Neurogenic nitric oxide release increases in mesenteric arteries from ouabain hypertensive rats

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Objectives We investigated whether chronic ouabain treatment changes the vasoconstrictor responses induced by electrical field stimulation (EFS) in endothelium-denuded rat superior mesenteric arteries and a possible role of neuronal nitric oxide (NO).

Method Mesenteric arteries from untreated and ouabain-treated rats (>8.0 μg/kg per day, for 5 weeks) were used in this study. Vascular reactivity was analyzed by isometric tension recording. Expression of the neuronal NO synthase isoform was analyzed by Western blot. Noradrenaline release was evaluated in segments incubated with [3H]noradrenaline.

Results Systolic (SBP) and diastolic (DBP) blood pressure were higher in ouabain-treated rats than in untreated rats (SBP, untreated: 120 ± 3.5 mmHg versus ouabain-treated: 150 ± 4.7 mmHg, P < 0.01; DBP, untreated: 87 ± 3.0 mmHg versus ouabain-treated: 114 ± 2.6 mmHg, P < 0.001). EFS-induced vasoconstrictions were smaller in arteries from ouabain-treated rats than in those from untreated animals, while the EFS-induced [3H]noradrenaline release and the vasoconstriction induced by exogenous noradrenaline (1 nmol/l–10 μmol/l) remained unmodified. The non-selective NO synthase (NOS) inhibitor, Nω-nitro-L-arginine methyl ester (100 μmol/l), increased the EFS-induced vasoconstriction in mesenteric arteries from both groups, although the effect was more pronounced in segments from ouabain-treated rats. The selective neuronal NOS inhibitor, 7-nitroindazole (7-NI; 100 μmol/l) increased EFS-induced contraction only in segments from ouabain-treated rats. Neuronal NOS expression was greater in the mesenteric arteries from ouabain-treated rats than in those from untreated animals. Sodium nitroprusside (0.1 nmol/l–10 μmol/l) induced a similar vasodilatation in segments from both groups.

Conclusions These results suggest that chronic ouabain treatment is accompanied by an increase in neuronal NO release that reduces EFS-induced vasoconstriction.


Keywords: ouabain, hypertension, neuronal nitric oxide, electrical field stimulation, Na+-pump

Introduction One of the characteristics of hypertension is increased vascular resistance caused by a higher smooth muscle tone. Several mechanisms have been proposed to explain this increment in vascular resistance, including endothelial dysfunction [1], increased sympathetic tone [2], structural changes [3,4] or alterations in Na+, K+-ATPase activity [5,6], among others.

It is well established that Na+, K+-ATPase participates in the modulation of vascular smooth muscle contractility and blood pressure [7]. Lowering the Na+-pump activity increases intracellular Na+ concentration, thus reducing the Na+/Ca2+ exchanging activity and consequently increasing intracellular Ca2+ concentration and tension [7]. Thus, changes in the functional activity of the vascular Na+-pump or its regulation may either contribute to the development of hypertension or be a compensatory mechanism against the elevated blood pressure [5,7]. Elevated plasma levels of the endogenous sodium pump inhibitor ouabain have been found in some experimental models of hypertension [6,8], as well as in essential human hypertension [9]. In addition, several reports have demonstrated that chronic administration of ouabain or its structurally related analogs can induce hypertension [10–17].

0263-6352 © 2004 Lippincott Williams & Wilkins

DOI: 10.1097/01.hjh.0000098300.36684.b7

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The hypertension induced by ouabain seems to be the result of a central mechanism associated with increased sympathetic tone, consequent to activation of the central renin–angiotensin system, and resetting of the baroreceptor reflex [11,18]. However, there is also a compensatory or adaptive peripheral mechanism, which we have demonstrated recently, whereby ouabain-induced hypertension is followed by a reduction in vascular reactivity to phenylephrine in the rat aorta and superior mesenteric artery, and increased activity and expression of Na\(^+\), K\(^+\)-ATPase in rat aorta [14,15]. This vascular alteration has been associated with increased endothelium-derived nitric oxide (NO) production, probably mediated by the increase in expression of endothelial (eNOS) and neuronal (nNOS) NO synthase isoforms induced by chronic ouabain administration [15]. Other authors [19] also showed, in aortic rings from chronically ouabain-treated Sprague–Dawley rats, a reduction in the contraction induced by phenylephrine and no changes in the depolarization induced by KCl. However, in renal arteries from chronically ouabain-treated Sprague–Dawley rats, an increase of K\(^+\)- and phenylephrine-induced vasoconstriction was observed [20]. The reason for these differences is not entirely clear, but contributing factors may be differences in the vascular bed studied.

