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Cyclooxygenase pathway is involved in the vascular reactivity and inhibition of the Na^+, K^+-ATPase activity in the tail artery from L-NAME-treated rats

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Abstract

L-NAME (LN) induces hypertension by blocking nitric oxide (NO) synthesis. It produces vascular hyperreactivity to phenylephrine (PHE) associated with a reduced vascular Na^+, K^+-ATPase activity. The aim of this work was to investigate whether products of the cyclooxygenase pathway are involved in alterations of vascular reactivity and Na^+-pump activity in the tail artery from LN-induced hypertension rats. Four groups of rats were used: Control (CT, normotensive), LN (50 mg/kg/day, hypertensive), indomethacin (Indo-4 mg/kg/day, normotensive), and LN plus Indo (LN + Indo, partially prevented hypertension). All drugs were administered in drinking water during 7 days. In isolated rat tail vascular beds; the reactivity to PHE, acetylcholine (ACh), sodium nitroprusside (SNP), the functional activity of the Na^+, K^+-ATPase (K^+-induced relaxation) and the modulation of PHE-induced vasoconstriction by constitutively available NO were evaluated. LN increased vascular sensitivity (pD_2) and reactivity (Emax) to PHE and Indo blocked the effect of LN on Emax without changing pD_2. Emax and pD_2 values for ACh were reduced by LN and partially reverted by Indo. SNP-induced vasodilatation was similar in all groups. LN reduced the activity of Na^+, K^+-ATPase and Indo prevented LN effects. LN also abolished NO ability to modulate PHE-induced contractions. This effect was partially prevented by Indo suggesting that products from the cyclooxygenase pathway might reduce NO actions. Indo itself did not affect vascular reactivity to PHE, ACh or SNP or the Na^+,K^+-ATPase activity. Results suggested that products from cyclooxygenase pathway are involved in the genesis or maintenance of LN-induced hypertension, playing

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a role in the increased vascular reactivity, in the reduction of the endothelium-dependent relaxation and in the inhibition of the functional activity of the Na^+, K^+-ATPase.

Keywords: Nitric oxide; Hypertension; Endothelium; Arachidonic acid-cyclooxygenase pathway; Na^+; K^+-ATPase and vascular reactivity

Introduction

Acute or chronic inhibition of the nitric oxide synthesis using L-arginine analogues, such as L^{ω}-Nitro-Arginine-Methyl-Ester (L-NAME), induces hypertension (Zatz and De Nucci, 1991; Ribeiro et al., 1992). This model of hypertension is associated with an increased sympathetic tone (Cunha et al., 1993; Sander et al., 1997), activation of the renin-angiotensin system (Ribeiro et al., 1992; Jover et al., 1993), increased vascular resistance (Küng et al., 1995; Moreau et al., 1997; Ruiz-Marcos et al., 2001) and activation of the arachidonic acid-cyclooxygenase pathway (da Cunha et al., 2000).

In agreement with these observations, we recently demonstrated a reduction of the vascular sodium pump activity in vascular preparations from rats made hypertensive by 7-day treatment with L-NAME (Rossoni et al., 2003). Other investigators also demonstrated that the chronic administration of L-NAME reduces the ion transporting function of the cardiac Na^+, K^+-ATPase (Vrbjar et al., 1999). It is well established that the Na^+, K^+-ATPase participates in the modulation of the vascular smooth muscle contractility and tone (Blaustein, 1993; Marín and Redondo, 1999). Changes in functional state of the vascular Na^+-pump or its regulation may contribute either to the development of hypertension or may be a compensatory mechanism (David-Dufilho et al., 1984; Songu-Mize et al., 1984; Blaustein, 1993; Rossoni et al., 2002a). Endothelium-derived nitric oxide is one of the factors that stimulate the Na^+, K^+-ATPase activity and, as cited above, acute or chronic treatment with L-arginine analogues, which inhibit NO synthesis, reduces the Na^+-pump activity (Gupta et al., 1994a,b; Rossoni et al., 1998; Vrbjar et al., 1999; Scavone et al., 2000; Rossoni et al., 2003).

According with these results, we speculated that changes in the vascular Na^+, K^+-ATPase activity could be associated with the hyperreactivity to vasopressor agents and with the development of hypertension induced by chronic treatment with L-NAME. Based on the observation that the cyclo-oxygenase inhibitor, indomethacin, reduced the increased blood pressure and the vascular hyperreactivity to phenylephrine in aorta from L-NAME-treated rats (da Cunha et al., 2000) and that arachidonic acid metabolites reduce the Na^+, K^+-ATPase activity (Marín and Redondo, 1999), we designed experiments to test whether the reduction of the blood pressure and vascular reactivity to phenylephrine produced by indomethacin in L-NAME-treated rats is accompanied by an improvement of the Na^+, K^+-ATPase activity in rat tail vascular beds.

Materials and methods

Experimental animals and treatment

Male albino Wistar rats, 12 weeks old (250–300 g) were used. Care and use of the laboratory animals were in accordance with NIH guidelines. During the study rats were housed at a constant room

temperature and 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 animals were randomly divided into four treatment groups: 1) Control (CT group), (2) L-NAME (50 mg/kg/day, LN group), (3) indomethacin (4 mg/kg/day, Indo group) and (4) L-NAME plus indomethacin (LN + Indo group). All drugs were administered for 7 days in drinking water and the actual doses of each group were calculated from the daily water intake.

