

Acute and Chronic Captopril, but Not Prazosin or Nifedipine, Normalize Alterations in Adrenergic Intracellular Ca^{2+} Handling Observed in the Mesenteric Arterial Tree of Spontaneously Hypertensive Rats

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ABSTRACT

The effect of hypertension and acute (36-h) or chronic (from age 6 to 16 weeks) antihypertensive treatment with prazosin (2 mg kg^{-1} per day), nifedipine (50 mg kg^{-1} per day), or captopril (50 mg kg^{-1} per day) on Ca^{2+} mobilization due to α_1 -adrenoceptor activation was analyzed in functional studies using arterial rings [four conductance/distributing vessels: aorta, main mesenteric, iliac, and tail arteries and two resistance vessels; first and second small mesenteric artery branches obtained from spontaneously hypertensive rats (SHR, 6 and 16 weeks old) and age-matched Wistar Kyoto rats (WKY)]. Maximal response to noradrenaline in the presence of extracellular Ca^{2+} is not affected by hypertension or by the antihypertensive treatment. The extracellular Ca^{2+} -independent contractile responses increased with age in iliac, tail, and small mesenteric arteries (SMA) and were further increased in SHR in SMA from both

young and adult animals and in the main mesenteric artery of adult SHR. In main mesenteric artery, this increased contraction in SHR was associated with a higher increase in cytosolic $[\text{Ca}^{2+}]$ mobilized by noradrenaline without changes in the total stored Ca^{2+} . Acute or chronic treatment with captopril abolished the differences observed between WKY and SHR in the noradrenaline-induced contraction in mesenteric arteries loaded in Ca^{2+} -free medium. In contrast, animals acutely treated with prazosin or chronically treated with either prazosin or nifedipine exhibit the same differences in Ca^{2+} handling than untreated rats. In conclusion, these differences are not a consequence of increased blood pressure but precede it and can only be normalized by inhibition of the rennin-angiotensin system.

High blood pressure in hypertensive humans or animals is caused by an elevation of systemic resistance, due to structural and functional changes in the wall of resistance vessels. However, at present, the mechanisms by which the most important vasoactive systems, such as sympathetic nervous system or renin-angiotensin system, contribute to the rise of vascular resistance in hypertension remain unclear (Schlaich et al., 2004).

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The crucial role of the increased sympathetic nervous system tone in the pathogenesis of hypertension is mediated not only by induction of cardiac hypertrophy and vascular remodeling, which, at least in spontaneously hypertensive rats (SHR), may even precede the rise of blood pressure but also by enhanced vasoconstriction (Zicha and Kunes, 1999). In hypertension, the postsynaptic β -adrenergic functions are attenuated, whereas α_1 -adrenergic functions become dominant, although the total number of α_1 -adrenoceptors remains unchanged in most vessels. The augmented vasoconstriction mediated by α_1 -adrenoceptors in SHR could be due not only to a higher norepinephrine release from sympathetic nerve fibers but also to the hyper-reactivity of vessels or their supersensitivity to α_1 -adrenoceptor agonists (Zicha and Kunes, 1999).

Differences in Ca^{2+} handling have been also invoked to

ABBREVIATIONS: SHR, spontaneously hypertensive rat(s); WKY, Wistar Kyoto rat(s); SMA, small mesenteric arteries; SMA-1, first branch of small mesenteric artery; SMA-2, second branch of small mesenteric artery; SBP, systolic blood pressure; NA, noradrenaline.

explain the increased adrenergic tone found in hypertension. There is previous evidence indicating that cytosolic Ca^{2+} is elevated in different cell lines, including platelets and smooth muscle cells in SHR in comparison with normotensive (WKY) as well as in hypertensive rats compared with normotensive human individuals (Cortes et al., 1997; Salomonsson and Arendshorst, 2001; Kisters et al., 2004). In addition, an increase in intracellular Ca^{2+} mobilization by adrenergic agonists has been found in renal resistance vessels from SHR (Salomonsson and Arendshorst, 2001). However, most of the previous studies have been performed in cultured smooth muscle cells obtained from large conduit vessels of animals with established hypertension, but an extensive analysis about changes in Ca^{2+} handling related to adrenoceptor activation, including young and adult spontaneously hypertensive rats as well as different vessels (conductance and resistance vessels) has not been previously performed.

The aim of the present work was to analyze the Ca^{2+} mobilization due to α_1 -adrenoceptor activation in different vessels and the possible changes due to hypertension and the antihypertensive treatment. Therefore, we tested noradrenaline-induced contractile response in a Ca^{2+} -containing and a Ca^{2+} -free medium. The study was performed in four conductance/distributing vessels: aorta, main mesenteric, iliac, and tail arteries and in two resistance vessels: first and second mesenteric branches. Vascular tissues were obtained from different groups of animals: young (6-week-old) SHR and WKY; adult (16-week-old) SHR, with the hypertensive syndrome; and controls (WKY). Simultaneous measurements of cytosolic Ca^{2+} and contraction were also performed in main mesenteric arteries from WKY and SHR. We have also analyzed whether antihypertensive agents acting through different mechanisms of action such as prazosin, nifedipine, and captopril modify Ca^{2+} handling in the SHR.

Materials and Methods

Male WKY and SHR aged 6 or 16 weeks were used (Harlan Interfauna Ibérica, Barcelona, Spain) and housed under a 12-h light/dark cycle at 22°C and 60% humidity. Starting at 6 weeks, some of the rats received prazosin (2 mg kg^{-1} per day), nifedipine (50 mg kg^{-1} per day), or captopril (50 mg kg^{-1} per day) in drinking water, until the age of 16 weeks (chronic treatment), whereas others were given no drugs. Systolic blood pressure was measured weekly from the tail of unanesthetized rats using a plethysmographic method (LE 5650/6; Leticia Scientific Instruments, Barcelona, Spain). Other groups of adult animals (16 weeks old) received prazosin (2 mg kg^{-1} per day), nifedipine (50 mg kg^{-1} per day), or captopril (50 mg kg^{-1} per day) in drinking water during 36 h (acute treatment), whereas others were given no drugs. Systolic blood pressure was also measured before receiving the treatment and 36 h later, shortly before isolating the vessels. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

Tissue Preparation

Rats were weighed and decapitated, and the selected vessels (thoracic aorta, tail, iliac, and mesenteric arteries) were removed. Vessels were placed in Krebs' solution, cleaned of adipose tissue, denuded of endothelium by gentle rubbing and suspended in a 10-ml organ bath containing Krebs' solution, maintained at 37°C and gassed with 95% O_2 and 5% CO_2 . An initial load of 1 g was applied to each preparation and maintained throughout a 75- to 90-min

equilibration period. Tension was recorded isometrically by Grass FTO3 force-displacement transducers.

