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Effects of small doses of ouabain on the arterial blood pressure of anesthetized hypertensive and normotensive rats

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Abstract

Ouabain increases vascular resistance and may induce hypertension by inhibiting the Na^+ pump. The effects of 0.18 and 18 µg/kg, and 1.8 mg/kg ouabain pretreatment on the phenylephrine (PHE; 0.1, 0.25 and 0.5 µg, in bolus)-evoked pressor responses were investigated using anesthetized normotensive (control and uninephrectomized) and hypertensive (1K1C and DOCA-salt treated) rats. Treatment with 18 µg/kg ouabain increased systolic and diastolic blood pressure in all groups studied. However, the magnitude of this increase was larger for the hypertensive 1K1C and DOCA-salt rats than for normotensive animals, while the pressor effect of 0.18 µg/kg ouabain was greater only in DOCA-salt rats. A very large dose (1.8 mg/kg) produced toxic effects on the normotensive control but not on uninephrectomized or 1K1C rats. Rat tail vascular beds were perfused to analyze the effects of 10 nM ouabain on the pressor response to PHE. In all animals, 10 nM ouabain increased the PHE pressor response, but this increase was larger in hypertensive DOCA-salt rats than in normotensive and 1K1C rats. Results suggested that a) increases in diastolic blood pressure induced by 18 µg/kg ouabain were larger in hypertensive than normotensive rats; b) in DOCA-salt rats, smaller ouabain doses had a stronger effect than in other groups; c) hypertensive and uninephrectomized rats were less sensitive to toxic doses of ouabain, and d) after treatment with 10 nM ouabain isolated tail vascular beds from DOCA-salt rats were more sensitive to the pressor effect of PHE than those from normotensive and 1K1C hypertensive rats. These data suggest that very small doses of ouabain, which might produce nanomolar plasma concentrations, enhance pressor reactivity in DOCA-salt hypertensive rats, supporting the idea that endogenous ouabain may contribute to the increase and maintenance of vascular tone in hypertension.

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Key words
- Pressor reactivity
- Ouabain
- Phenylephrine
- 1K1C rats
- DOCA-salt
- Uninephrectomy

Introduction

Previous reports have shown that ouabain pretreatment of normotensive rats enhances arterial blood pressure and vasopressor responses to vasoconstrictor agents *in vivo* and *in vitro* (1,2). *In vivo*, the sensitization induced by ouabain was also observed in spontaneously hypertensive rats (SHR) (1). However, the increment of pressor responses showed a difference between normotensive rats and SHR. The SHR presented enhanced pressor responses after administration of very low doses of ouabain, which

were not enough to induce the same effect in normotensive rats (1).

In vitro, we also demonstrated that both 100 μM and 10 nM ouabain enhance the vasoactive responses elicited by phenylephrine in rat tail arteries (1,2). In these preparations the enhancement of the vasoconstrictor responses to phenylephrine was greater in SHR (1).

The sensitization produced by ouabain is caused by the inhibition of the Na^+ pump (3). A reduction in Na^+ pump activity leads to Na^+ accumulation in the myoplasm (4). The Na^+ increment affects the Na^+/Ca^{2+} exchange mechanism and consequently increases the amount of activator Ca^{2+} necessary for contraction of vascular smooth muscle (4,5). Therefore, disturbances in such mechanisms can produce contractile changes, which may be relevant for arterial blood pressure homeostasis. Indeed, the consequence of the inhibition of the sarcolemmal Na^+ pump is an enhanced responsiveness of vascular smooth muscle to constrictor agents (2).

It is now known that all three isoforms of the Na^+,K^+-ATPase α subunit have distinct sensitivities for cardiac glycosides and all are expressed in rat tail arteries and aorta (6). The presence of high-affinity isoforms in the vasculature raises the possibility that, even at low levels, endogenous ouabain may act as a modulator increasing tone or pressor responses to endogenous vasoactive agents (5). This possibility is reinforced by the description of ouabain as an endogenous compound existing in human plasma (7) and in several other animal species (8,9).

The purpose of the present study was to determine whether ouabain at concentrations similar to the endogenous ones can increase vascular tone and enhance phenylephrine-induced vasoconstriction *in vivo* and *in vitro* in normotensive control, uninephrectomized, hypertensive one kidney, one clip (1K1C) rats and in deoxycorticosterone acetate (DOCA)-salt rats.

Material and Methods

Experimental animals

Studies were performed on 129 male Wistar rats weighing 200 to 380 g, divided into five main groups: 22 control rats (normotensive rats), 58 uninephrectomized rats, 21 DOCA-salt-treated rats, and 28 1K1C rats. The care and use of the laboratory animals were in accordance with NIH guidelines. All rats had free access to water and rat chow *ad libitum*.

Hypertension models

Renovascular hypertension. With rats under ether anesthesia, renovascular hypertension (Goldblatt 1K1C model) was produced by application of a silver clip (0.2-mm internal diameter) to the left renal artery, combined with nephrectomy of the right kidney through an abdominal incision. Weight-matched control rats were uninephrectomized and submitted to right-side nephrectomy without the clip application. After these procedures, muscle layers were reconnected, and the skin incision was closed. Rats were placed in plastic cages with supplies of water and chow for 30 days.

DOCA-salt hypertension. Male 45-day-old rats were uninephrectomized under ether anesthesia. Four days later the animals were treated with either DOCA (8 mg/kg twice a week) or vehicle (soybean oil, 0.25 ml/animal). This treatment was repeated twice a week for 30 days. The vehicle-treated uninephrectomized rats drank water and the DOCA-salt rats drank saline solution (0.9% NaCl) during the treatment.