Blood pressure, heart rate and body weight determinations

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

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

Isolated rat tail vascular bed preparation

Isolated rat tail vascular beds were used in this study as previously reported (França et al., 1997). Briefly, the rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and after loss of the righting reflex, heparin (500 UI, i.p.) was administered. Ten min after the administration of heparin, 1 cm strip of the tail artery was dissected free and cannulated with an intracath (Nipro 24 G 3/4, Sorocaba, SP, BR) near the base of the tail. The vascular bed was flushed with Krebs-Henseleit buffer (KHB in mM) (120 NaCl, 5.4 KCl, 1.2 $MgCl_2$, 1.25 $CaCl_2$, 2.0 NaH_2PO_4, 27 $NaHCO_3$, 11 glucose and 0.03 EDTA) bubbled with 5% CO_2-95% O_2, at 36 $\pm$ 0.5 °C. The tail was then severed from the body and placed in a tissue bath and perfused with KHB at a constant flow of 2.5 ml/min with a peristaltic pump (Milan, Colombo, PR, BR). After a 30 to 45 min equilibration period, the experimental protocol was initiated. Mean perfusion pressure was measured by using a pressure transducer (TPS-2, InCor, São Paulo, SP, BR) and the data recorded using an interface and software for computer data acquisition (model MP100A, BIOPAC Systems, Inc., Santa Barbara, CA, 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.

After 30–45 min equilibration period, the following protocols were used.

Effects of treatments on the vascular reactivity to phenylephrine

The vascular sensitivity to phenylephrine was evaluated by generating dose-response curves to phenylephrine (PHE, 0.01 to 100 μg) administered as bolus injections of 100 μl in all preparations obtained from all groups.

Endothelium-dependent and -independent relaxation

Acetylcholine was used to evaluate endothelium-dependent relaxation. In these preparations, vasoconstriction was induced by perfusion of KHB containing 45 mM KCl. KCl produced approxi-

mately 60 to 80% of the contraction produced by phenylephrine (100 μg). Once a plateau was attained, dose-response-curves to graded concentrations of acetylcholine (10^{-10} to 10^{-3} M) were generated, each one infused for a duration of 4 min.

Sodium nitroprusside was used to evaluate endothelium-independent relaxation. The preparations were precontracted with 45 mM KCl and dose-responses curves to sodium nitroprusside (10^{-9} to 10^{-2} M) were generated, in the same manner as those for acetylcholine.

Measurement of the functional activity of the Na^+, K^+-ATPase

The functional activity of Na^+, K^+-ATPase was measured using K^+-induced relaxation as described by Webb and Bohr (Webb and Bohr, 1978). The functional activity of the Na^+, K^+-ATPase was determined in isolated tail vascular bed preparations from all treatment groups. After a 30–45 min equilibration period in normal KHB, the perfusion buffer was changed to one containing no added potassium. The tails were perfused for 30 min and then the preparations were precontracted with phenylephrine (3×10^{-6} M) that produced 60–80% of the contraction induced by phenylephrine (100 μg). Once a plateau was attained, the concentration of KCl in the perfusate was increased in steps (1, 2, 4 and 6 mM), each one with 5 min duration and relaxing responses to increased concentrations of potassium were measured. It is important to emphasize that potassium concentration higher than 6 mM induces depolarization and contraction in the isolated tail vascular bed preparations different to that observed in isolated tail rings or aorta (Rossoni et al., 2002a, 2003). For this reason, the response produced by 6 mM KCl was assumed to be the maximum response.

Influence of endogenous nitric oxide in the contractions induced by phenylephrine

This was evaluated by dose-response curves to phenylephrine (0.1 to 10 μg, as bolus injections, 100 μl) performed before and after blockade of nitric oxide production with 10^{-4} M L-NAME added to the KHB.

Tail artery media layer thickness determination

At the end of the experimental protocol the preparations were perfused with normal KHB until baseline pressure was attained. The tail artery was dissected and two or three segments of 3 mm length were used for morphometric determination of muscular layer. The segments were fixed, in the relaxed state, in 10% buffered formalin, embedded in paraffin and sectioned (5 μm sections), and stained with hematoxylin-eosin. Media layer thickness was determined using a light microscope equipped $STUPAR^{LAB}$ (Mentripex Hungary in cooperation with PZO-Labimex made in Poland) with a micrometer of 1 μm resolution.

Drugs and reagents

The following drugs were used: L-NAME, acetylcholine chloride, l-phenylephrine hydrochloride, TRIS ((hydroxymethyl) aminomethane) and sodium nitroprusside were purchased from Sigma (St. Louis, MO, USA), indomethacin from Merck (Whitehouse Station, NJ, USA), heparin from Roche (São Paulo, SP, BR) and sodium pentobarbital from Fontoveter (São Paulo, SP, BR).

For chronic treatment, L-NAME was dissolved directly in water and indomethacin was previously dissolved in 0.1 M TRIS (hydroximethyl aminomethane) buffer and after dissolved in distilled water or water plus L-NAME. For "in vitro" studies, all stock solutions of the compounds used were dissolved in bidistilled water. All solutions were freshly prepared before use and protected from light.