It is already known that the activity of the Na\(^+\)-pump affects the release of noradrenaline (NA) from sympathetic nerve endings. Electrical field stimulation (EFS) is widely used to study the influence of neurotransmitters released from nerve endings on vasomotor responses [21]. In rat mesenteric arteries, EFS produces a vasoconstrictor response. This response is a balance between the effects resulting from the release of different neurotransmitters, such as NA from adrenergic, neuronal NO from nitricergic and calcitonin gene-related peptide (CGRP) from sensory innervation [22–24]. In addition, evidence of increased neuronal NO release in mesenteric arteries from hypertensive rats has also been reported [24].

Based on the observations cited above, the goal of the present study was to investigate whether ouabain-induced hypertension was associated with changes in vasoconstrictor responses induced by EFS in rat mesenteric arteries, and the possible role of neuronal NO in these changes.

Materials and methods

Animals

Six-week-old male Wistar rats were obtained from the Animal Quarters of the Facultad de Medicina of the Universidad Autónoma de Madrid. Animals were housed at a constant room temperature, humidity and a 12 h light/dark cycle. Rats had free access to tap water and were fed with standard rat chow ad libitum. The entire study was conducted in accordance with current Spanish and European laws (RD 223/88 MAPA and 609/86).

Pellet implantation

Rats were anesthetized with diethyl ether and a small incision was made in the back of the neck to implant a subcutaneous controlled time-release pellet (Innovative Research of America, Sarasota, Florida, USA) containing ouabain (0.5 mg/pellet) or vehicle (placebo), as described previously [10]. These pellets are designed to release approximately 8.0 μg/day of ouabain for 60 days.

Blood pressure and heart rate measurements

Five weeks after implantation of the pellets, the rats were anesthetized with ether and the right carotid artery was cannulated with PE-50 tubing filled with heparinized saline. The catheter was tunneled subcutaneously, exteriorized at the nape, and secured to the skin. On the day of the experiments, 24 h later, blood pressure was measured in conscious rats by connecting the arterial catheter to a pressure transducer attached to a data acquisition system (Uni Graph 50; Letica, Barcelona, Spain). Heart rate was determined from the intra-beat intervals of arterial pulses during the experiment.

Tissue preparation

The rats were anesthetized with ether and killed by exsanguination. The superior mesenteric arteries were carefully dissected out, cleaned of connective tissue and placed in Krebs–Henseleit solution (KHS) at 4°C. For experiments of vascular contractility, the arteries were divided into segments of 4 mm length (wet weight: untreated, 4.8 ± 0.32 mg; ouabain-treated, 4.7 ± 0.53 mg; P > 0.05). For analysis of nNOS expression, arteries were frozen rapidly in liquid nitrogen and kept at −70°C until the day of analysis.

Constrictor responses

Each arterial segment was mounted between parallel wires (75 μm in diameter) in tissue baths at 37°C containing KHS gassed with 95% O\(_2\) and 5% CO\(_2\) to maintain the pH at 7.4. One wire was fixed to the organ bath wall, and the other was connected vertically to the strain gauge for isometric tension recording, according to the method described by Nielsen and Owman [25]. Isometric tension was recorded using an isometric force displacement transducer (Grass FT03C; Massachusetts, USA) connected to a polygraph (Grass Model 7D). For EFS experiments, the segments were mounted between two platinum electrodes, 0.5 cm apart, connected to a stimulator (Gibertec Model CS9; Madrid, Spain), modified to supply adequate current strength. The segments from untreated and ouabain-treated rats were stretched to an optimal resting tension of 0.5 g, which
was re-adjusted every 15 min during a 90-min equilibration period before drug administration. Next, the vessels were exposed to 75 mmol/l K⁺ to check their functional integrity. All experiments were performed in endothelium-denuded segments. Endothelium was removed by gently rubbing the intimal surface with a stainless-steel rod. The effectiveness of endothelium removal was confirmed by the absence of the relaxation induced by acetylcholine (ACh, 10 μmol/l) in segments precontracted with 1 μmol/l NA.