The day before the experiments the systolic blood pressure of all rats was measured using a tail-cuff method (IITC Model 29 Pulse Amplifier, IITC Inc., Santa Barbara, CA, USA). Animals which did not develop hypertension were excluded.

Anesthetized animals

At the end of the treatments, rats were anesthetized with urethane (1.8 g/kg, *ip*), 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 polygraphic recorder (RG 300, FUNBEC). ECG was also measured using standard procedures.

The following protocol was used for all groups. Pressor reactivity was investigated by measuring arterial blood pressure and by injecting (*iv*) increasing doses of phenylephrine (0.1, 0.25 and 0.5 µg) administered in small volumes (5 µl or less) before and 1 h after ouabain treatment. All groups of rats were divided into three subgroups and each subgroup was treated with either 0.18 µg/kg, 18 µg/kg or 1.8 mg/kg ouabain. Ouabain treatments were employed based on the human loading doses, but taking into consideration that the rat is more resistant to ouabain. In humans, acute digitalization with ouabain is obtained with 4 to 5 µg/kg. We then administered ouabain covering the range of toxic (1.8 mg/kg) and non-toxic doses (18 µg/kg), including a very small dose (0.18 µg/kg) which is expected to produce plasma concentrations at the nanomolar level. Systolic and diastolic blood pressure and the ECG were monitored continuously for heart rate evaluation.

Isolated rat tail vascular bed preparation. Isolated rat tail vascular beds were used in this study as previously reported (10). Briefly, the rats were anesthetized with sodium pentobarbital (65 mg/kg, *ip*) and heparin (500 IU, *ip*) was administered after loss of the righting reflex. Ten minutes after the administration of heparin, 1 cm of the tail artery was dissected free and cannulated with an intracath (Gelco 24) near the base of the tail. The vascular bed was flushed with Krebs-Henseleit buffer (KHB) (120 mM NaCl, 5.4 mM KCl, 1.2 mM $MgCl_2$, 1.25 mM $CaCl_2$, 2.0 mM NaH_2PO_4, 27 mM $NaHCO_3$, 11 mM glucose, and 0.03 mM EDTA) 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 with a pressure transducer (TP-200T, Nihon-Kohden connected to an MP-100 pressure amplifier, FUNBEC) and the data were recorded using an interface and software for computer data acquisition, BIOPAC MP100A (Biopac System, Inc., Santa Barbara, CA, USA) with a sample rate of 500 Hz per channel. Because a constant flow was maintained, changes in the perfusion pressure represented changes in vascular resistance. The following protocol was used.

After 30 min stabilization bolus injections of increasing doses of phenylephrine (0.5, 1, 2, 5 and 10 µg, in 100 µl) were administered in the absence or in the presence of 10 nM ouabain. The second dose-response curve was constructed 1 h after continuous infusion of KHB containing 10 nM ouabain. At the end of the experiment, preparations were contracted by continuous infusion of phenylephrine (100 nM) and endothelial integrity was tested using bolus injection of acetylcholine (5 µg in 100 µl). Endothelial integrity of the preparations was considered adequate when relaxation in response to acetylcholine attained 50% or more. This protocol was performed in preparations from normotensive (control, N = 6, and uninephrectomized, N = 8) and hypertensive (1K1C, N = 7, and DOCA-salt, N = 7) rats.

Statistical analysis

Results are reported as means ± SEM.

Results regarding perfusion pressure measurements are presented as changes in the mean perfusion pressure calculated by subtracting peak pressure from baseline pressure. Data were analyzed by the Student *t*-test and ANOVA. When the ANOVA showed a significant treatment effect, the Tukey *post hoc* test was used to compare the different treatment groups. The level of significance was set at $P<0.05$.

Drugs and reagents used

Deoxycorticosterone acetate, urethane, L-phenylephrine hydrochloride, acetylcholine chloride, and ouabain octahydrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Heparin was purchased from Roche (São Paulo, SP, Brazil) and pentobarbital sodium was purchased from Fontoverter (Itapira, SP, Brazil).

Results

On the day before the experiments the systolic blood pressure was measured in awake unrestrained animals and the DOCA-salt and 1K1C hypertension was confirmed (Table 1). The systolic blood pressure measured after 30 days was higher in 1K1C than in DOCA-salt rats.

Anesthetized animals

After urethane anesthesia, systolic and diastolic blood pressure were reduced in all models and this reduction was significantly larger in the 1K1C and DOCA-salt groups (Table 1).

Figure 1 shows the time-course changes of systolic blood pressure for 1 h after 18 µg/kg ouabain administration (*iv*) in all groups. Between 1 to 3 min after injection, systolic blood pressure increased and then decreased back to control values (approximately 15 to 20 min) after which it increased slowly again. It is interesting to note that this systolic blood pressure increment became significant only after 40 min. Heart rate did not change after ouabain treatment and the changes produced by phenylephrine injections were similar both before and after ouabain (data not shown).

The administration of 18 µg/kg ouabain caused a similar increase in systolic blood pressure after 1 h in all groups studied (Figure 2). Diastolic blood pressure did not change significantly after this dose of ouabain in control rats but increased in the other groups, including uninephrectomized rats (Figure 3). Analysis of the changes in diastolic pressure before and 1 h after 18 µg/kg ouabain showed that hypertensive rats had a significant enhancement of diastolic pres-

Table 1. Systolic (SBP) and diastolic (DBP) blood pressure in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) awake and anesthetized rats.