Mesenteric arterial trees were dissected and a ring segment (2 mm in length) from first (SMA-1) or second (SMA-2) branch was mounted in a myograph (J.P. Trading, Aarhus, Denmark) with separate 5-ml organ baths. After a 30-min stabilization period, the internal diameter of each vessel was set to a tension equivalent to 0.9 times the estimated diameter at 100 mm Hg effective transmural pressure ($I_{100} = 252\text{--}457 \mu\text{m}$ for SMA-1 and $I_{100} = 182\text{--}401 \mu\text{m}$ for SMA-2) according to the standard procedure (Mulvany and Halpern, 1977). Data were recorded on a disc (Mac Lab ADInstruments Pty Ltd., Castle Hill, Australia).

Evaluation of the Contractile Response to Noradrenaline

Two different protocols have been followed:

Contractile Response to Noradrenaline in Ca^{2+} -Containing Medium. A concentration-response curve to noradrenaline (0.1 nM–30 μM) was performed in each vessel until the maximal response was obtained. From these curves, pD_2 and E_{max} were calculated using a nonlinear regression plot (GraphPad Software Inc., San Diego, CA). Maximal contractions were expressed in milligrams of developed tension.

Contractile Response to Noradrenaline in Ca^{2+} -Free Medium. The experimental procedure designed to analyze the participation of internal Ca^{2+} stores in the contractile response to noradrenaline was performed according to previous works (Noguera and D'Ocon, 1993). Initially, a maximal contractile response to noradrenaline was obtained using a different concentration of noradrenaline in each vessel: 1 μM in the aorta; 10 μM in iliac, tail, and main mesenteric arteries; and 30 μM in SMA. After drug washout, the preparations were placed in a Ca^{2+} -free solution (containing 0.1 mM EDTA) for 20 min, which led to a weak loss in tension (<10–15%). Finally, vessels were exposed to the same concentration of noradrenaline in Ca^{2+} -free solution for 10 min. The contractile response was measured 1 min (phasic response) and 10 min (tonic response) after agonist addition. Contractions in Ca^{2+} -free medium were expressed as a percentage of the maximal noradrenaline-induced contractions obtained in Ca^{2+} -containing solution.

In another set of experiments using the specific α_1 -agonist phenylephrine instead of noradrenaline, similar phenylephrine-induced contractions were obtained in Ca^{2+} -free medium in the same vessels from Wistar rats, ruling out a possible role for β - or α_2 -adrenoceptors in this response. In addition, the fact that prazosin, a specific α_1 -adrenoceptor blocker, completely inhibits this response confirms that α_1 -adrenoceptors are responsible for this contraction (data not shown).

Simultaneous Measurements of $[\text{Ca}^{2+}]_i$ and Tension

Main mesenteric rings were incubated for 1.5 to 2 h at room temperature in Krebs' solution containing the fluorescent dye Fura-2 acetoxyethyl ester (5 μM). The castor oil derivative Cremophor EL (final concentration in Krebs' 0.05%) was used to solubilize and facilitate Fura-2 acetoxyethyl ester penetration. Arterial vessels were then suspended under 1 g of tension in a 5-ml organ bath containing Krebs' solution, maintained at 37°C and gassed with 95% O_2 and 5% CO_2 . The bath was part of a fluorimeter (CAF 110; Jasco, Tokyo, Japan) that allows the estimation of changes in the fluorescence intensity of Fura-2 simultaneously with force development (Kanaide, 1999; Perez-Vizcaino et al., 1999). Rings were alternatively illuminated (128 Hz) through the adventitial side with two excitation wavelengths (340 and 380 nm) from a xenon lamp coupled with two monochromators. The emitted fluorescent light at the two excitation wavelengths (F340 and F380) was measured by a photomultiplier through a 510-nm filter and recorded by using data acquisition hardware (Mac Lab, model 8e; ADInstruments Pty Ltd.) and data recording software (Chart version 3.2; ADInstruments Pty Ltd.). Force data were recorded simultaneously by an isometric force-

displacement transducer coupled to the Mac Lab data acquisition system as described previously (Gisbert et al., 2003). In these experiments, after equilibration for 45 min, an initial response to 10 μM noradrenaline was obtained. After drug washout, the preparations were placed in a Ca²⁺-free solution (containing 0.1 mM EDTA) for 20 min and exposed again to noradrenaline in Ca²⁺-free solution. After washing in Ca²⁺-free solution, vessels were exposed to 14 μM ionomycin. Finally, 2 mM CaCl₂ and 8 mM EGTA were sequentially added to calibrate the signal as described previously (Kanaide, 1999; Gisbert et al., 2003). Peak increases in [Ca²⁺]_{cytosolic} induced by noradrenaline in the absence of extracellular Ca²⁺ are expressed as a percentage of the total Ca²⁺ content (noradrenaline plus ionomycin-induced Ca²⁺ release).

Data Analysis

The results are presented as the mean \pm S.E.M. for *n* determinations obtained from different animals. The concentration ($-\log$ [M]) of noradrenaline required to produce 50% of the maximal response (pD₂) was obtained from a nonlinear regression plot (GraphPad Software Inc.). Statistically significant differences between groups were calculated by an analysis of variance test. Where analysis of variance showed significant differences ($P < 0.05$), the results were further analyzed using the Student-Newman-Keuls test, and differences were considered significant when $P < 0.05$.