Data analysis and statistics

Results of perfusion pressure measurements are presented as changes in the mean perfusion pressure (MPP) subtracting peak pressure from baseline pressure and expressed as mmHg. Relaxation responses to KCl, acetylcholine or sodium nitroprusside were expressed as a percentage of relaxation in relation to precontracted MPP.

Dose-response-curves and data presented in Table 1 and Figs. 1 and 2 were analyzed by iterative nonlinear regression analyses (GraphPad Prism software, San Diego, CA, USA) for fits to the equation:

$$E = E_{max}/[1 + (10^{C}/10^{X})]$$

where E is the response produced by the drug; E_{max} is the calculated maximal response produced by the drug; X is the log of the dose; and C is the log of the dose that produces one-half maximal response, $\log ED_{50}$. In the text, values for $-1 \cdot \log ED_{50}$ (pD_2) are used.

Dose-response data for phenylephrine from the studies on the participation of endogenous nitric oxide in modifying contractions induced by phenylephrine are expressed as a percentage of the contraction induced by 10 μg phenylephrine before L-NAME (10^{-4} M) infusion. The participation of endogenous nitric oxide in the phenylephrine-induced contractions was deter-emined by comparing the areas under the normalized dose-response curves for phenylephrine in the absence and in the presence of L-NAME (GraphPad Prism software, San Diego, CA, USA). The results are expressed as the differences between area under the curve (AUC) before and after L-NAME acute treatment. The difference between areas represents the nitric oxide-dependent endothelial modulation.

Table 1
Effect of treatment with L-NAME, indomethacin, and L-NAME + indomethacin on the dose-response parameters (E_{max} and pD_2 values) for phenylephrine and acetylcholine in the isolated perfusated tail artery preparation

Treatment group	Phenylephrine		Acetylcholine	
	E_{max} (mmHg)	pD_2	E_{max} (%)	pD_2
Control	194 ± 10.2 (10)	2.29 ± 0.09 (10)	88.6 ± 3.6 (7)	6.31 ± 0.15 (7)
L-NAME	311 ± 28.6* (10)	2.77 ± 0.19* (10)	42.2 ± 5.0* (7)	4.34 ± 0.25* (7)
Indomethacin	186 ± 16.4 (8)	2.16 ± 0.08 (8)	84.1 ± 5.6 (6)	6.36 ± 0.32 (6)
LN + INDO	217 ± 8.6[#] (8)	2.71 ± 0.17* (8)	77.5 ± 4.8[#] (8)	5.26 ± 0.21*,[#] (8)

Each value represents the mean ± SEM and number (N) of experiments. ANOVA (1-way).
*P < 0.01 LN and LN + Indo vs. CT.
[#] P < 0.01 LN + Indo vs. LN.

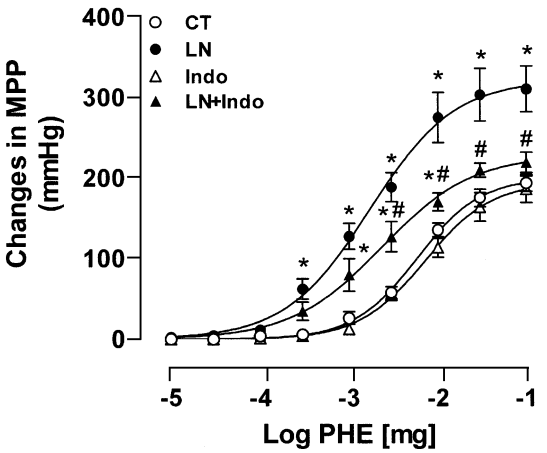

Fig. 1. Dose-response curves to phenylephrine (PHE) in rat isolated tail vascular beds from control (CT, n = 10), L-NAME (LN, n = 10), indomethacin (Indo, n = 8) and L-NAME plus indomethacin (LN + Indo, n = 8) groups. Symbols represent mean ± SEM of changes in MPP. 2-way ANOVA, completely randomized. *P < 0.01 LN and LN + Indo vs. CT and # P < 0.01 LN + Indo vs. LN.

Results are expressed as mean ± standard error of the mean (SEM) and were compared by using simple linear regression and one- or two-way analysis of variance (ANOVA), completely randomized, followed by Tukey's post hoc test. Statistical significance was established at P < 0.05.