Frequency–response curves to EFS or concentration–response curves to NA (1 nmol/l–10 μmol/l) were performed. The parameters used for EFS were 200 mA/0.3 ms/1–8 Hz for 30 s, and a 1-min interval between stimuli, the time required to recover basal tone. A washout period of at least 1 h between consecutive curves was necessary to avoid desensitization. Two successive frequency–response or NA concentration–response curves, separated by 1 h intervals, induced similar contractile responses.

To determine the participation of nitricergic innervation in EFS-induced responses, 100 μmol/l NG-nitro-L-arginine methyl ester (L-NAME, a non-selective NOS inhibitor) or 100 μmol/l 7-nitroindazole (7-NI, an nNOS inhibitor) was added to the bath 40 min before recording the second frequency–response curve. The effect of L-NAME or 7-NI on the concentration–response curve to NA was also evaluated.

The ability of sodium nitroprusside (SNP, 0.1 nmol/l–10 μmol/l) to induce relaxation in arteries from untreated and ouabain-treated hypertensive rats was assessed in NA (1 μmol/l) precontracted segments.

**Tritium overflow**

Rat mesenteric artery segments of 4 mm length, from untreated and ouabain-treated rats, were set up in a nylon net and immersed for 90 min in 1 ml of oxygenated KHS at 37°C containing (±)-[3H]NA (0.5 μmol/l, 5 μCi/ml, specific activity 12.8 Ci/mmol). Afterwards, the arteries were transferred to a superfusion chamber with two parallel platinum electrodes, 0.5 cm apart, connected to a stimulator (Gibertec model CS9, modified to supply the adequate current strength) for EFS. The arteries were superfused at a rate of 2 ml/min with oxygenated KHS at 37°C for 60 min, during which the steady-state level of basal tritium efflux was reached. Then, two electrical stimulation periods of 60 s (200 mA, 0.3 ms, 4 Hz; S₁ and S₂) were applied to the arteries at a 60-min interval, and the superfuse was collected in vials (10 in total) at 30-s intervals. These vials were distributed in the following manner: three before stimulation, to determine the basal level of tritium efflux, two during and five after the stimulation; these five vials were enough to recover a basal level of tritium efflux. OptiPhase HiSafe solution (Fisons Chemicals, Loughborough, Leicestershire, UK) was added to the vials and the radioactivity measured in a scintillation counter (Beckman LS 5000 TD; Beckman Instruments, Fullerton, California, USA). 7-NI (100 μmol/l) administered 30 min before S₂ was used to assess if this drug interferes with tritium overflow.

**Western blot analysis of nNOS protein expression**

Segments of superior mesenteric arteries were homogenized in ice-cold Tris–EDTA buffer (50 mmol/l Tris, 1.0 mmol/l EDTA, pH = 7.4). Homogenates (50 μg protein per lane) and pre-stained molecular weight sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) standards (Bio-Rad, Hercules, California, USA) were separated electrophoretically on 7.5% SDS-PAGE and then transferred to polyvinyl difluoride membranes overnight at 4°C in the Mini Trans-Blot Cell system (Bio-Rad) containing 25 mmol/l Tris, 190 mmol/l glycine, 20% methanol, and 0.05% SDS. Rat pituitary was used for the nNOS-positive control. The membrane was then blocked for 60 min at room temperature in Tris-buffered solution (10 mmol/l Tris, 100 mmol/l NaCl and 0.1% Tween 20) with 5% powdered non-fat milk. Next, the membrane was incubated for 1 h at room temperature with mouse monoclonal antibody for nNOS (1:2500), purchased from Transduction Laboratories (Lexington, UK). After washing, the membrane was incubated with a 1:2000 dilution of antimouse IgG antibody conjugated to horseradish peroxidase (Transduction Laboratories). The membrane was thoroughly washed, the immunocomplexes were detected with an enhanced horseradish peroxidase/luminol chemiluminescence system (ECL Plus; Amersham International, Little Chalfont, UK) and then subjected to imaging (Hyperfilm ECL; Amersham International). Signals on the immunoblot were quantified using a National Institutes of Health Image V1.56 computer program. The same membrane was used to determine α-actin expression, by means of a monoclonal antibody anti-α-actin (1:300 000 dilution, Boehringer Mannheim, Mannheim, Germany) and the content of the latter was used to correct nNOS expression in each sample.