	Awake rats	Anesthetized rats	
	SBP (mmHg)	SBP (mmHg)	DBP (mmHg)
CT	127 ± 3.5	101 ± 1.8	74 ± 2.6
UNR	126 ± 2.3	98 ± 1.8	72 ± 1.9
1K1C	184 ± 6.7$^{+\#}$	104 ± 3.5$^{\#*}$	76 ± 3.6$^{\#}$
DOCA-salt	141 ± 6.5^{+}	94 ± 2.3^{+*}	60 ± 2.3^{+}

$^{+}P<0.01$, comparison between hypertensive and normotensive rats;
$^{\#}P<0.01$, comparison between 1K1C and DOCA-salt rats;
$^{*}P<0.05$, comparison between hypertensive anesthetized and awake rats (*t*-test).

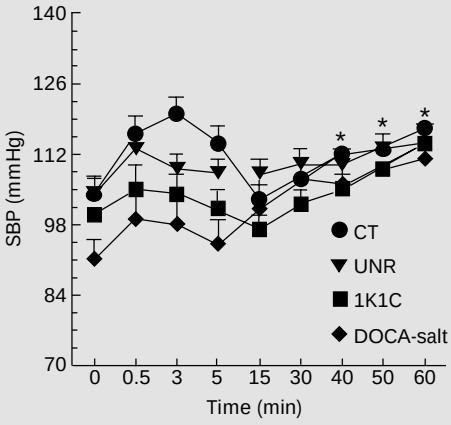

Figure 1. Time course of systolic blood pressure (SBP) changes during 1-h treatment with 18 µg/kg ouabain in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats after anesthesia. Points represent the means ± SEM. *P<0.05 compared to before ouabain treatment (time zero) for all groups (ANOVA).

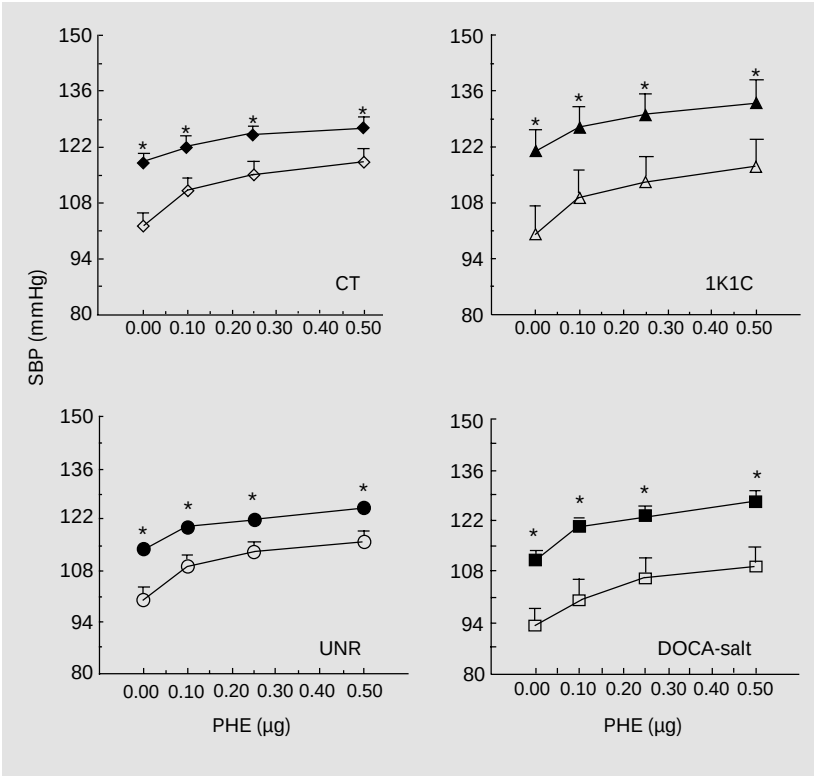

Figure 2. Changes in systolic blood pressure (SBP) in response to increasing doses of phenylephrine (PHE) in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats before (open symbols) and after (closed symbols) treatment with 18 µg/kg ouabain. Points represent the means ± SEM. *P<0.05 compared to before ouabain treatment (ANOVA).

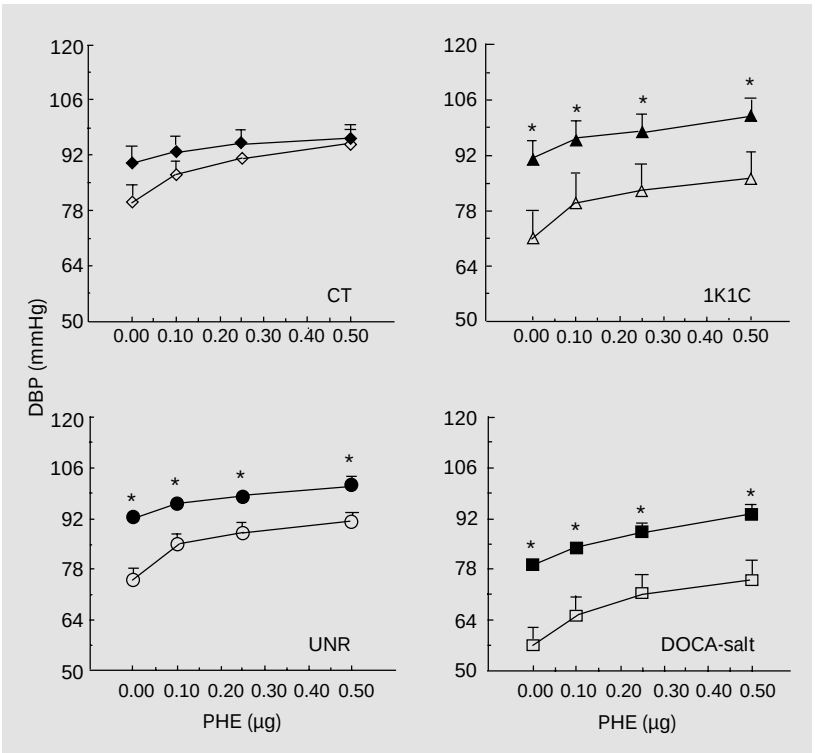

Figure 3. Changes in diastolic blood pressure (DBP) in response to increasing doses of phenylephrine (PHE) in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats before (open symbols) and after (closed symbols) treatment with 18 µg/kg ouabain. Points represent the means ± SEM. *P<0.05 compared to before ouabain treatment (ANOVA).