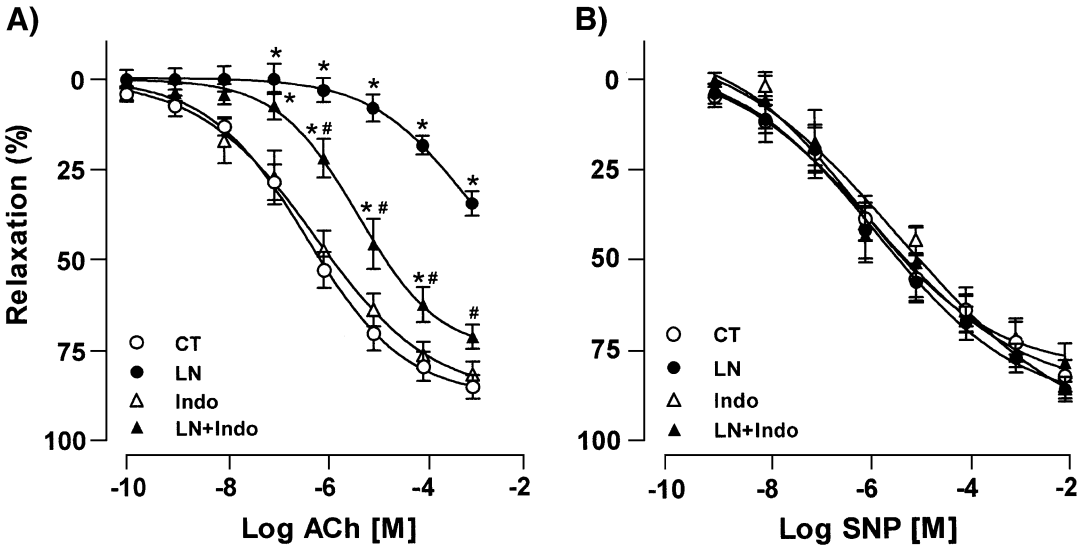

Fig. 2. Endothelium-dependent and -independent relaxation induced by acetylcholine (ACh, Fig. 2A) and sodium nitroprusside (SNP, Fig. 2B), respectively, in tail vascular beds from control (CT, n = 7–6), L-NAME (LN, n = 6–7), indomethacin (Indo, n = 6) and L-NAME plus indomethacin (LN + Indo, n = 8–6) groups. Results are represented as mean ± SEM percentage of relaxation. 2-way ANOVA, completely randomized. *P < 0.01 LN and LN + Indo vs. CT and # P < 0.01 LN + Indo vs. LN.

Results

Blood pressure, heart rate, body weight and tail artery media layer thickness

L-NAME treatment induced a significant elevation of systolic (CT: 124 ± 2 vs. LN: 211 ± 6 mmHg, $P < 0.01$) and diastolic blood pressure (CT: 97 ± 4 vs. LN: 161 ± 4 mmHg, $P < 0.01$) when compared to the control group. In the L-NAME plus indomethacin group systolic (LN: 211 ± 6 vs. LN + Indo: 154 ± 3 mmHg, $P < 0.01$) and diastolic blood pressure (LN: 161 ± 4 vs. LN + Indo: 121 ± 4 mmHg, $P < 0.01$) were lower when compared to the L-NAME-treated rats, but still significantly higher than the control group (Systolic, LN + Indo: 154 ± 3 vs. CT: 124 ± 2 mmHg, $P < 0.01$ and diastolic, LN + Indo: 121 ± 4 vs. CT: 97 ± 4 mmHg, $P < 0.01$). The treatment only with indomethacin did not change blood pressure levels (data not shown).

No differences in body weight, heart rate and tail artery media layer thickness were observed among the groups (data not shown).

Effects of treatments on the vascular reactivity to phenylephrine

Fig. 1 and Table 1 show that the chronic treatment with L-NAME increased significantly the tail vascular bed sensitivity (pD_2) and maximal response (Emax) to phenylephrine compared to the control group. Cotreatment with indomethacin prevented the increment of the vascular responsiveness to phenylephrine produced by L-NAME, but did not prevent the increase in the sensitivity (Fig. 1 and Table 1). Indomethacin only did not alter the dose-response curve to phenylephrine compared to the control group (Fig. 1 and Table 1).

Endothelium-dependent and -independent relaxation

Fig. 2A and Table 1 show the endothelium-dependent relaxation of the tail vascular bed induced by acetylcholine in all groups studied. L-NAME treatment reduced both sensitivity and maximal relaxation to acetylcholine compared to the control group. This effect of L-NAME on the actions of acetylcholine was partially prevented by cotreatment with indomethacin; the maximal response to acetylcholine was totally restored while the sensitivity was only partially improved (Fig. 2A and Table 1). Indomethacin only did not change the acetylcholine-induced relaxation compared to control (Fig. 2A and Table 1). As shown in the Fig. 2B, responses to sodium nitroprusside were similar after all treatments.

Precontraction produced by 45 mM KCl increased the mean perfusion pressure in a similar manner in all groups (changes in mean perfusion pressure (in mmHg): CT: 157 ± 15 vs. Indo: 151 ± 23 vs. LN: 216 ± 47 vs.LN + Indo: 186 ± 16, $P > 0.05$).

Measurement of the functional activity of the Na^+, K^+-ATPase

Fig. 3A shows the concentration-response curve for KCl-induced relaxation displaced upwards in L-NAME group when compared to the control (Emax CT: 78.6 ± 1.9 vs. LN: 29.8 ± 3.6%, $P < 0.01$), suggesting a reduction of the functional activity of the Na^+, K^+-ATPase caused by L-NAME treatment. The maximal KCl-induced relaxation of the L-NAME plus indomethacin group was