Drugs and Solutions

The composition of the Ca²⁺-containing solution (Krebs' solution) was 118 mmol/l NaCl, 4.75 mmol/l KCl, 1.8 mmol/l CaCl₂, 1.2 mmol/l MgCl₂, 1.2 mmol/l KH₂PO₄, 25 mmol/l NaHCO₃, and 11 mmol/l glucose. In Ca²⁺-free solution, CaCl₂ was omitted and 0.1 mM EDTA was added. The following drugs were obtained from Sigma-Aldrich (St. Louis, MO): (-)-noradrenaline, Cremophor EL, ionomycin, prazosin, nifedipine, and captopril. Fura-2 acetoxyethyl ester (1 μM solution in dimethyl sulfoxide) was from Calbiochem (San Diego, CA). Other reagents were of analytical grade. All compounds were dissolved initially in distilled water except ionomycin, which was dissolved in absolute ethanol. Prazosin and captopril were dissolved in drinking water for the treatment of the animals. Nifedipine was prepared in absolute ethanol and diluted in drinking water until a final concentration of 0.02% ethanol.

Results

Systolic Blood Pressure

In young animals (6 weeks old), systolic blood pressure (SBP) values were not significantly different in WKY and SHR (Table 1). In untreated adult animals (16 weeks old), SBP was significantly higher ($P < 0.001$) in SHR than in WKY (Table 1). However, SBP values in SHR treated with

TABLE 1

Mean systolic blood pressure (mm Hg) in young (6-week-old), adult (16-week-old), and adult pretreated with prazosin (2 mg kg⁻¹ per day), nifedipine (50 mg kg⁻¹ per day), and captopril (50 mg kg⁻¹ per day) during 36 h (acute treatment) or 10 weeks (chronic treatment from 6 to 16 weeks old)

Values were determined 1 h before the animals were sacrificed and are expressed as the mean \pm S.E.M. of *n* = 8 to 15 animals.

	Treatment	WKY	SHR
Young		116 \pm 3	125 \pm 10
Adult		119 \pm 7	184 \pm 6***
+ prazosin	Acute	99 \pm 3*	118 \pm 2
	Chronic	118 \pm 3	125 \pm 6
+ nifedipine	Acute		
	Chronic	121 \pm 4	119 \pm 5
+ captopril	Acute	110 \pm 4	107 \pm 3
	Chronic	104 \pm 2*	106 \pm 2

* $P < 0.05$ and *** $P < 0.001$ versus nontreated WKY.

prazosin, captopril, or nifedipine (acute or chronic) were not significantly different from those in treated WKY (Table 1).

The treatments had no effect on SBP in WKY, except in the group of acute prazosin and chronic captopril treatments, in which a slight decrease in the SBP values was obtained in WKY compared with their untreated controls (Table 1).

Noradrenaline-Induced Contractile Response in Ca²⁺-Containing Solution

In aortic rings from young or adult SHR, a significantly smaller maximal aortic contraction was observed compared with WKY (Table 2). In the rest of the vessels, no significant differences were found between strains. In addition, in tail and SMA noradrenaline-induced maximal contractile response significantly increased with age in both normotensive and hypertensive rats (Table 2).

Comparative analysis of the maximal contractile response obtained in each group of animals that received acute or chronic antihypertensive treatment evidenced that these treatments did not change the maximal response to noradrenaline in iliac, tail, main mesenteric, or small mesenteric branches (data not shown). The same occurs in aorta obtained from rats that received acute treatments with the antihypertensive agents (Fig. 1). In contrast, noradrenaline-induced contractions in aorta of chronically treated WKY were significantly smaller than those obtained in control animals ($P < 0.001$); therefore, no differences were detectable between treated WKY and SHR (Fig. 1).

In adult animals, the potency of noradrenaline (pD₂) was significantly higher in aorta than in the other vessels, but no significant differences were found between SHR and WKY groups. None of the antihypertensive agents significantly changed this parameter (Table 3).

Noradrenaline-Induced Contractile Response in Ca²⁺-Free Medium

Noradrenaline-induced contractions in the absence of Ca²⁺ were tested following the experimental procedure described under *Materials and Methods*. The profile of these contractile responses is different depending on the vessel, the age, the hypertensive state, and the treatment with antihypertensive agents.

Untreated Animals. In young animals (6 weeks old), NA induced a phasic contractile response in all vessels. Only in aorta and mesenteric artery was the initial peak (phasic response) followed by a smaller plateau phase (tonic response) that persisted until the tissue was washed (10 min later). The contractile response to noradrenaline in Ca²⁺-free medium was significantly higher in SMA from young SHR relative to WKY (Table 4). The response to NA in Ca²⁺-free medium changed with age and hypertension. If we compare the magnitude of contraction between adult and young WKY, we can observe an increase in the response in adult rats, except in aorta and main mesenteric artery (Table 4). The maximal phasic response observed in SMA obtained from adult hypertensive SHR was significantly higher relative to that obtained in the same vessels from normotensive WKY, and persisted until the tissue was washed (tonic component). Moreover, a similar change was observed in main mesenteric artery from adult SHR (Table 4; Fig. 2). In iliac and tail arteries, maximal phasic responses did not change significantly, although a slight increase in the duration of the

TABLE 2

Maximal contractile response to noradrenaline in Ca^{2+} -containing medium in the arterial vasculature of young (6-week-old) and adult (16-week-old) WKY or SHR

Values are expressed in milligrams of contraction (mean \pm S.E.M.). Values in parentheses represent the number of experiments.

	Young		Adult	
	WKY	SHR	WKY	SHR
Aorta	809.0 \pm 47.1 (7)	445.5 \pm 60.1*** (5)	836.1 \pm 68.2 (16)	379.6 \pm 24.9*** (18)
Iliac	262.0 \pm 42.1 (6)	435.1 \pm 88.5 (7)	372.7 \pm 53.8 (11)	385.2 \pm 54.9 (8)
Tail	152.5 \pm 27.4 (5)	160.2 \pm 26.1 (3)	439.5 \pm 106.6 ^a (8)	489.8 \pm 51.2 ^a (15)
MMA	402.5 \pm 29.4 (9)	360.4 \pm 63.7 (5)	379.7 \pm 38.0 (14)	358.5 \pm 19.5 (20)
SMA-1	786.7 \pm 102.2 (6)	708.0 \pm 81.6 (3)	1320.4 \pm 98.3 ^a (12)	1495.9 \pm 98.85 ^a (20)
SMA-2	435.7 \pm 163.3 (4)	581.6 \pm 51.0 (4)	721.4 \pm 58.2 ^a (12)	941.8 \pm 77.6 ^a (20)

MMA, main mesenteric artery.

