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European Journal of Pharmacology 412 (2001) 273–279

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Mechanisms of the increased pressor response to vasopressors in the mesenteric bed of nitric oxide-deficient hypertensive rats

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Received 21 September 2000; received in revised form 13 October 2000; accepted 17 October 2000

Abstract

In the present study we analyzed mesenteric vascular reactivity of chronic nitric oxide (NO)-deficient hypertensive rats (N^{W}-nitro-L-Arginine Methyl Ester, L-NAME, 50 mg/kg/day, oral, 3 weeks). Perfusion pressure changes in response to cumulative additions of methoxamine and KCl were significantly increased in the mesenteric vessels of the L-NAME-treated as compared with vessels of the controls. Verapamil reduced the responses to methoxamine, but those of the hypertensive rats were still enhanced. In contrast, responses to KCl were almost completely abolished by verapamil. In mesenteric vessels perfused with zero calcium and high-potassium Krebs, pressor responses to the re-addition of calcium were also significantly enhanced in the hypertensive rats compared to the controls. Vasodilator responses to acetylcholine in KCl-preconstricted vessels, while still significant, were reduced in the L-NAME-treated rats. In this case, acute inhibition of NO blocked the vasodilator responses to acetylcholine and abolished the differences between the two groups. In methoxamine-preconstricted vessels and in the presence of acute inhibition of NO and prostaglandins, vasodilator responses to acetylcholine were significantly greater in the hypertensive vessels than in controls. In conclusion, the mesenteric vessels of L-NAME hypertensive rats show an enhanced response to vasopressors which is related to calcium entry. These data also reveal the existence of an enhanced role of a NO and prostaglandin-independent vasodilator factor, probably endothelium-derived hyperpolarizing factor that may play a compensatory role in the deficiency of NO.

Keywords: Nitric oxide (NO); Vascular reactivity; Arterial hypertension; Methoxamine; Acetylcholine; EDHF (endothelium-dependent hyperpolarizing factor)

1. Introduction

Chronic inhibition of nitric oxide (NO) synthase with drugs such as N^{G}-nitro-L-arginine methyl ester (L-NAME) has been shown to result in a very reproducible model of arterial hypertension (Ribeiro et al., 1992). To date, a number of neural (Matsuoka et al., 1994; Zanchi et al., 1995), reno-humoral (Navarro et al., 1994; Fernández-Rivas et al., 1995; García-Estañ et al., 1996; Navarro-Cid et al., 1996; Vargas et al., 1996) and structural (Deng et al., 1993; Li and Schiffrin, 1994; Bryant et al., 1995; Kung et al., 1995; Dowell et al., 1996; Henrion et al., 1996) mechanisms have been described as important mediators of this hypertension. However, there is no general agreement on the mechanisms that may be responsible for the elevation in blood pressure and associated abnormalities secondary to the chronic deficiency of NO. Thus, the effects of chronic NO inhibition of NO on vascular reactivity have not been fully investigated, and in some instances divergent results have been reported. Aortic rings of chronically L-NAME-treated rats have reduced response to most vasoconstrictors (Kung et al., 1995; Henrion et al., 1996). In contrast, isolated kidneys of L-NAME-treated rats have an increased response to vasoconstrictors and to increases in flow (Bryant et al., 1995; Vargas et al., 1996). Moreover, in rings of second and third order mesenteric vessels, increased (Deng et al., 1993; Li and Schiffrin, 1994) and decreased (Dowell et al., 1996) responses to α-agonists have been reported. Navarro-Cid et al. (1996) found enhanced pressor responses of isolated and perfused mesenteric beds to receptor-dependent agonists. These discrepancies may be due to the type of vessel studied, conduit, such as the aorta, or resistance, such as the mesenteric and renal vessels. Another reason for these discrepancies could be

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0014-2999/01/$ - see front matter
PII: S0014-2999(00)00795-0

related to the different roles of NO and other vasodilators, such as endothelium-derived hyperpolarizing factor (EDHF), in the various tissues examined. Thus, the role of EDHF seems to be much more important in tissues such as the mesenteric or renal vessels than in the aorta (Chen et al., 1988; Nagao et al., 1992; Nagao and Vanhoutte, 1993). In fact, Vargas et al. (1996) have reported the existence of an increased role for EDHF in the isolated kidney of L-NAME hypertensive rats.