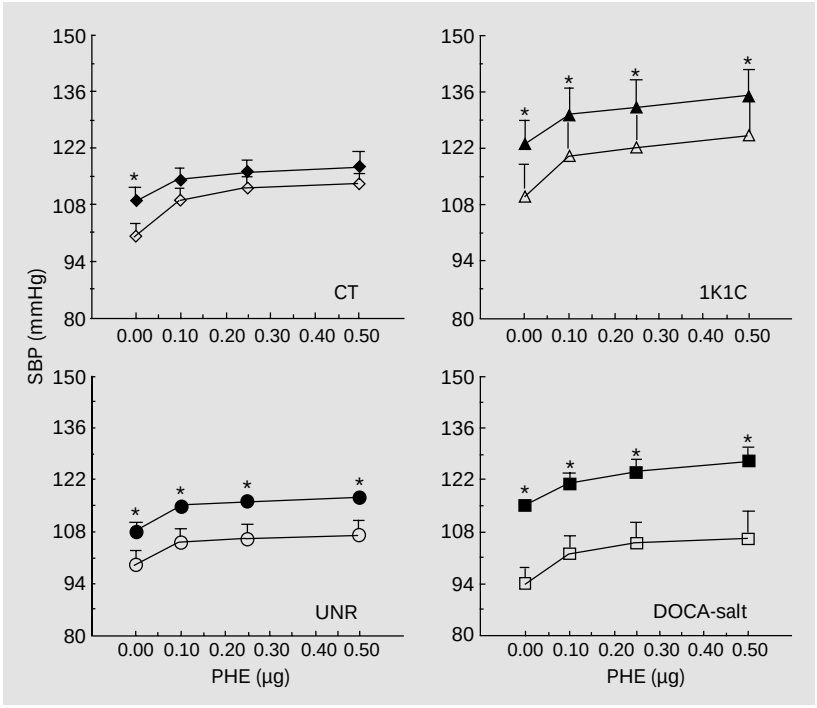

Figure 4. Changes in systolic blood pressure (SBP) in response to increasing doses of phenylephrine (PHE) in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats before (open symbols) and after (closed symbols) treatment with 0.18 µg/kg ouabain. Points represent the means ± SEM. *P<0.05 compared to before ouabain treatment (ANOVA).

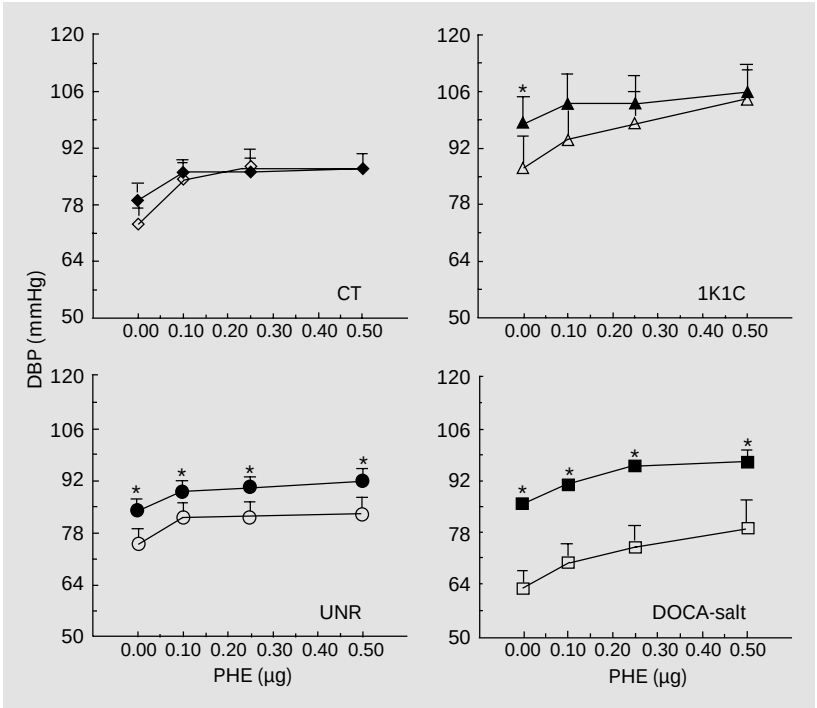

Figure 5. Changes in diastolic blood pressure (DBP) in response to increasing doses of phenylephrine (PHE) in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats before (open symbols) and after (closed symbols) treatment with 0.18 µg/kg ouabain. Points represent the means ± SEM. *P<0.05 compared to before ouabain treatment (ANOVA).

sure compared to the normotensive controls (normotensive - control: 8.50 ± 2.62 mmHg; uninephrectomized: 16.41 ± 3.47 mmHg, P>0.05 *vs* control, *t*-test, and hypertensive - 1K1C: 20.10 ± 3.13 mmHg, P<0.02 *vs* control, *t*-test; DOCA-salt: 21.71 ± 4.20, P<0.03 *vs* control, *t*-test). When pressor reactivity was tested (Figures 2 and 3), it could be seen that dose-dependent phenylephrine-evoked systolic and diastolic blood pressure responses were shifted upwards. However, ouabain did not change the gain of this response.