^a Value versus young rats.

*** $P < 0.001$ SHR versus WKY.

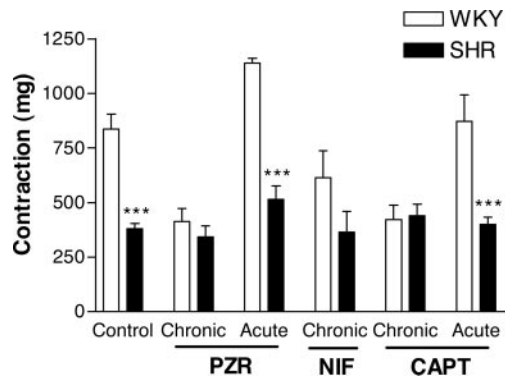


Fig. 1. Effect of chronic (from age 6 to 16 weeks) or acute (36-h) treatment with prazosin (2 mg kg^{-1} per day), nifedipine (50 mg kg^{-1} per day), or captopril (50 mg kg^{-1} per day) on noradrenaline-induced contractile response in the presence of Ca^{2+} in aorta from adult (16-week-old) WKY (white columns) and SHR (black columns). *, $P < 0.05$; ***, $P < 0.001$ versus WKY.

response was observed in adult SHR, i.e., the response was biphasic in these vessels, whereas a monophasic response was observed in WKY (Table 4). In aorta, no appreciable changes were observed.

Animals Pretreated with Different Antihypertensive Agents. In animals acutely treated with prazosin or chronically treated with either prazosin or nifedipine, no changes were observed in the noradrenaline-induced contraction in Ca^{2+} -free medium in any vessel. Difference between WKY and SHR remained as in untreated controls (Table 5). In aorta, iliac, and tail arteries from acutely (36-h) captopril-treated rats, NA-induced contractions were also similar to those obtained in untreated animals, i.e., no differences were found between SHR and WKY as in control experiments (Table 5). In main mesenteric artery from SHR, a slight, not significant decrease in the response to NA was observed (Fig. 2). In SMA obtained from acutely captopril-treated SHR, contractile responses to NA in Ca^{2+} -free medium were significantly decreased compared with untreated animals (Fig. 2). Thus, the differences between SHR and WKY animals on NA-induced contractile responses in SMA were abolished by acute captopril treatment, and these responses were not significantly different from control WKY (Table 5). In chronically captopril-treated animals, a decrease in the response to NA in Ca^{2+} -free medium was observed in all vessels from both WKY and SHR (Table 5). This decrease was statistically significant in arteries from the mesenteric tree (Fig. 2). Moreover, in these arteries, no differences in the noradrenaline-

induced response in Ca^{2+} -free medium were detectable between SHR and WKY (Table 5).

Simultaneous Measurements of $[\text{Ca}^{2+}]_i$ and Tension

To analyze whether the increase in the contractile responses to noradrenaline in Ca^{2+} -free medium in SHR depends on alterations in intracellular Ca^{2+} handling, experiments of simultaneous measurements of $[\text{Ca}^{2+}]_i$ and tension were performed in main mesenteric artery from WKY and SHR.

The results on contractile force in the arteries mounted in the fluorimeter (Fig. 3, A and B) were similar to those in the conventional organ bath. The maximal response to 10 μM noradrenaline in the presence of Ca^{2+} was not significantly different in WKY and SHR (457 \pm 36 mg, $n = 7$, and 383 \pm 53 mg, $n = 5$, respectively). The contractile responses induced by noradrenaline in the presence of Ca^{2+} were accompanied by a sustained increase in $[\text{Ca}^{2+}]_i$, reflecting both Ca^{2+} release from the intracellular stores plus additional Ca^{2+} entry from the extracellular medium. Upon washing in Ca^{2+} -free medium, there was a decrease in $[\text{Ca}^{2+}]_i$ below the baseline in both strains with minor effects on resting tone (Fig. 3, A and B).

In Ca^{2+} -free medium, mesenteric arteries developed a significantly higher contractile response induced by noradrenaline in SHR than in WKY (Fig. 3B) as has been described in the above-mentioned experiments. The increases in $[\text{Ca}^{2+}]_i$ induced by noradrenaline in Ca^{2+} -free medium, which represent only the magnitude of noradrenaline-induced Ca^{2+} released from the intracellular stores, were transient (Fig. 3). Subsequent addition of ionomycin also induced a transient increase in $[\text{Ca}^{2+}]_i$, which represents an estimation of the Ca^{2+} remaining in the intracellular stores. Thus, the total intracellular Ca^{2+} content can be estimated following this protocol by the Ca^{2+} released by noradrenaline plus the remaining released by ionomycin. The total intracellular Ca^{2+} content, estimated by the sum of these two responses in absolute values (i.e., $\Delta [\text{Ca}^{2+}]_{\text{noradrenaline}} + \Delta [\text{Ca}^{2+}]_{\text{ionomycin}}$), was similar in WKY and SHR (191 \pm 57 nM, $n = 7$, and 171 \pm 50 nM, $n = 5$, respectively). However, consistent with the increased contractile response-induced by noradrenaline in Ca^{2+} -free medium, the fraction of the total Ca^{2+} content released by noradrenaline was significantly higher in SHR than in WKY (Fig. 3C).

Discussion

The present study was designed to analyze the extracellular Ca^{2+} -dependent and -independent contractile responses induced by stimulation of α_1 -adrenoceptors with noradrena-

TABLE 3

pD₂ values for noradrenaline in the arterial vasculature of adult (16-week-old) WKY and SHR untreated or pretreated with prazosin (2 mg kg⁻¹ per day), nifedipine (50 mg kg⁻¹ per day), and captopril (50 mg kg⁻¹ per day) during 10 weeks (chronic treatment from 6 to 16 weeks old). Values are expressed in milligrams of contraction (mean ± S.E.M., *n* = 6–10 experiments).