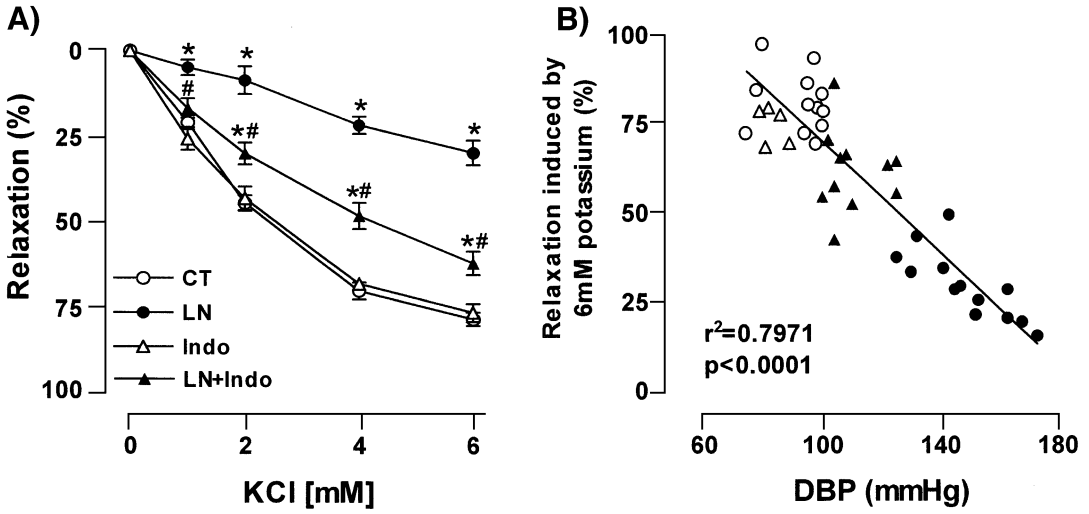

Fig. 3. (A) Effects of the treatments on the functional activity of the Na^+, K^+-ATPase evaluated by relaxation to potassium. Data represent percentage decrease in mean perfusion pressure (MPP) produced by varying concentrations of KCl in the tail vascular beds of control (CT, n = 8), L-NAME (LN, n = 10), indomethacin (Indo, n = 7) and L-NAME plus indomethacin groups (LN + Indo, n = 11). 2-way ANOVA, completely randomized. *P < 0.01 LN and LN + Indo vs. CT and, # P < 0.01 LN + Indo vs. LN. (B) Correlation between diastolic blood pressure (DBP) and percentage of relaxation produced by 6 mM potassium. Simple linear regression, $r^2 = 0.7971$, P < 0.0001.

significantly larger compared to the L-NAME group. However, this relaxation was still smaller than that in the control group (LN + Indo: 62.2 ± 3.4 vs. CT: 78.6 ± 1.9% and LN: 29.8 ± 3.6%, P < 0.01, Fig. 3A). This result suggested that treatment with indomethacin partially prevented the reduction of the functional activity of the Na^+, K^+-ATPase produced by L-NAME. The KCl-induced relaxation of the indomethacin treated group was not altered when compared to the control group (Indo: 76.7 ± 2.6 vs. CT: 78.6 ± 1.9%, P > 0.05, Fig. 3A).

Fig. 3B shows the relationship between the relaxation induced by 6 mM KCl and diastolic blood pressure obtained in all groups. A continuous distribution was found among variables and the linear regression analysis showed a significant negative correlation ($r^2 = 0.7971$ and P < 0.0001).

Precontraction produced by perfusion with 3 μM phenylephrine increased the mean perfusion pressure in a similar manner in all studied groups (changes in mean perfusion pressure (in mmHg): CT: 164 ± 13 vs. Indo: 162 ± 26 vs. LN: 217 ± 13 vs. LN + Indo 184 ± 6, P > 0.05).

Influence of endogenous nitric oxide in the contractions induced by phenylephrine

Fig. 4 shows the effect of acute inhibition of NO-synthesis by L-NAME (10^{-4} M) on the contraction induced by phenylephrine. The acute perfusion of Krebs plus L-NAME increased the pressor responses to phenylephrine in control and in indomethacin treated rats (Fig. 4A and 4C). However, in the L-NAME-treated group, the pressor response to phenylephrine was not modified after perfusion with L-NAME added to the bath (Fig. 4B). In contrast, in preparations from the L-NAME plus indomethacin group, in vitro L-NAME administration partially increased the pressor response to phenylephrine to the control level (Fig. 4D). Fig. 4E summarizes the differences between areas under the curves for