Treatment with 0.18 µg/kg ouabain produced a smaller but significant increment in systolic blood pressure and had no significant effect on diastolic blood pressure in the control rats (Figures 4 and 5). This dose is expected to produce nanomolar levels of circulating ouabain if we assume that the drug is being diluted in 40 ml of extracellular fluid per each 100 g of rat. In normotensive rats, phenylephrine-evoked pressor effects were similar before and after ouabain treatment. However, this very low dose of ouabain increased both systolic and diastolic blood pressure in the hypertensive groups (1K1C and DOCA-salt), and in the uninephrectomized rats (Figures 4 and 5). When pressor reactivity was tested (Figures 4 and 5) it could be seen that dose-dependent phenylephrine-evoked systolic and diastolic blood pressure responses were shifted upwards. Moreover, analysis of the magnitude of systolic and diastolic pressure increment before and 1 h after 0.18 µg/kg ouabain showed that DOCA-salt rats were more responsive to the effects of ouabain than normotensive and 1K1C hypertensive rats (Table 2).

Another dose of ouabain was used. Since we knew from previous experiments that SHR are more resistant to toxic doses of ouabain, another protocol was performed using 1.8 mg/kg ouabain to test this behavior in another hypertensive group. 1K1C rats and their uninephrectomized controls were investigated and both behaved like SHR, with an increase in systolic and diastolic blood pressure after ouabain treatment (Table 3).

Isolated rat tail vascular bed preparation

Phenylephrine produced a dose-dependent increase in mean perfusion pressure in the rat tail vascular bed from all animals (Figure 6). This increment did not differ between normotensive and 1K1C hypertensive rats but was smaller in DOCA-salt rats compared to the other groups (Figure 6).

After treatment with 10 nM ouabain for 60 min, the baseline perfusion pressure was reduced in all animals (normotensive: control, from 74 ± 3.24 to 66 ± 1.60 mmHg, and uninephrectomized, from 79 ± 4.88 to 64 ± 3.13 mmHg; hypertensive: 1K1C, from 82 ± 6.85 to 67 ± 2.12 mmHg, and DOCA-salt, from 77 ± 6.13 to 61 ± 4.93 mmHg; $P<0.05$ for all groups, paired *t*-test). Perfusion with ouabain-free Krebs solution for 1 h also reduced the baseline perfusion pressure but did not enhance the pressor response to phenylephrine (data not shown). After ouabain treatment the pressor response to phenylephrine was significantly enhanced only at the 5- and 10-µg doses in normotensive and 1K1C hypertensive rats (Figure 6). However, in DOCA-salt hypertensive rats the increment of the pressor response obtained after ouabain treatment became significant at a lower dose of phenylephrine, i.e., 1 µg (Figure 6).

Table 2. Changes in systolic (SBP) and diastolic (DBP) blood pressure before and after 0.18 µg/kg ouabain in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats.

	ΔSBP (mmHg)	ΔDBP (mmHg)
CT	7.6 ± 3.6	3.7 ± 3.6
UNR	9.3 ± 2.7	9.2 ± 3.1
1K1C	12.7 ± 4.2	10.7 ± 3.8
DOCA-salt	24.3 ± 2.6*+	27.2 ± 3.5*+

*P<0.001, comparison between hypertensive and normotensive rats;
+P<0.001, comparison between DOCA-salt and 1K1C rats (*t*-test).

Table 3. Changes in systolic (SBP) and diastolic (DBP) arterial blood pressure (in mmHg) in response to the vasopressor effects of increasing doses of phenylephrine (PHE) before and after 1.8 mg/kg ouabain treatment in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C) rats.

		PHE (0)		PHE (0.1 µg)		PHE (0.25 µg)		PHE (0.5 µg)	
		SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
CT	Before	109 ± 9.4	79 ± 10.6	120 ± 9.4	90 ± 10.4	120 ± 6.9	93 ± 8.1	124 ± 6.7	98 ± 7.9
	After	88 ± 4.4*	78 ± 6.1	98 ± 7.7*	85 ± 5.9	102 ± 10.5*	87 ± 7.2	100 ± 10.2*	90 ± 7.6
UNR	Before	99 ± 4.9	72 ± 5.3	104 ± 4.5	78 ± 4.6	107 ± 3.9	81 ± 4.6	110 ± 3.7	85 ± 4.6
	After	114 ± 5.3*	93 ± 5.3*	119 ± 5.2*	97 ± 5.1*	120 ± 4.7*	97 ± 5.2*	123 ± 5.1*	102 ± 5.2*
1K1C	Before	112 ± 6.1	81 ± 5.9	121 ± 6.4	92 ± 6.5	123 ± 6.5	94 ± 6.3	127 ± 6.5	98 ± 6.3
	After	141 ± 7.7*	116 ± 7.8*	145 ± 7.6*	118 ± 7.6*	147 ± 7.8*	120 ± 7.8*	150 ± 8.0*	123 ± 8.0*

*P<0.05 compared to before ouabain treatment (ANOVA).