	Untreated		Prazosin		Nifedipine		Captopril	
	WKY	SHR	WKY	SHR	WKY	SHR	WKY	SHR
Aorta	8.3 ± 0.1	8.0 ± 0.1	8.2 ± 0.2	8.3 ± 0.2	8.5 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	7.9 ± 0.2
Iliac	7.2 ± 0.2	7.0 ± 0.1	7.2 ± 0.3	7.1 ± 0.2	6.8 ± 0.2	6.8 ± 0.1	6.7 ± 0.3	6.8 ± 0.1
Tail	6.3 ± 0.1	6.0 ± 0.1	6.2 ± 0.2	5.7 ± 0.2	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1
MMA	7.2 ± 0.2	6.8 ± 0.1	6.8 ± 0.2	7.1 ± 0.2	6.5 ± 0.3	6.6 ± 0.1	6.4 ± 0.1	6.6 ± 0.2
SMA-1	5.9 ± 0.1	5.8 ± 0.2	5.9 ± 0.2	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
SMA-2	5.9 ± 0.2	6.1 ± 0.2	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	6.1 ± 0.1

MMA, main mesenteric artery.

TABLE 4

Maximal contractile responses to noradrenaline in Ca²⁺-free medium in the arterial vasculature of young (6-week-old) and adult (16-week-old) WKY or SHR

Values are expressed as a percentage of the contractile response to noradrenaline in Ca²⁺-containing medium (mean ± S.E.M.). Values in parentheses represent the number of experiments.

		Young		Adult	
		WKY	SHR	WKY	SHR
Aorta	Phasic	27.3 ± 2.5	30.1 ± 7.8	33.9 ± 4.1	34.4 ± 4.0
	Tonic	15.7 ± 3.4 (8)	18.0 ± 7.2 (6)	16.6 ± 1.7 (15)	17.6 ± 2.3 (15)
Iliac	Phasic	1.1 ± 0.7 (8)	2.3 ± 0.8 (5)	9.4 ± 2.8 [†] (8)	16.7 ± 2.2 ^{††} (16)
	Tonic				6.5 ± 1.8 (9)
Tail	Phasic	7.6 ± 2.7 (6)	11.3 ± 4.3 (3)	22.3 ± 3.4 ^{†††} (7)	26.1 ± 2.4 [†] (9)
	Tonic				5.0 ± 1.1 (9)
Main mesenteric	Phasic	22.8 ± 2.9 (7)	21.6 ± 3.0 (9)	18.2 ± 2.4 (14)	32.7 ± 3.0 ^{***†} (17)
	Tonic	3.5 ± 0.7 (7)	9.6 ± 2.7 (9)	5.3 ± 1.5 (14)	11.4 ± 1.3 ^{**} (17)
SMA-1	Phasic	3.0 ± 1.9 (6)	9.7 ± 4.4* (3)	9.8 ± 1.9 [†] (10)	31.5 ± 3.9 ^{***†} (11)
	Tonic				7.0 ± 1.4 (11)
SMA-2	Phasic	0.9 ± 0.6 (5)	5.9 ± 1.9* (3)	9.1 ± 2.2 [†] (9)	19.5 ± 2.1 ^{***†} (16)
	Tonic				3.2 ± 0.4 (16)

* *P* < 0.05, ** *P* < 0.01, and *** *P* < 0.001 SHR versus WKY.

[†] *P* < 0.05, ^{††} *P* < 0.01, and ^{†††} *P* < 0.001 versus young rats.

line in several conductance and resistance arteries and its changes with hypertension. Vessels were obtained from young and adult spontaneously hypertensive rats, young and adult normotensive WKY, and adult SHR and WKY in which blood pressure was normalized with antihypertensive agents as shown by measurement of tail blood pressure.

Noradrenaline contracts vascular smooth muscle through the activation of α₁-adrenoceptors (Guimaraes and Moura, 2001; Piascik and Perez, 2001). Activation of these receptors induces an increase in cytosolic Ca²⁺ due to both Ca²⁺ entry from the extracellular medium and Ca²⁺ release from intracellular stores (Zhong and Minneman, 1999; Piascik and Perez, 2001; Gisbert et al., 2003). Therefore, higher and sustained contractions were observed in the presence of extracellular Ca²⁺ in all vessels, whereas lower and, in some vessels, transient responses were obtained in a Ca²⁺-free medium.

In some vessels such as tail artery or SMA (first and second branch), maximal response to noradrenaline increases with age in both normotensive and hypertensive animals. Similar results were described previously in the contractile response to phenylephrine, but not to caffeine, in SMA from old (21–22 months) versus young (3–4 months) rats (Rubio et al., 2002), suggesting that differences in Ca²⁺ handling, i.e., an increase in the total number of α₁-adrenoceptors or a higher

efficiency of coupling to the transduction systems, takes place with age. In young and adult animals, no significant changes in the potency and maximal contractile response to noradrenaline in the presence of extracellular Ca²⁺ were observed in any vessel between SHR and WKY, except in the aorta, where a decreased maximal response to noradrenaline without changes in the potency was observed in hypertensive animals. In addition, this lower aortic contraction observed in SHR was not modified by acute treatment with the anti-hypertensive agents, even when they normalized the values of blood pressure. Paradoxically, the difference in noradrenaline response between strains was corrected by the chronic treatment with prazosin, captopril, or nifedipine, i.e., a decrease in noradrenaline-induced response was only observed in WKY, suggesting that the different response observed could be a characteristic of the WKY strain not related to arterial blood pressure. Therefore, these results exclude a hyper-reactivity or a supersensitivity of isolated arterial vessels to adrenergic stimulus associated to genetic hypertension.