**Solutions, drugs and statistical analysis**

The composition of KHS was as follows (mmol/l): NaCl 115, CaCl₂ 2.5, KCl 4.6, H₂PO₄ 1.2, MgSO₄ × 7H₂O 1.2, NaHCO₃ 25, glucose 11.1 and Na₂ EDTA 0.03. Drugs used were NA, ACh, SNP, L-NAME and 7-NI, (Sigma, St. Louis, Missouri, USA). Stock solutions (10 mmol/l) of drugs were made in distilled water, except for NA, which was dissolved in a NaCl (0.9%)–ascorbic acid (0.01% w/v) solution, and 7-NI, which was dissolved in a water–ethanol solution (50% w/v). These solutions were kept at −20°C and appropriate dilutions were made on the day of the experiment.
Contractile responses are expressed as a percentage of the maximum response produced by 75 mmol/l KCl. Relaxation caused by SNP was expressed as a percentage of the initial contraction induced by 1 μmol/l NA. In order to compare the magnitude of effects of 7-NI on the responses to NA in segments from untreated or ouabain hypertensive rats, some results were expressed as ‘differences’ of area under the concentration–response curves (dAUC) in control and experimental situations. AUC were calculated from the individual concentration–response curves and the differences were expressed as a percentage of the difference to the AUC of the corresponding control situation.

The EFS-induced tritium overflow was calculated by subtracting the basal tritium release from that evoked by electrical stimulation. Ratios of the net tritium overflow between S2 and S1 were calculated to eliminate differences between the arteries. The action of the 7-NI on the evoked overflow was expressed as its effects on this ratio. The amount of radioactivity released is indicated in dpm/mg of tissue.

For nNOS expression, results are expressed as the ratio between signals on the immunoblot corresponding to isoforms of nNOS and α-actin. To compare the results for protein expression within the same experiment and with others, we assigned a value of one to the ratio in arteries from untreated rats and used that value to calculate the relative density of other bands from the same gel.

All values are expressed as means ± SEM. ‘n’ represents the number of animals used in each experiment. Results were analyzed using Student’s t-test, by completely randomized two-way ANOVA for comparison between groups, or by repeated-measure ANOVA to compare treatments in the same group. When ANOVA showed a significant treatment effect, Bonferroni’s post hoc test was used to compare individual means. Differences were considered statistically significant at P < 0.05.

**Results**

After 5 weeks’ treatment the systolic (SBP) and diastolic (DBP) blood pressures were significantly increased in rats treated with ouabain, when compared to untreated rats (SBP: untreated, 120 ± 3.5 mmHg versus ouabain-treated, 150 ± 4.7 mmHg, P < 0.01; DBP: untreated, 87 ± 3.0 mmHg versus ouabain-treated, 114 ± 2.6 mmHg, P < 0.001). There were no significant differences in heart rate (HR) between the two groups of rats (untreated, 391 ± 13 bpm versus ouabain-treated, 400 ± 18 bpm, P > 0.05).

Body weight after 5 weeks of treatment was similar in all groups (untreated, 287 ± 11 g versus ouabain-treated, 293 ± 15 g, P > 0.05).

**Vascular reactivity**

A reduction in vasoconstrictor responses induced by EFS (1, 2, 4 and 8 Hz) was observed in de-endothelialized mesenteric arteries from ouabain-treated rats (Fig. 1a). However, the vascular responses to 75 mmol/l K⁺ (untreated, 1241 ± 101 mg versus ouabain-treated, 1257 ± 165 mg, P > 0.05) or NA (1 nmol/l–10 μmol/l)
Influence of preincubation with N^G^-nitro-L-arginine methyl ester (L-NAME, LN, 100 μmol/l) on the frequency-dependent responses of mesenteric artery segments from untreated (a, n = 7) and ouabain-treated rats (b, n = 7). (c) compares the electrical field stimulation-induced contraction in the two groups in the presence of L-NAME. Results (means ± SEM) are expressed as a percentage of response elicited by 75 mmol/l K^+. ANOVA: +P < 0.05, ++P < 0.01, untreated (control) versus untreated (LN). **P < 0.01, ***P < 0.001 ouabain-treated (control) versus ouabain-treated (LN).