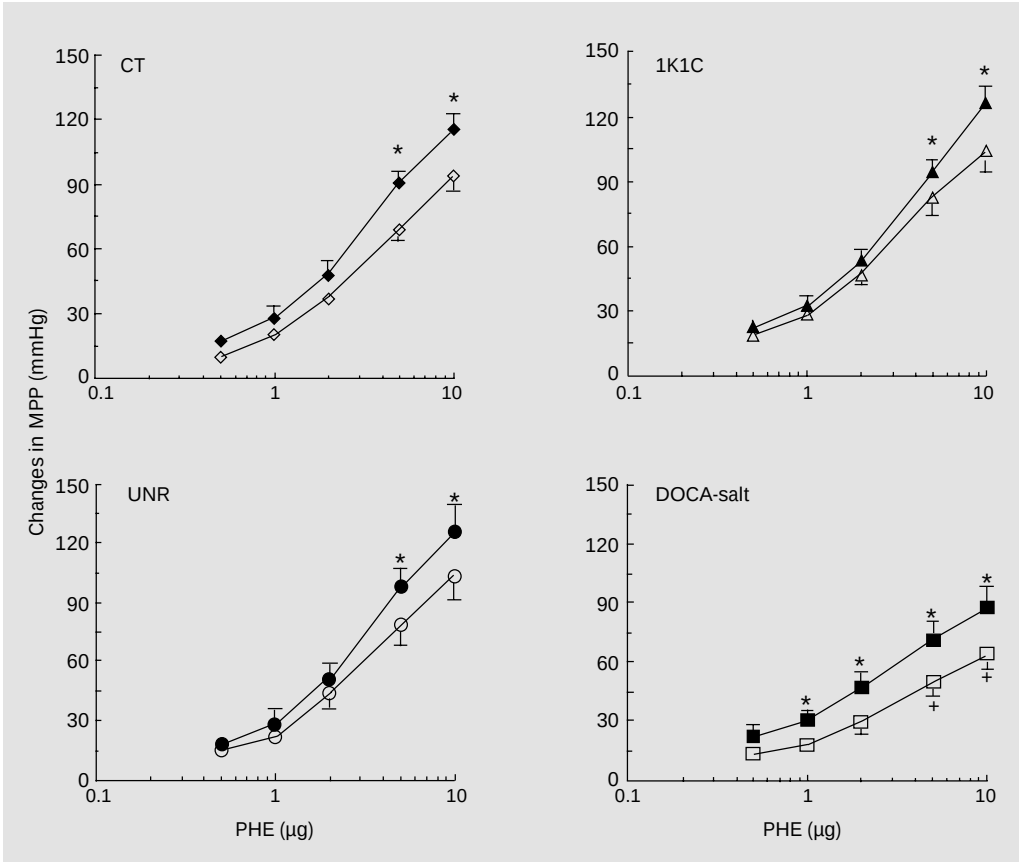

Figure 6. Changes in mean perfusion pressure (MPP) in response to increasing doses of phenylephrine (PHE) in normotensive (control, CT; uninephrectomized, UNR) and hypertensive (1K1C and DOCA-salt) rats before (open symbols) and after (closed symbols) infusion of 10 nM ouabain. Points represent means ± SEM. *P<0.05 compared to before ouabain treatment; +P<0.05 compared to normotensive control, uninephrectomized and hypertensive 1K1C rats rats (ANOVA).

Discussion

The present results suggest that DOCA-salt rats were more sensitive to pressor effects after treatment with small doses of ouabain (0.18 µg/kg) than normotensive and 1K1C rats *in vivo*. When using higher doses of ouabain (18 µg/kg) the hypertensive 1K1C and DOCA-salt rats showed similar behavior regarding the pressor effects induced by this digitalis compound. Although ouabain did not increase sensitivity to phenylephrine *in vivo*, this effect was observed *in vitro* in isolated vessels obtained from DOCA-salt rats. These findings suggest that the increase of arterial pressure caused by small doses of ouabain in DOCA-salt rats seems to be the result of an increased sensitization of vascular smooth muscle to pressor α-agonist agents. Our results also suggest that the pressor response to ouabain depends on the dose and on the hypertensive animal model used.

Ouabain inhibits Na^+,K^+-ATPase by binding to the α subunits of the enzyme in several tissues like the heart, vessels, kidneys and the nervous system (3,6,11). The cell membrane enzyme Na^+,K^+-ATPase is the biochemical expression of the electrogenic Na^+ pump and exchanges 3 Na^+ for 2 K^+, maintaining the resting potential of excitable cells (3). A reduction in Na^+ pump activity leads to Na^+ accumulation in the myoplasm (4). Inhibition of the pump also affects the Na^+/Ca^{2+} exchange mechanism and, consequently, the amount of activator Ca^{2+} necessary for contraction in several tissues such as vascular smooth muscle (4). By inhibiting the Na^+ pump, ouabain may have effects on the homeostatic mechanisms affecting the regulatory control of blood pressure, there-

fore playing a central role in the development and maintenance of hypertension (4). Indeed, many reports have suggested such a role since ouabain can produce hypertension during long-term administration in rats (9,12,13).

Recently, ouabain has been reported to be an adrenocortical hormone present in plasma at nanomolar or subnanomolar concentrations (4,8,9). There are results indicating that a circulating ouabain-like substance is increased in DOCA-salt rats and in low-renin rat models such as 1K1C (14,15). A recent study using anesthetized normotensive and SHR rats has reported that the pressor effects induced by ouabain were operative *in vivo* (1). In SHR, sensitization was present at doses of ouabain reaching the nanomolar level. This effect was observed when vascular smooth muscle was challenged by phenylephrine stimulation after ouabain treatment. Therefore, the possibility of sensitization by ouabain may manifest *in vivo* since vascular smooth muscle is continuously stimulated by the sympathetic drive or by the presence of endogenous vasopressor substances. The present study was designed to investigate the vascular reactivity of anesthetized 1K1C and DOCA-salt rats after several ouabain treatments.