Interestingly, experiments in a Ca²⁺-free medium yielded different results. In this case, addition of noradrenaline induces a contraction that can be used as an index of the content of agonist-sensitive intracellular Ca²⁺ stores. Except in aorta, appreciable differences in this response were found

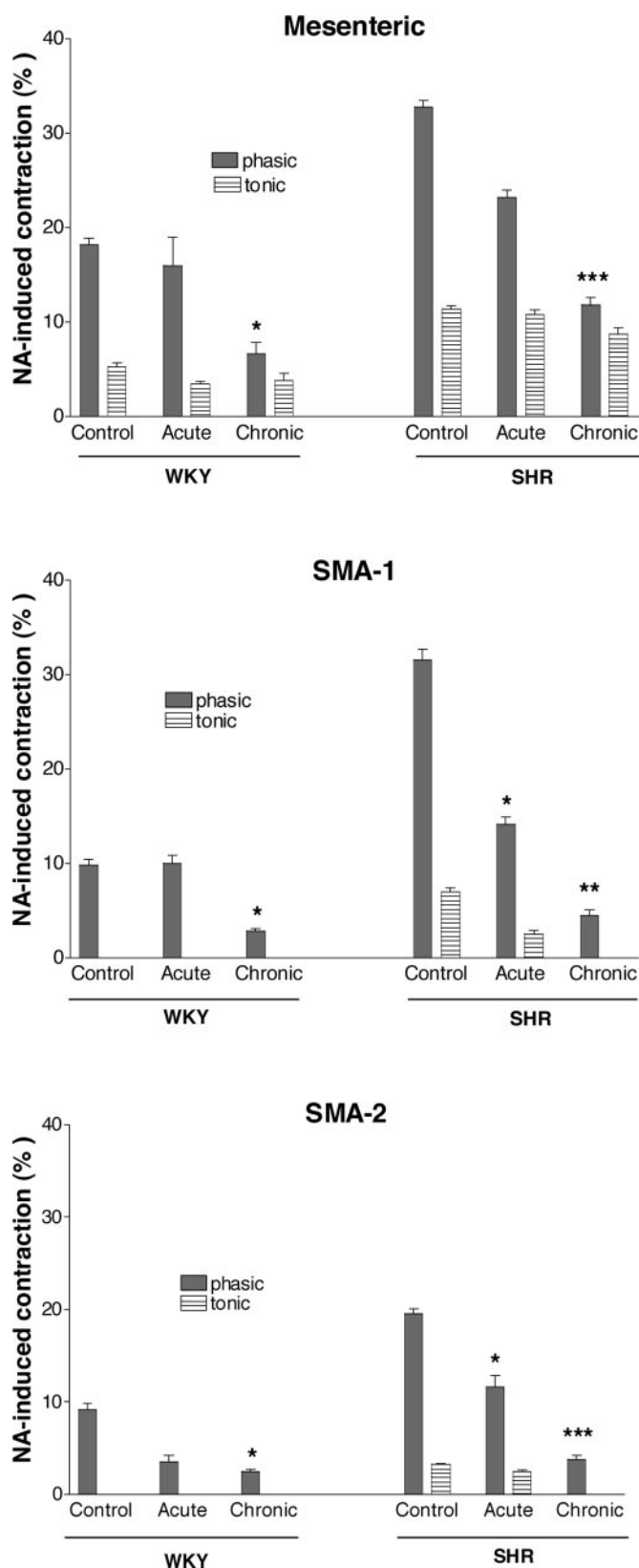


Fig. 2. Effect of chronic (from age 6 to 16 weeks) or acute (36-h) treatment with captopril (50 mg kg^{-1} per day) on the phasic and tonic component of the contractile response to noradrenaline in Ca^{2+} -free medium in the arterial vasculature of adult (16-week-old) WKY and SHR. Values are expressed as a percentage of the contractile response to noradrenaline in Ca^{2+} -containing medium. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus WKY or SHR controls.

depending on age and between strains. In iliac, tail, and SMA, an age-dependent increase in noradrenaline-induced contractions was observed, suggesting that internal Ca^{2+} stores mobilized by α_1 -adrenergic stimulus increases with age. With regard to the hypertension, in SMA (first and second branch) obtained from young normotensive SHR, we observed an increased maximal adrenergic response compared with WKY. This increased response in SHR versus WKY was observed even in young animals, which show blood pressure values in the normal range. Thus, changes in Ca^{2+} handling precede the increment in blood pressure in SHR.

In the adult animals with high blood pressure, we observed similar results in SMA but, the increased responsiveness was also extended to mesenteric arteries. Thus, the present results suggest a difference in Ca^{2+} handling of the intracellular Ca^{2+} stores sensitive to adrenergic stimulation and between SHR and WKY mesenteric arterial vessels both in young and adult rats. Similar results were found by Salomonsson and Arendshorst (2001) in renal resistance vessels: these authors found an increased intracellular Ca^{2+} mobilization from an inositol-1,4,5-triphosphate-sensitive pool stimulated by α -adrenoceptors in arterioles from young SHR compared with WKY.

The higher contractile response in Ca^{2+} -free medium observed in vessels from SHR might reflect an increase in the total amount of stored Ca^{2+} , an increase in the Ca^{2+} pool released by α_1 -adrenoceptor activation in these vessels, or a different efficacy of cytosolic Ca^{2+} to induce a contractile response (i.e., different Ca^{2+} sensitivity). Therefore, we analyzed the changes in $[\text{Ca}^{2+}]$ simultaneously with the recording of contractile force in main mesenteric arteries from adult WKY and SHR. Noradrenaline-sensitive Ca^{2+} pool was estimated by the peak increase in $[\text{Ca}^{2+}]$ induced by this agonist in the absence of extracellular Ca^{2+} and the remaining noradrenaline-insensitive pool by the peak $[\text{Ca}^{2+}]$ increase induced by the ionophore ionomycin that fully releases Ca^{2+} from intracellular stores (Morgan and Jacob, 1994). Although a previous study (Kisters et al., 2004) indicates that the total amount of intracellular Ca^{2+} is higher in aortic smooth muscle cells from SHR, our present results indicate that the total releasable Ca^{2+} in main mesenteric arteries, i.e., the sum of NA released plus ionomycin released, was similar in both strains. However, noradrenaline-sensitive Ca^{2+} release was higher in SHR than in WKY. Therefore, in SHR, an increased fraction of the total intracellular Ca^{2+} pools was available to be released by noradrenaline. These results explain the differences observed in noradrenaline-induced contraction in Ca^{2+} -free medium and confirm the difference in Ca^{2+} handling between strains previously described by other authors (Neusser et al., 1994; Cortes et al., 1997) in cultured vascular smooth muscle cells. Corroborating previous results (Bian and Bukoski, 1995), enhanced myofilament Ca^{2+} sensitivity is unlikely to contribute to the increased contraction observed in SHR vessels.