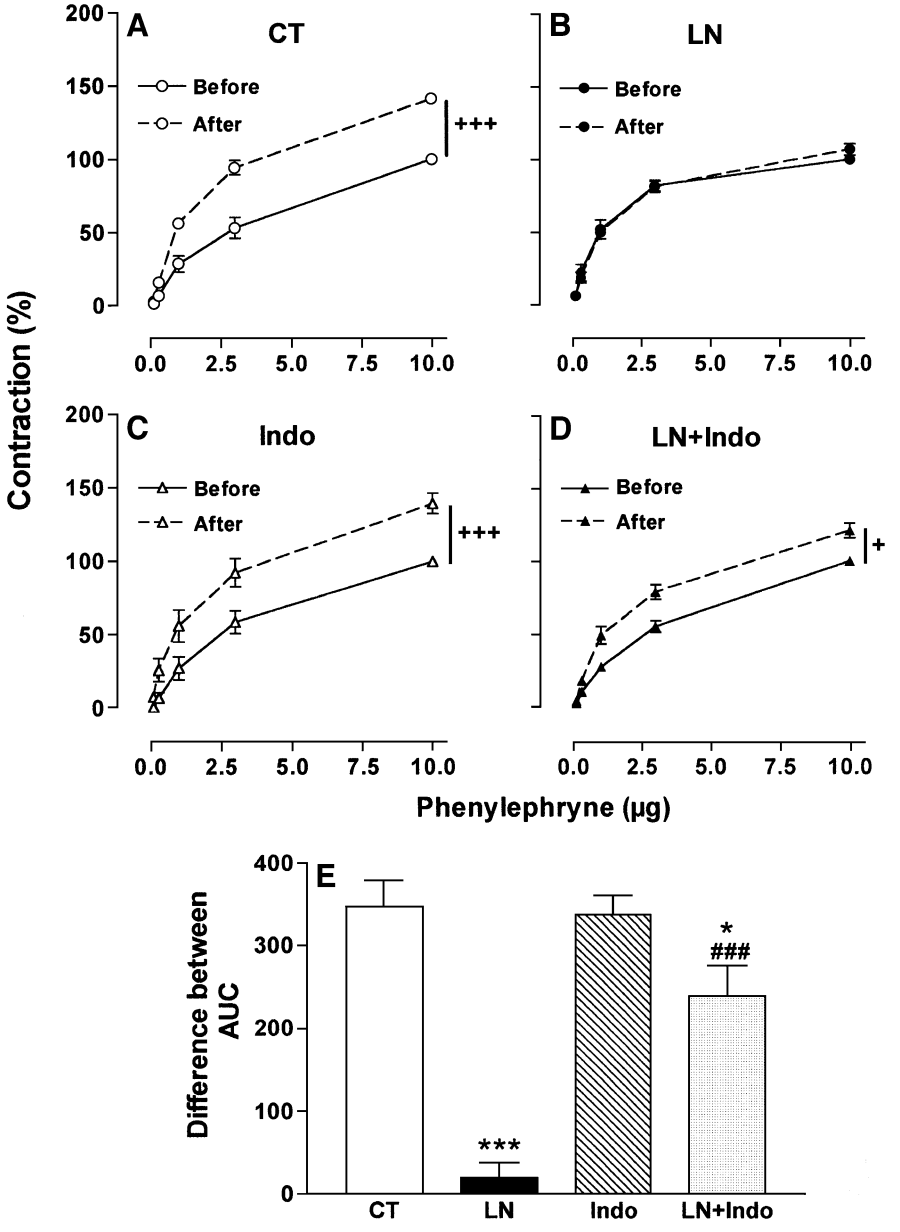

Fig. 4. Effects of acute perfusion of L-NAME (10^{-4} M) on the contractile response to phenylephrine (PHE) in tail vascular beds from control (A, CT, n = 6), L-NAME (B, LN, n = 6), indomethacin (C, indo, n = 6) and L-NAME plus indomethacin (D, LN + Indo, n = 6) groups. Results are presented as mean ± SEM percentage of contraction. Continuous lines represent responses in the absence of L-NAME (before) and the dashed lines represent responses in the presence of L-NAME (after). 2-way ANOVA, completely randomized. $^{+}P < 0.01$ and $^{+++}P < 0.0001$, after vs. before. Fig. 4E shows the difference between areas under the curves (AUC) for phenylephrine in the absence and in the presence of L-NAME in the perfusate. Columns represent mean and the bars, SEM. One-way ANOVA, completely randomized. *P < 0.01 and ***P < 0.0001, LN and LN + Indo vs. CT and $^{###}P < 0.0001$, LN + Indo vs. LN.

phenylephrine in the absence and in the presence of L-NAME in the perfusate for each group. In preparations from L-NAME-treated animals, the addition of L-NAME to the perfusate produced no additional effects on the vasoconstriction produced by phenylephrine. The cotreatment with L-NAME plus indomethacin partially restored the response to the L-NAME added to the perfusate (Fig. 4E). The treatment with indomethacin only did not alter the effects of added L-NAME when compared to the control group (Fig. 4E).

Discussion

Results presented here reinforce previous results (da Cunha et al., 2000) showing that the development of L-NAME-induced hypertension was partially prevented by cotreatment with the cyclooxygenase inhibitor indomethacin. This treatment also induced a reduction of the vascular hyperreactivity to phenylephrine and improved both endothelial function and functional activity of the Na^+, K^+-ATPase in the rat tail vascular bed. Moreover, cotreatment with indomethacin unmasked the inhibition of the negative endothelium-dependent modulation induced by nitric oxide on the phenylephrine-induced contraction produced by L-NAME treatment.

The chronic inhibition of nitric oxide synthase (NOS) by L-NAME or other L-arginine analogues produces hypertension (Ribeiro et al., 1992). This effect is due to several factors like: inhibition of the nitric oxide production (Ribeiro et al., 1992), increase in sympathetic tone (Cunha et al., 1993; Sander et al., 1997), increase in the renin-angiotensin system activity (Ribeiro et al., 1992; Jover et al., 1993) and increase in the vascular resistance (Küng et al., 1995; Ruiz-Marcos et al., 2001). Also, the reactivity to vasoconstrictor agents is increased and the responsiveness to endothelial dependent vasodilators is reduced in this model of hypertension (Küng et al., 1995; Moreau et al., 1997; Maeso et al., 1999; da Cunha et al., 2000). Similar changes were reported for humans with essential hypertension (Panza et al., 1990; Taddei et al., 1993) and for spontaneously hypertensive rats (Lüscher and Vanhoutte, 1986). In agreement with these observations, we also found in the rat tail vascular bed an enhanced sensitivity and reactivity to phenylephrine and a decreased endothelium-dependent relaxation produced by acetylcholine, without changes in the relaxation induced by sodium nitroprusside.