As seen in Table 3, a toxic dose of ouabain (1.8 mg/kg) produced a hypotensive effect in normotensive rats but not in the other groups. It has been reported that normotensive rats show a reduction of systolic blood pressure after this ouabain treatment while SHR show an increase in systolic blood pressure (1). Diastolic blood pressure did not change significantly after 1.8 mg/kg ouabain treatment in normotensive rats but increased in the others. Also, confirming previous findings (1), this large dose of ouabain frequently produced arrhythmias in normotensive rats, characterizing a toxic effect. The hypotension was probably caused by a reduction in cardiac output produced by the presence of arrhythmias in the normotensive group or by a putative calcium overload causing diastolic dysfunction. This suggests that hypertensive and uninephrectomized rats are more resistant to the toxic effects of ouabain than normotensive rats.

The arrhythmogenic effect of ouabain is also related to the inhibition of Na^+,K^+-ATPase. This inhibition increases intracellular sodium concentration and depolarizes the cell. The Na^+/Ca^{2+} exchange activity is also reduced and Ca^{2+} accumulates inside the cell. Once intracellular Ca^{2+} increases, the Na^+/Ca^{2+} exchanger is stimulated and, since this is an electrogenic exchange, depolarizing currents are generated causing arrhythmias (16). The resistance to the toxic effects of ouabain in the hypertensive group has been explained by the fact that Na^+,K^+-ATPase activity is reduced in hypertrophic hearts from hypertensive animals (17,18). Moreover, some of these reports show that the α_2 isoform of Na^+,K^+-ATPase is reduced (18) while the α_3 isoform increases (19). Since the α_3 isoform is less sensitive to intracellular Na^+ (19), this could explain the increased resistance of the hypertrophied myocardium to the effects of this digitalis compound.

The intermediate dose of ouabain (18 µg/kg) increased arterial systolic blood pressure after 1 h. It is interesting to note that ouabain, even at the largest dose, never increased significantly the diastolic blood pressure of normotensive rats, which reflects the lack of effects on systemic peripheral resistance. However, a significant diastolic blood pressure increment was observed after all ouabain treatments in other groups. It should be emphasized that, although we did not observe differences among groups, when the changes in systolic pressure were evaluated after 18 µg/kg ouabain, this dose was able to increase diastolic pressure in hypertensive animals when compared to normotensive animals. This result was similar to what was reported before for SHR (1), indicating that the effect of ouabain on arterial blood pres-

sure is larger in these animals. Ouabain treatment with the smaller dose of 0.18 µg/kg was able to increase arterial pressure. However, in contrast to what occurred when 18 µg/kg ouabain was used, this pressure increment was larger in DOCA-salt rats than in normotensive and 1K1C rats. These data show that not all hypertension models have the same sensitivity to ouabain at very small doses. Treatment with ouabain increased arterial pressure and shifted upwards the vasopressor responses to phenylephrine in all models at all doses studied. However, no changes in the gain of phenylephrine pressor responses were observed. An interesting result was obtained with the uninephrectomized rats whose response to ouabain reached an intermediate level between hypertensive and normotensive control rats. This was not the only difference they showed compared to normal rats, since a reduction of cardiac contractility was also reported following uninephrectomy (20). These findings led us to conclude that uninephrectomized animals cannot be considered as a normal control. Our results, however, cannot discriminate the underlying mechanisms responsible for this sensitization effect but suggest that a) ouabain increases blood pressure and maintains phenylephrine-evoked pressor responses in all groups studied when acute digitalization doses are used, b) these effects are still present even when smaller doses of ouabain are used in all groups of rats except the normotensive ones, c) the increment in systolic and diastolic blood pressure is more efficient in DOCA-salt hypertensive rats, and d) hypertensive and uninephrectomized rats are less sensitive to toxic doses of ouabain.

One possible mechanism that explains the changes in pressor responses caused by ouabain in these models of hypertension is a vascular mechanism. Previous reports have demonstrated that higher doses of ouabain sensitize the contractile response to α-adrenergic agonists and to KCl in 1K1C rats (21,22). The same occurs with DOCA-salt rats in which ouabain treatment increased even more the pressor responses (23). This would help to explain why 18 µg/kg ouabain was capable to produce a significant increase of diastolic pressure in hypertensive, but not normotensive rats. However, these results were obtained with higher doses of ouabain and this may mask a different effect obtained with lower doses of ouabain. Indeed, the effect obtained in DOCA-salt rats after 0.18 µg/kg ouabain could be explained by such enhanced sensitization of the vascular bed.

It has been reported that low plasma concentrations of ouabain may modulate vascular reactivity without directly affecting vascular smooth muscle tone (2,24,25). For example, low ouabain concentrations (10 nM) increase caffeine-evoked contractions in rat aortic rings (26). Using a perfused rat tail vascular bed preparation we showed (2,24) that 10 nM ouabain did not alter baseline perfusion pressure but increased the sensitivity to phenylephrine-evoked pressor responses both in normotensive rats and SHR. The same was observed in our normotensive control rats. Ouabain was able to sensitize the pressor response to phenylephrine in the tail vascular bed from all models studied, without causing changes in baseline perfusion pressure.