The next questions we addressed were whether the change in noradrenaline-sensitive Ca^{2+} was associated with the elevated blood pressure and/or whether it was a consequence of the vascular remodeling characteristic of the SHR vessels (Arribas et al., 1997; Zicha and Kunes, 1999; Safar et al., 2001). To answer these questions, we analyzed the effects of three antihypertensive drugs extensively used therapeutically and that have proven effects of not only decreasing

TABLE 5

Phasic contractile responses to maximal concentrations of noradrenaline in Ca²⁺-free medium in the arterial vasculature of adult WKY and SHR untreated or pretreated with prazosin (2 mg kg⁻¹ per day), nifedipine (50 mg kg⁻¹ per day), or captopril (50 mg kg⁻¹ per day) from age 6 to 16 weeks (chronic treatment)

Values are expressed as a percentage of the contractile response to noradrenaline in Ca²⁺ containing medium (mean ± S.E.M. of n = 6–12 animals).

		Untreated	Prazosin		Nifedipine	Captopril	
			Acute	Chronic	Chronic	Acute	Chronic
Aorta	SHR	34.4 ± 4.0	48.4 ± 5.5	37.3 ± 3.2	29.9 ± 4.9	35.5 ± 3.8	26.2 ± 2.9
	WKY	33.9 ± 4.1	41.6 ± 3.5	33.6 ± 2.9	37.9 ± 1.8	29.1 ± 2.3	22.8 ± 4.8
Iliac	SHR	16.7 ± 2.2	15.4 ± 4.0	12.2 ± 1.2	18.2 ± 3.9	16.0 ± 4.9	6.3 ± 1.8
	WKY	9.4 ± 2.8	11.0 ± 1.6	7.5 ± 2.9	9.7 ± 3.1	9.6 ± 3.3	5.9 ± 2.0
Tail	SHR	26.1 ± 2.4	33.5 ± 5.7	27.0 ± 6.7		30.6 ± 8.4	14.7 ± 2.2
	WKY	22.3 ± 3.4	26.2 ± 6.2	16.2 ± 4.0		22.0 ± 6.1	11.3 ± 2.9
MMA	SHR	32.7 ± 3.0**	44.3 ± 7.1*	25.3 ± 1.7	23.6 ± 1.5*	23.2 ± 2.1	11.8 ± 2.0
	WKY	18.2 ± 2.4	25.5 ± 2.4	18.8 ± 3.4	11.1 ± 0.7	16.0 ± 5.9	6.7 ± 2.9
SMA-1	SHR	31.5 ± 3.9***	30.1 ± 4.9*	29.8 ± 7.6**	27.7 ± 7.5*	14.1 ± 1.9	4.5 ± 1.2
	WKY	9.8 ± 1.9	6.2 ± 3.3	6.0 ± 2.6	10.3 ± 3.6	10.0 ± 1.8	2.8 ± 0.7
SMA-2	SHR	19.5 ± 2.1**	43.0 ± 8.0***	24.5 ± 3.5*	17.8 ± 4.0*	11.6 ± 2.7	3.8 ± 0.7
	WKY	9.1 ± 2.2	5.9 ± 3.7	9.7 ± 2.9	7.6 ± 2.1	3.5 ± 1.5	2.4 ± 0.7

MMA, main mesenteric artery.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus WKY.