In segments from both groups precontracted with 1 μmol/l NA, SNP (0.1 nmol/l–10 μmol/l) induced a similar concentration-dependent relaxation (Fig. 5), which was unmodified by the presence of L-NAME or 7-NI (results not shown).

\[ ^3H \]NA release experiments

In [3H]NA-preincubated mesenteric arteries from untreated and ouabain-treated rats, two successive electrical pulses induced tritium overflows, which remained unchanged by chronic ouabain treatment: (untreated: S_1 = 318 ± 34 dpm/mg and S_2 = 277 ± 46 dpm/mg versus ouabain-treated: S_1 = 310 ± 35 dpm/mg and S_2 = 289 ± 87 dpm/mg; P > 0.05, n = 6 in each group). Addition of 100 μmol/l 7-NI, 30 min before S_2, did not modify the basal or stimulated tritium overflow in arteries from both untreated and ouabain-treated rats (untreated: control, S_2/S_1 = 0.89 ± 0.08; 7-NI, S_2/S_1 = 0.82 ± 0.11; P > 0.05; ouabain-treated: control, S_2/S_1 = 0.88 ± 0.11; 7-NI, S_2/S_1 = 0.82 ± 0.15, P > 0.05).

Western blot analysis of nNOS expression

Figure 6 shows that ouabain treatment increased the expression of nNOS in superior mesenteric arteries.

Discussion

The main and new finding of this work is that hypertension induced by chronic ouabain treatment is accompanied by a reduction in vasoconstrictor response to EFS in de-endothelialized rat mesenteric arteries. This effect seems to be mediated by an increase in neuronal NO release.
The endogenous digitalis-like compound ouabain is found in the plasma of several mammals and has been associated with the development and/or maintenance of some cardiovascular diseases, including hypertension [6,8,9]. Chronic administration of ouabain leads to increased blood pressure [12,13,17], which has been associated with an increased activity in sympathoexcitatory pathways and a resetting of the baroreceptor reflex [10,11,16]. However, we have recently demonstrated that chronic ouabain treatment reduces the contractile responses to the α-adrenergic agonist phenylephrine in isolated endothelium-intact thoracic aorta and superior mesenteric arteries, a reduction which has been associated with the greater release of endothelial NO [14,15]. Additionally, we have also demonstrated that ouabain-induced hypertension is accompanied by an increase in eNOS and nNOS protein expression in thoracic aorta [15].

The EFS technique is widely used to study the influence of neurotransmitters released by nervous endings on vasomotor response; NA released from perivascular...
nerve endings is the main neurotransmitter implicated in the vasoconstrictor response induced by EFS in these arteries [26]. However, the entire response depends on the balance between relaxing and contracting agents [26]. Perivascular nitricergic innervation has been described in some vascular beds, including rat mesenteric arteries, as an important source of NO, a substance that induces vasodilatation and participates in the regulation of the vascular tone [26–28]. Since hypertension induced by ouabain seems to be associated with increases in vascular NO production [15] as well as with sympathetic nerve activity [29], we analyzed the possible alterations of EFS-induced responses in mesenteric arteries from ouabain-treated rats and the possible role of neuronal NO production in these alterations.

The contractile responses induced by EFS were reduced in endothelium-denuded segments from ouabain-treated rats, compared to untreated rats. Based on previous results obtained by our group [14] the changes observed in the current work after ouabain treatment do not seem to be due to alterations in the expression of the α1 or α2 isoforms of the catalytic subunit of Na⁺, K⁺-ATPase, or in the activity of this enzyme, because in the mesenteric arteries both parameters remained unmodified by ouabain treatment. On the other hand, this reduction was not due to alterations in the NA release from perivascular nerve endings or its effects on vascular smooth muscle, since both [³H]NA release and NA-induced vasoconstrictor responses remained unmodified after ouabain chronic treatment. To determine whether alterations in NO release could account for the changes in vasoconstrictor responses induced by EFS in segments from ouabain-treated rats, arterial segments from both groups were preincubated with the non-selective NOS inhibitor L-NAME. Inhibition of NOS significantly increased the vasoconstrictor response to EFS in segments from both groups, indicating the participation of non-endothelial NO in the contractile responses induced by EFS. However, the effect of L-NAME was greater in mesenteric arteries from ouabain-treated rats than in those from untreated rats. Since the differences in EFS-induced responses between untreated and ouabain-treated rats were abolished by preincubation with L-NAME and ouabain treatment did not change NA-induced contraction, our results reflect the existence of constitutively available non-endothelial NO that would modulate EFS-induced contractile responses by vascular smooth muscle in segments from ouabain-treated rats. Similar results were observed in mesenteric arteries from spontaneously hypertensive rats (SHR), where the neuronal NO release induced by EFS is also increased [24]. These results lead us to suggest that increased neuronal NO release can be one of the mechanisms of adaptation developed to compensate for the increased total peripheral resistance subsequent to hypertension. However,
the results with respect to the alteration of NO synthesis or breakdown in hypertension are controversial. Thus, evidence of attenuated [30], unaltered [20,31] or enhanced [15,24,32,33] NO synthesis has been demonstrated in several vascular beds from hypertensive animals. In hypertension induced by ouabain, no change in the endothelial NO release induced by ACh in renal, aorta, mesenteric and caudal arteries has been reported [14,20]. However, the role of endothelial NO in the vasoconstrictor responses to phenylephrine were greater in arteries from rats treated with ouabain [15], suggesting that ouabain-induced hypertension is accompanied by increased NO release derived from endothelial NOS.