Recently, it has been shown that small increments of myoplasmic Ca^{2+} may increase vascular reactivity because of the amplifier action of the sarcoplasmic reticulum (4,5). Thus, low concentrations of ouabain can augment Ca^{2+} transients in vascular smooth muscle without increasing cytosolic sodium (25). This mechanism explains why low concentrations of ouabain increase the contractile response to phenylephrine without causing contraction by a direct action on vascular smooth muscle. However, by comparing the sensitization of the phenylephrine pressor response among the studied models it was possible to see that *in vitro* treatment with 10 nM ouabain is more effective in DOCA-salt

rats when compared with normotensive and hypertensive 1K1C rats. This result also explains why low doses of ouabain caused a larger pressor response in DOCA-salt rats compared to 1K1C. Possible mechanisms that could be modified in these models of hypertension and could explain the results described here are changes in the expression and activity of the Na^+ pump.

The expression and activity of Na^+,K^+-ATPase and its α isoform are regulated by multiple factors such as developmental stage (27), hormones (28), endothelial factors (29) and the occurrence of hypertension (30,31). Furthermore, it has been shown that α subunits of Na^+,K^+-ATPase consist of different α_1, α_2 and α_3 subunits with different affinities for digitalis (11). Since the Na^+,K^+-ATPase isoforms may change under several situations the maintenance of the sensitizing mechanism could be of clinical relevance. Recently, Blaustein et al. (5) suggested that a special distribution of the α_2 and α_3 isoforms of Na^+,K^+-ATPase exists in the sarcolemma that binds ouabain preferentially and they are located near the vesicles of the sarcoplasmic reticulum. This special localization enables the increment of cytosolic calcium near the reuptake sites, facilitating the operation of the proposed mechanism. Then, when vascular smooth muscle is stimulated the final result is an "amplification" of the calcium released and an increase in the response of the vasculature to agents that produce constriction (1,2,24,26). This is an amplification mechanism that operates even at low ouabain concentrations.

These results lead us to speculate that there might be differences in Na^+,K^+-ATPase isoforms in these normotensive and hypertensive rats. One possible explanation for this difference would be the up-regulation in hypertensive groups. Recently, Liu et al. (31) reported up-regulation of both α_1 and α_2 isoforms after a 2- or 4-day stretch in cultured vascular smooth muscle cells. Similar results have been obtained in several models of cardiac hypertrophy (18,19,32). Increase of Na^+ pump activity and up-regulation of the expression of α_1 mRNA in the heart, together with a down-regulation of the α_2 mRNA as a result of DOCA-salt hypertension, has been reported (6,30). However, increased α_1 isoform activity would result in low levels of inhibition by ouabain sufficient to make 1K1C and uninephrectomized rats more resistant to the toxic effects of the drug. In the aorta, it has been reported that corticosteroids regulate Na^+,K^+-ATPase isoform expression and activity in vascular tissue, suggesting a mineralocorticoid-dependent modulation of the α_2 isoform (28). Also, up-regulation of the α_2 isoform in DOCA-salt hypertensive rats may facilitate ouabain binding, further increasing its sensitizing effects.

The ability of ouabain to elevate arterial pressure in anesthetized rats might be the result of a peripheral and central pathway. Ouabain is reported to activate the sympathetic autonomic nervous system and to reduce the efficiency of the baroreflex, which in turn, contributes to an increase in arterial pressure (33). In addition, hypertension has been reported to occur with enhanced sympathetic activity and a blunted baroreflex in 1K1C and DOCA-salt rats (34,35). Thus, a putative effect of ouabain potentiating this mechanism cannot be ruled out. Another important finding supported by our results is the fact that ouabain directly enhances the release of norepinephrine from nerve terminals (36). Moreover, greater vascular reactivity to vasopressor agents, increased endothelin levels and an increased activity of the renin-angiotensin system have been reported to occur in both 1K1C and DOCA-salt rats (37,38). Thus, sensitization of the vascular smooth muscle by ouabain, associated with the greater release of norepinephrine by nerve terminals, might explain the increase of diastolic pressure produced by ouabain. Although ouabain sensitizes the vessels to the pressor effect of phenylephrine *in vitro*, this action did not occur *in vivo*. *In vivo*, the

pressor effects of phenylephrine were displaced upwards but no sensitization was observed. Part of this response can be explained by the action of ouabain or by the arterial pressure elevation stimulating the baroreflex. Also, there are reports showing that ouabain, instead of blunting the baroreflex, can enhance it in normotensive animals, an effect that is amplified in SHR (39). On this basis, we may suppose that a putative *in vivo* sensitization might be masked by this effect.

Another interesting aspect is related to the fact that the *in vitro* pressor responses to phenylephrine are reduced in DOCA-salt rats compared to the other models studied. Previous reports have shown that in DOCA-salt rats the chronic increment of sympathetic tone in vascular beds with more sympathetic innervation, like the mesenteric arteries, might reduce the response of these arteries to norepinephrine. Prolonged exposure to high levels of norepinephrine may reduce the action of α-adrenergic agonists via stimulation of the vascular endothelium, leading to an increased production of vasodilator factors like nitric oxide (40). This mechanism should explain the reduction of the pressor response to phenylephrine of the tail vascular bed from DOCA-salt rats since the tail artery is highly innervated. In addition, previous results from our laboratory have suggested that endothelial modulation of the pressor response to phenylephrine is enhanced in DOCA-salt rats.

In conclusion, these results suggest that ouabain treatment increases blood pressure and that this increment is more marked in hypertensive rats. The pressor effects after the administration of low ouabain concentrations were more pronounced in hypertensive DOCA-salt rats *in vivo* and *in vitro*. These increments still occurred with very low doses of ouabain in hypertensive rats, suggesting an enhanced pressor reactivity which may contribute to the genesis and maintenance of hypertension.

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