In the present study, we have evaluated in the isolated and perfused mesenteric arterial bed the vascular reactivity to receptor-dependent (methoxamine) and independent (KCl) drugs in a model of arterial hypertension induced by chronic inhibition of NO synthesis. Moreover, the possible roles of the release of catecholamines from the mesenteric nerve endings and that of Ca^{2+} entry as mechanisms contributing to the vascular response to KCl were also studied. Finally, the possible involvement of EDHF as a compensatory vasodilator mechanism was evaluated by comparing dose–response curves to acetylcholine in methoxamine- and KCl-preconstricted preparations.

2. Methods

Male Sprague–Dawley rats born and raised in the Animal House of the Universidad de Murcia were used in the present study. All the experiments were performed according to the ethical rules of the European Union for the treatment of laboratory animals.

2.1. Experimental groups

Animals weighing around 200 g were given the NO synthase inhibitor, L-NAME, in the drinking water for 3 weeks at the dose of 50 mg/kg/day. The dose of the inhibitor was adjusted daily according to water intake and body weight. Control animals were also concurrently maintained with no treatment. Normal rat chow was offered with no restrictions.

2.2. Experimental protocols

At the end of the 3-week period, the animals were anesthetized (Inactin, 100 mg/kg, i.p., RBI, Natick, MA, USA) and mean arterial pressure was measured by catheterization of the right femoral artery (Hewlett-Packard transducers, amplifiers and polygraph). Then, the mesenteric bed was isolated and Krebs-perfused as described below and the following protocols were performed.

2.2.1. Vasoconstrictor responses

In this protocol, cumulative dose–response curves to methoxamine (1–1000 μM, five controls, six L-NAME-treated) and to KCl (20–120 mM) were made. Regarding KCl, two different experiments were performed. The first one with normally perfused mesenteric beds (eight controls, six L-NAME-treated), and the second with mesenteric beds perfused with Krebs containing prazosin and propranolol (1 μM each) to block the effects of catecholamines released from adrenergic nerve endings (eight controls, five L-NAME-treated). To analyze the role of Ca^{2+} entry, methoxamine and KCl pressor responses were also studied in the presence of verapamil (3 μM in the Krebs throughout the experiment, $n = 4$ in each group). Finally, the pressor response to the addition of Ca^{2+} (0.25–5 mM) in high potassium and zero-Ca^{2+} Krebs was also evaluated (four controls, four L-NAME-treated). In this last experiment, the concentration of K^+ was chosen from the previous dose–response curves as the dose that produced an 80% of the maximum pressor response.

2.2.2. Vasodilator responses

In the first set of experiments, vasodilator responses to acetylcholine (10^{-9} to 10^{-3} M, six controls, eight L-NAME-treated) were obtained in mesenteric beds preconstricted with KCl (at a dose which resulted in 80% of the pressor response obtained in the previous protocol). The first curve was obtained in normally perfused beds, and the second curve after acute inhibition of NO synthesis with L-NAME (100 μM). In the second set of experiments, the acetylcholine vasodilator effect was studied in mesenteric beds preconstricted with methoxamine (four controls, four L-NAME-treated) and with metoxamine plus L-NAME and indomethacin to acutely inhibit NO and prostaglandin synthesis (100 and 5 μM, respectively; six controls, six L-NAME-treated). Finally, the response to sodium nitroprusside (10^{-9} to 10^{-3} M, four controls, four L-NAME-treated) was also evaluated in KCl-preconstricted mesenteric beds. Appropriate time-control experiments were performed with both protocols. Three animals per group were used in these experiments to ascertain that the precontraction could be maintained for the time necessary to perform the vasodilator perfusions (around 30 min).