Another mechanism that may be associated with an increased vascular resistance is a change in the Na^+, K^+-ATPase activity. It is well established that the Na^+, K^+-ATPase participates in the modulation of the vascular smooth muscle contractility and tone (Blaustein, 1993; Marín and Redondo, 1999). Lowering the Na^+-pump activity, intracellular Na^+ concentration increases, thus reducing the Na^+/Ca^{2+} exchanger activity and consequently increasing intracellular Ca^{2+} concentration and tension (Blaustein, 1993). Then, changes of 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 (David-Dufilho et al., 1984; Songu-Mize et al., 1984; Blaustein, 1993; Marín and Redondo, 1999; Rossoni et al., 2002a). Nitric oxide stimulates the activity of the Na^+, K^+-ATPase via mechanisms involving cyclic GMP- and cyclic AMP-dependent protein kinases (Gupta et al., 1994a,b; Marín and Redondo, 1999; Scavone et al., 2000). Therefore, the inhibition of nitric oxide synthesis might cause a reduction in the Na^+, K^+-ATPase activity contributing to the hypertension produced by L-NAME. Webb and Bohr (1978) characterized the potassium-induced relaxation as an index of Na^+, K^+-ATPase activity in arteries based on several evidences. Among those evidences they also showed that the Na^+, K^+-ATPase activity is dependent on extracellular K^+, intracellular Na^+ and

magnesium concentration, temperature and is also inhibited by ouabain (Webb and Bohr, 1978). Previous studies (Rossoni et al., 1998; Vrbjar et al., 1999; Rossoni et al., 2003) and the results presented here using a similar approach suggested that there is a reduction of the functional activity of the vascular Na^+, K^+-ATPase in the L-NAME hypertensive rats.

Vascular alterations induced by L-NAME treatment might be explained by a reduced bioavailability of nitric oxide, since it is associated with changes in nitric oxide production, and by an increased production of endothelium-derived vasoconstrictors (Lüscher and Vanhoutte, 1986; Carvalho et al., 1997; Taddei et al., 1997). In human essential hypertension and in some experimental models of hypertension, an increase of cyclooxygenase-derived vasoconstrictor factors released by the endothelium, has been associated with the development and/ or maintenance of hypertension and vascular dysfunction (Lüscher and Vanhoutte, 1986; Carvalho et al., 1997; Taddei et al., 1997). Also, it has been shown that nitric oxide inhibits both cyclooxygenase- and lipoxygenase-dependent lipid peroxidation (Kanner et al., 1992; Gryglewski, 1993). Moreover, the vasoconstriction induced by L-NAME in vitro is partially dependent on TxA_2/PGH_2 generation (Ziyyat et al., 1996), pointing towards the involvement of prostanoids on the vascular effects caused by NO inibition. In agreement to our previous results with thoracic aorta (da Cunha et al., 2000), the current study suggests the involvement of prostanoids on the development of hypertension and on the increase vascular reactivity to phenylephrine in the tail vascular bed, since chronic treatment with indomethacin attenuated the effects of L-NAME treatment.

The effects of indomethacin on L-NAME treated rats could also be associated with an improvement in either endothelium-dependent vasodilatation and/ or Na^+, K^+-ATPase activity. As reported here, the vasodilatation induced by acetylcholine in tail vascular bed preparations seems to be dependent only on the nitric oxide production and not to either PGI_2 or an endothelium-derived hyperpolarizing factor (EDHF). This conclusion results from the fact that the relaxation induced by acetylcholine in animals treated with indomethacin (that blocks PGI_2) and precontracted with 45 mM KCl (that blocks EDHF) did not change when compared to control animals (Adeagbo and Triggle, 1993). In the hypertension induced by L-NAME, as well as in human essential hypertension and other models of hypertension, the effects of an endothelium-dependent agonist, such as acetylcholine, are not only dependent upon changes in nitric oxide, EDHF or PGI_2 production, but also an enhanced production of endothelium-derived vasoconstrictor factors (Lüscher and Vanhoutte, 1986; Moreau et al., 1997; Carvalho et al., 1997; Taddei et al., 1997). The ability of indomethacin to prevent the effects of L-NAME on the actions of acetylcholine in the rat tail vascular bed suggests that vasoconstrictor metabolites derived from the arachidonic acid-cyclooxygenase pathway contribute to the impaired endothelium-dependent relaxation induced by chronic NO inhibition. However, this effect of indomethacin on the actions of L-NAME might be a characteristic of this vascular bed since a similar effect was not seen in isolated aortic rings (da Cunha et al., 2000).