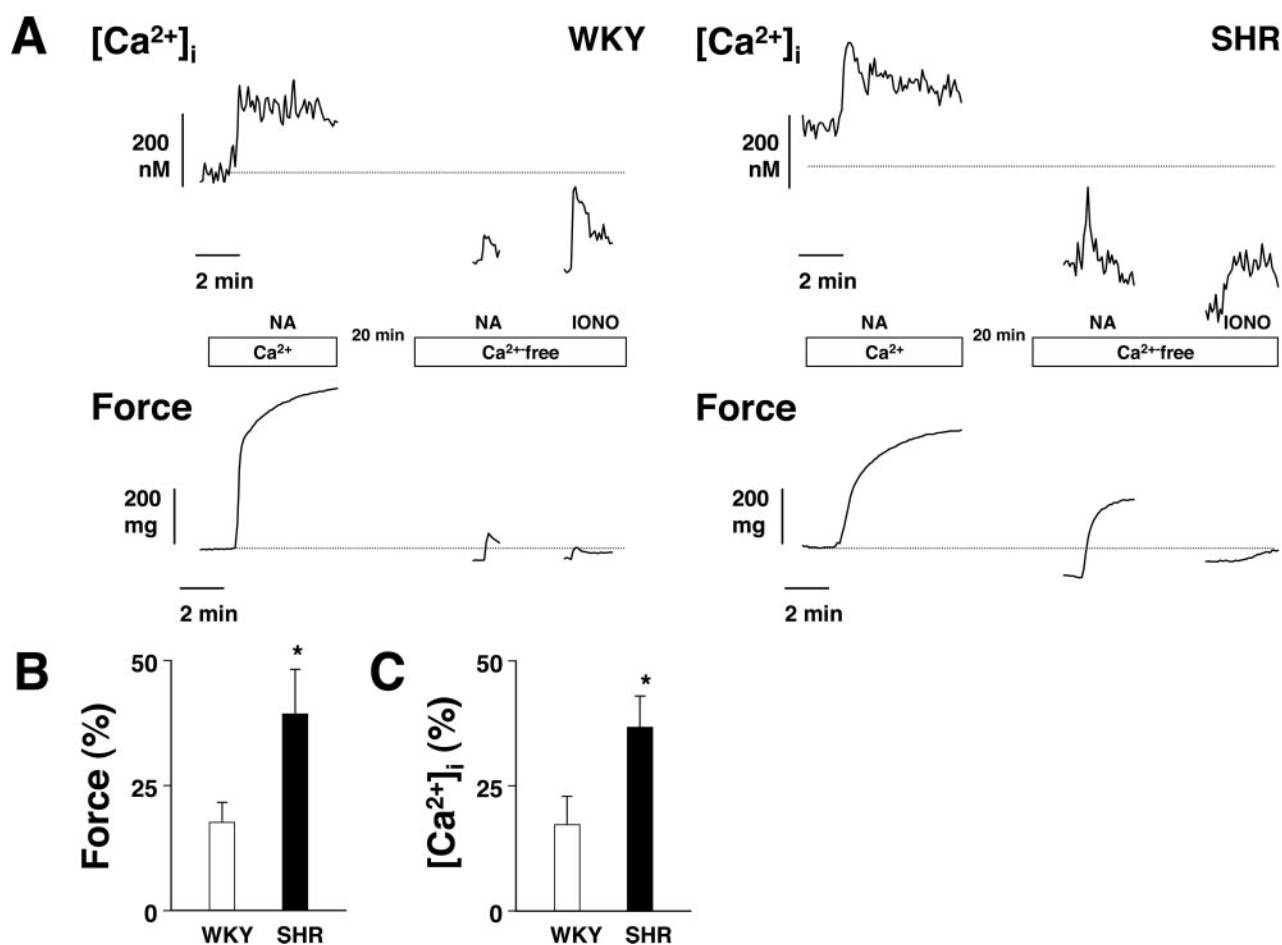


Fig. 3. A, simultaneous recordings of [Ca²⁺]_i (top traces) and contractile force (bottom traces) in main mesenteric arteries isolated from WKY (left) and SHR (right) loaded with fluorescent Ca²⁺ dye Fura-2. B, contractile responses induced by noradrenaline in the absence of extracellular Ca²⁺ expressed as a percentage of the response in Ca²⁺-containing medium. C, peak increases in [Ca²⁺]_i induced by noradrenaline in the absence of extracellular Ca²⁺ expressed as a percentage of the total Ca²⁺ content (noradrenaline- plus ionomycin-induced Ca²⁺ release). Results in B and C are means ± S.E.M. of five to seven experiments as those shown in A.

blood pressure but also of inducing the regression of cardiac hypertrophy, the vascular remodeling, and the endothelial dysfunction characteristics of the hypertensive state in humans and animals (Strauer 1988; Christensen et al., 1989; Lee et al., 1991; Onaka et al., 1998; Intengan et al., 1999; Tea

et al., 1999; Bravo et al., 2001; Ruilope and Schiffrin, 2001; Pontremoli et al., 2001; Farkas et al., 2001; Raasch et al., 2002; Hale et al., 2003). We used prazosin, a selective α_1 -adrenoceptor antagonist; nifedipine, a Ca²⁺ channel blocker; and captopril, an angiotensin-converting enzyme inhibitor,

and we designed two different treatments, acute and chronic. The goal of the acute treatment was to normalize blood pressure in adult hypertensive animals, whereas with the chronic treatment we aimed to prevent or minimize the structural and functional changes resulting from a sustained hypertensive state as has been cited previously.

Our results show that prazosin and nifedipine, which normalized blood pressure after acute treatment and avoided the hypertensive state after chronic administration, did not modify the increased noradrenaline-induced contraction in mesenteric vessels from SHR. However, acute treatment with captopril normalizes blood pressure and the contractile response to noradrenaline in these vessels. In addition, chronic captopril treatment significantly decreased the noradrenaline-induced contraction in Ca^{2+} -free medium in both WKY and SHR. From these results, we can draw three interesting conclusions. First, the increased Ca^{2+} pool sensitive to noradrenaline is not related to vascular remodeling because acute treatment with captopril for 36 h, which is not sufficient to normalize the vessel structure, reduced this response. Second, it is not a consequence of the increased blood pressure because it was present in young SHR in which blood pressure was still similar to young WKY and in adult SHR with normalized blood pressure by chronic treatment with prazosin or nifedipine. And third, the noradrenaline-sensitive Ca^{2+} pool is modulated by angiotensin and not directly by the α_1 -adrenoceptor since acute and chronic treatment with the selective α_1 -antagonist prazosin had no effect but the angiotensin-converting enzyme inhibitor captopril normalized (acute treatment) or even decreased it (chronic treatment). In addition, the lack of effect of chronic nifedipine suggests that inhibition of Ca^{2+} entry through L-channels is not a decisive factor regulating the Ca^{2+} content of this pool.

The fact that changes in Ca^{2+} handling can be dissociated to the elevated blood pressure (e.g., young SHR and adult nifedipine- or prazosin-treated SHR show increased Ca^{2+} release but normal blood pressure) clearly indicates that increased Ca^{2+} release is not acutely responsible for the elevated blood pressure in SHR. In fact, differences in contractile responses were only observed when vessels were "artificially" incubated in a Ca^{2+} -free solution. Therefore, these data by themselves do not explain why SHR are hypertensive. What these data clearly indicate is that the mechanisms regulating Ca^{2+} homeostasis are different in WKY and SHR. In spite of similar contractile responses in Ca^{2+} -containing medium in SHR and WKY, the different mechanisms for Ca^{2+} regulation suggest a different subcellular distribution of Ca^{2+} that might modulate the activity of other Ca^{2+} -dependent proteins and even alter gene expression. This different Ca^{2+} handling is clearly not a consequence of elevated blood pressure, but it is triggered by the same stimulus, i.e., the renin-angiotensin system, and is only prevented or normalized by inhibition of this system and not by other antihypertensive strategies. Whether in the long term these altered mechanisms for Ca^{2+} handling contribute to the pathophysiology of hypertension or it is just an epiphenomenon is presently unknown.

In summary, our study presents novel findings regarding differences in internal Ca^{2+} handling during stimulation of α_1 -adrenoceptors in conductance and resistance mesenteric vessels from SHR and WKY. These differences are not a consequence of increased blood pressure, but precede it, and

they can only be normalized by inhibition of the renin-angiotensin system.

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