2.3. Isolation and perfusion of the mesenteric bed

This was performed as previously described (Atucha et al., 1996). Briefly, the superior mesenteric artery was cannulated using a PE-60 catheter and gently perfused with 15 ml of warmed Krebs solution to eliminate blood. After the superior mesenteric artery was isolated with its mesentery, the gut was cut off near its mesenteric border. The mesenteric bed was then put in a 37°C water-jacketed container and perfused at a constant rate (4 ml/min) with oxygenated 37°C Krebs solution (95% O_2, 5% CO_2) using a roller pump (Masterflex, Cole-Parmer, Barrington, IL). The Krebs solution had the following composition (mM): NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; EDTA, 0.026; and glucose, 11.0; pH 7.4. The preparation was covered with a piece of Parafilm (American National Can, Greenwich, CT) to prevent dry-

ing. Perfusion pressure was measured with a transducer (Hewlett-Packard 1280) on a side arm just before the perfusing cannula and continuously recorded on a polygraph inscriber (Hewlett-Packard 8805D). Since flow rate was kept constant throughout the experiment, pressure changes reflect vascular resistance changes. The preparation was allowed to recover for at least 30 min and then the experimental protocol was performed. Perfusion pressure at each concentration was allowed to plateau before the addition of the next higher concentration. Only one concentration–response curve was performed in each preparation.

2.4. Drugs

All the products used were from Sigma (Madrid, Spain). Stock solutions were prepared in distilled water and kept frozen (−20°C). Appropriate dilutions were prepared freshly every day in normal Krebs.

2.5. Statistical analysis

Data are expressed as the means ± S.E. Pressor responses are shown as absolute increases in pressure from the basal, while vasodilator responses are expressed as percentage decreases in perfusion pressure from the preconstricted value. The dose–response curves were analyzed by two-way analysis of variance for repeated measures. The differences in ED_{50}, obtained from the individual curves, and the maximum responses were analyzed by Student's *t*-test.

3. Results

Mean arterial pressure was increased in all the L-NAME-treated animals studied and averaged 145.9 ± 1.4 mm Hg ($n = 55$). This value was significantly different from that observed in the control animals (102.5 ± 1.4, $n = 57$).

3.1. Vasoconstrictor responses (Fig. 1 and Table 1)

The basal perfusion pressures of the mesenteric bed were significantly increased in all the groups of L-NAME-treated rats and averaged 14.9 ± 0.6 mm Hg, being 11.7 ± 0.4 in the control vessels. The changes in perfusion pressure in response to methoxamine were significantly greater

