic pressure. This is the first suggestion that ouabain may have a mechanism of action on diastolic pressure that is not dependent on the Na^+ pump. Previous reports have demonstrated that ouabain and a series of structurally related analogs have hypertensinogenic activity, regardless of their potency as Na^+ pump inhibitors (28). An additional explanation by Ward et al. (29) suggested that adrenocortical cells express ouabain receptors that are distinct from the Na^+ pumps. These findings could explain why the pressor effect of ouabain on the baseline DAP seems not to be dependent on its action on the Na^+ pump.

We also tested the effects of canrenone on the pressor reactivity to phenylephrine of diastolic and systolic pressure. When analyzing dAUCs for diastolic pressure, we observed that, after the administration of canrenone plus ouabain, the effects on diastolic pressure were enhanced. This finding suggests that the actions of ouabain on diastolic pressure are not dependent on binding to the Na^+ pump. This finding also reinforces our results regarding the increase in baseline diastolic pressure, since, in spite of the blockade of the Na^+ pump by canrenone, ouabain continued to exert its pressor effect.

In addition, studies from our laboratory have demonstrated that acute administration of low ouabain concentrations stimulates local ACE activity and increases angiotensin II release in the tail vascular bed of SHR (17). On the other hand, the central mechanism already described suggests that ouabain stimulates the brain renin-angiotensin system, leading to the production of angiotensin II that further increases sympathetic tone and ultimately increases arterial blood pressure (14). Indeed, it has been reported that acute central administration of ouabain causes sympathoexcitatory and pressor effects in rats (30). In addition, the acute hypertensinogenic effect of ouabain is attenuated in transgenic rats that are deficient in brain angiotensinogen (31).

The participation of the renin-angiotensin system was then investigated, and the administration of enalapril and losartan abolished the effect of ouabain on baseline arterial pressure. Therefore, our results suggest that to increase baseline systolic pressure ouabain also stimulates the renin-angiotensin system. Thus, an operational renin-angiotensin system is necessary for the effect of ouabain. Both findings, which involved the Na^+ pump and renin-angiotensin system, might explain the increase in baseline systolic pressure

induced by ouabain.

Regarding the baseline diastolic pressure, the action of ouabain could be mediated only by the stimulation of the renin-angiotensin system (14,17). The release of angiotensin II may act on vascular smooth muscle, promoting vasoconstriction and increasing peripheral resistance and, consequently, the diastolic pressure (32). Considering that ouabain stimulates angiotensin II release (15,17) and increases sympathoexcitatory activity (14), the sharp reduction of the pressor reactivity to phenylephrine after blockade of the renin-angiotensin system could be explained by a reduced stimulation of the sympathetic nervous system.

Although no changes in phenylephrine reactivity were observed, after pharmacological interventions, it was possible to show the participation of the renin-angiotensin system, the Na^+ pump and the autonomic reflexes in the effects of ouabain. In addition, other mechanisms, possibly central ones, might blunt ouabain actions when phenylephrine concentration-response curves are constructed. Regarding the changes in baseline blood pressure, the effect of ouabain on SAP seems to be dependent on both inhibition of the Na^+ pump and activation of the renin-angiotensin system. The effect of ouabain on DAP, reflecting the peripheral resistance, seems to be dependent only on the renin-angiotensin system.

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