Our results suggested a role for neuronal NO in EFS-induced responses, and the nNOS inhibitor, 7-NI, was used to confirm this possibility. Although 7-NI has been used widely as a selective nNOS inhibitor [34], it has been suggested that this drug could have the same potency to inhibit eNOS and nNOS activity [34]. In rat superior mesenteric arteries, 100 μmol/l 7-NI reduced the ACh-induced relaxation, but to a lesser extent than that observed with 100 μmol/l L-NAME (unpublished results). Similar results have been found by other investigators in rat aorta [35], indicating that these drugs do not have the same potency to inhibit endothelial and neuronal NO release. On the other hand, in our experiments evaluating the role of NO on EFS-induced vasoconstrictor responses in untreated and ouabain-treated rats, we used de-endothelialized arteries, eliminating the possibility that 7-NI was affecting the NO synthesis induced by eNOS. In addition, Ayajiki et al. [36] reported that 100 μmol/l 7-NI completely abolished the neuronal NO-dependent relaxation induced by EFS or nicotine in de-endothelialized monkey cerebral arteries. 7-NI increased the vasoconstriction induced by EFS in segments from ouabain-treated rats but did not modify EFS responses in segments from untreated rats. This effect of 7-NI is not due to alterations of NA release, since this drug did not modify the tritium overflow in segments from both experimental groups. However, 7-NI reduced vasoconstrictor responses to exogenous NA to a similar extent in both groups. These results could help to explain the absence of an increase of EFS-induced contraction in segments from untreated rats, as that observed in L-NAME preincubated arteries, and support the hypothesis that an increased NO release from nNOS could be responsible for the decreased EFS-induced vasoconstriction in mesenteric arteries from ouabain-induced hypertensive rats. Reinforcing this suggestion, nNOS expression was increased in segments from ouabain hypertensive rats. Similarly increases in nNOS expression have been reported in aorta from ouabain-treated rats [15], as well as in resistance mesenteric arteries and carotid arteries from SHR [37,38].

The mechanism involved in the non-specific effects of 7-NI on NA-induced contraction is not known. However, other investigators [39,40] have reported similar results related with an alteration of cellular calcium influx [39]. In the present work, the K⁺-induced contraction was not changed by 7-NI treatment, which suggests that the calcium influx through voltage-dependent calcium channels is not inhibited. However, inhibition by 7-NI of the other calcium mobilization pathway activated by NA cannot be ruled out.

The relaxation induced by SNP was similar in NA-precontracted mesenteric arteries from ouabain-treated and untreated rats, and was unaffected by L-NAME or 7-NI. These results rule out the possibility that the greater potentiation induced by L-NAME or 7-NI in segments from ouabain-treated rats could be due to changes in the sensitivity of vascular smooth muscle to NO.

Since EFS can liberate other neurotransmitters as well as NA and NO, it is possible that other neurotransmitters release will also be altered in the mesenteric arteries from ouabain-treated rats. However, these possible alterations do not seem to have functional implications, since after NOS inhibition no differences were detected in EFS-induced responses between the two study groups.

In summary, the results presented here suggest that chronic ouabain treatment reduces vasoconstriction induced by EFS in rat mesenteric arteries. This alteration seems to be a consequence of an increased neuronal NO release, probably associated with increased nNOS protein expression. These neuronal alterations seem to constitute a counter-regulatory mechanism against the elevated blood pressure present in these animals.

Acknowledgements
We are grateful to Dr M.C. Fernández-Criado for the care of animals and Miss Rocío Baena Porras and Marta Miguel for their technical assistance.

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