An improvement of the bioavailability or an unmasked effect of constitutively available nitric oxide might be another mechanism by which indomethacin reduced the hyperreactivity to phenylephrine and increased the relaxation produced by acetylcholine in L-NAME-treated rats. To test this hypothesis we studied the actions of constitutively available nitric oxide that modulates the contractile responses of the vascular smooth muscle (Hayashi et al., 1992). The modulatory action of constitutively available nitric oxide in the response induced by phenylephrine was studied perfusing the tail vascular bed with the NO-synthase inhibitor L-NAME. The differences between the areas under the dose-response curves to phenylephrine, generated in the absence and the presence of acute treatment with L-NAME, were compared as an index of nitric oxide modulation in response to phenylephrine (Rossoni et al.,

2002b). The increase of the area under the curve generated in the presence of L-NAME added in vitro suggested the participation of constitutively available nitric oxide modulation on contractions produced by phenylephrine (Rossoni et al., 2002b). The present results suggest that chronic L-NAME treatment abolished the modulatory actions of nitric oxide on phenylephrine-induced contractions. Indomethacin treatment only, had no effects on these actions of nitric oxide. However, indomethacin cotreatment prevents in approximately 70% the constitutively available nitric oxide effect on the phenylephrine contraction in the tail vascular bed. In this situation the effect of constitutively available nitric oxide was unmasked when compared to L-NAME-hypertensive rats. The masked effect of the modulatory actions of constitutively available nitric oxide in response to phenylephrine in the tail vascular bed from L-NAME rats may represent an increased degradation of nitric oxide by the free oxygen radicals or an increased production of endothelium-derived vasosoconstrictor products (Usui et al., 1999; Ziacikova et al., 1999; Kitamoto et al., 2000). The inhibition of cyclooxygenase products with indomethacin unmasked this effect, suggesting that products of this pathway are able to modulate the constitutively available nitric oxide actions that occur only in the hypertensive state. The pathway by which cyclooxygenase products are able to modulate the effects of constitutively available nitric oxide is not know and new studies are necessary.

As described above, it is already known that the endothelium-derived nitric oxide stimulates the activity of the Na^+, K^+-ATPase (Gupta et al., 1994a,b; Marín and Redondo, 1999). Rossoni et al. (1998) showed that the acute perfusion of the tail vascular bed from normotensive Wistar rats with L-NAME, reduces Na^+, K^+-ATPase activity. As reported here, this reduction was greater when the rats were treated for 7-days with L-NAME, suggesting that alterations induced by the hypertensive state also affect the sodium pump activity. It is also known that cyclooxygenase metabolites, such as PGE_2, are able to negatively modulate Na^+, K^+-ATPase activity (Satoh et al., 1993; Marín and Redondo, 1999). These observations are reinforced by our results, which show that treatment with a cyclo-oxygenase inhibitor, indomethacin, partially prevents the effects of L-NAME treatment on the functional activity of the sodium pump and partially prevented the development of hypertension. These results also suggest a role of the cyclooxygenase products regulating the Na^+, K^+-ATPase activity and the vascular tone of the tail bed from L-NAME hypertensive rats. Then, the improvement of the Na^+, K^+-ATPase functional activity in the L-NAME plus indomethacin treated group could be explained by an increased nitric oxide bioavailability or by a reduction of cyclooxygenase products that negatively modulate Na^+, K^+-ATPase activity.

As a second approach to investigate the role of Na^+, K^+-ATPase in the development of the hypertension induced by L-NAME we determined if there was a correlation between the ability of 6 mM KCl, which produced maximal relaxation of the tail vascular bed by activating the Na^+-pump, and the diastolic pressure of the animal, which reflects the peripheral resistance. A significant negative correlation between these two parameters was found, suggesting that there is an inverse relationship between the severity of hypertension and the functional Na^+, K^+-ATPase activity. Inhibition of the Na^+-pump activity, according to the mechanism proposed by Blaustein (1993), increases calcium and ultimately the contractile response of the vascular smooth muscle. Hence, the reduction of the Na^+, K^+-ATPase activity, in addition to the impairment of nitric oxide synthesis and the increased amount of vasoconstrictor factors derived from cyclooxygenase pathway, might be associated with hyperreactivity to phenylephrine and decreased in the endothelial-dependent relaxation in the tail vascular beds from L-NAME-treated rats. Therefore, these alterations might be associated with the development and/ or maintenance of hypertension in L-NAME-treated rats.

The response to vasoconstrictor agents could be also affected by changes in the wall structure. However, in agreement to our previous studies with thoracic aorta (da Cunha et al., 2000), no differences in the tail artery medial thickness were found among the experimental groups. Therefore, it is unlikely that arterial smooth muscle hypertrophy, at least during the 7 day L-NAME treatment, contributed to the altered contractility.

In conclusion, our findings confirmed previous reports showing that 7 day chronic treatment with L-NAME induces hypertension and this hypertension was partially prevented by cotreatment with indomethacin. Cotreatment with L-NAME plus indomethacin partially prevents the vascular hyperreactivity to phenylephrine, improves the endothelial vasodilator function and reduces the functional impairment of the Na^+, K^+-ATPase activity. These results suggest that products derived from the cyclooxygenase pathway participate as an important additional mechanism changing the vascular reactivity and the Na^+-pump activity that might contribute to the L-NAME-induced hypertension.

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