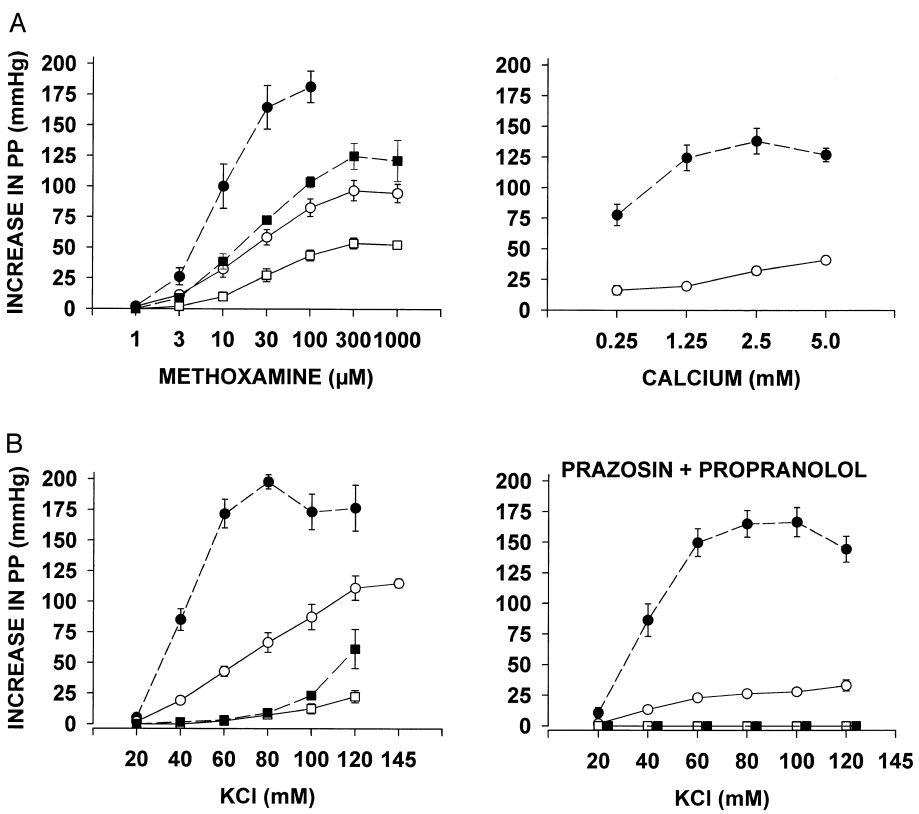

Fig. 1. (A) Increase in perfusion pressure (PP) in mesenteric arterial beds in response to the cumulative addition of methoxamine (controls, $n = 5$, open circles; L-NAME-treated, $n = 6$, closed circles), methoxamine plus verapamil (squares, $n = 4$ in each group) and calcium ($n = 4$ in each group). (B) Increase in perfusion pressure (PP) in mesenteric arterial beds in response to the cumulative addition of KCl alone (controls, $n = 8$, open circles; L-NAME-treated, $n = 6$, closed circles), KCl in the presence of prazosin and propranolol (controls, $n = 8$; L-NAME-treated, $n = 5$), and KCl in the presence of verapamil (squares, $n = 4$ for each group and set of conditions).

Table 1
Maximum pressor response (MAX, mm Hg) and ED_{50} ($-\log M$) in the protocol of vasoconstrictor responses

Dose–response curve to	n	MAX	ED_{50}
MTX			
Control	5	101.1 ± 7.75	4.67 ± 0.04
L-NAME hypertension	6	189.7 ± 11.2^{a}	4.98 ± 0.09^{a}
MTX + verapamil			
Control	4	55.0 ± 3.5^{b}	4.48 ± 0.05^{b}
L-NAME hypertension	4	$127.5 \pm 6.8^{a,b}$	$4.60 \pm 0.08^{a,b}$
KCl			
Control	8	111.2 ± 9.9	4.21 ± 0.01
L-NAME hypertension	6	203.3 ± 5.5^{a}	4.39 ± 0.02^{a}
KCl + PRAZ + PROP			
Control	8	33.3 ± 4.5^{b}	4.35 ± 0.02^{b}
L-NAME hypertension	5	$170.3 \pm 11.9^{a,b}$	4.41 ± 0.02

Data are means ± E.E. MTX, methoxamine; KCl, potassium chloride; PRAZ, prazosin; PROP, propranolol.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.05$ vs. respective group under untreated conditions.

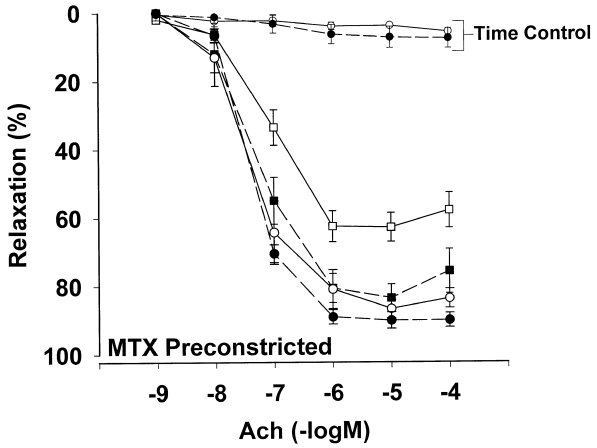

Fig. 3. Percentage relaxations in response to acetylcholine (Ach) in mesenteric beds preconstricted with methoxamine (MTX) under basal conditions ($n = 4$ in each group, control, open circles and L-NAME-treated, closed circles) and after acute pretreatment with L-NAME and indomethacin (Indo, $n = 6$ in each group, squares).

in the vessels from hypertensive rats than in those of the controls. Verapamil did not alter significantly baseline perfusion pressure in controls (12.1 ± 0.9 in untreated vessels and 12.5 ± 0.5 in verapamil-treated) or in the L-NAME-treated animals (14.5 ± 1.3 vs. 16.3 ± 1.4, respectively). Pretreatment with verapamil significantly reduced the methoxamine pressor responses in both groups, but the responses of the L-NAME-treated rats were still significantly higher than those of the controls (Fig. 1A, left panel). When the mesenteries were perfused with high K^+-Krebs in the absence of Ca^{2+}, perfusion pressure increased in both groups (controls, 32.0 ± 1.6 mm Hg; L-NAME-treated, 49.3 ± 4.2), but the vessels from the L-NAME-treated rats still showed higher perfusion pressure. Then, there-addition of Ca^{2+} resulted in significantly higher increases in perfusion pressure in the vessels from the L-NAME-treated rats than in the controls (Fig. 1A,

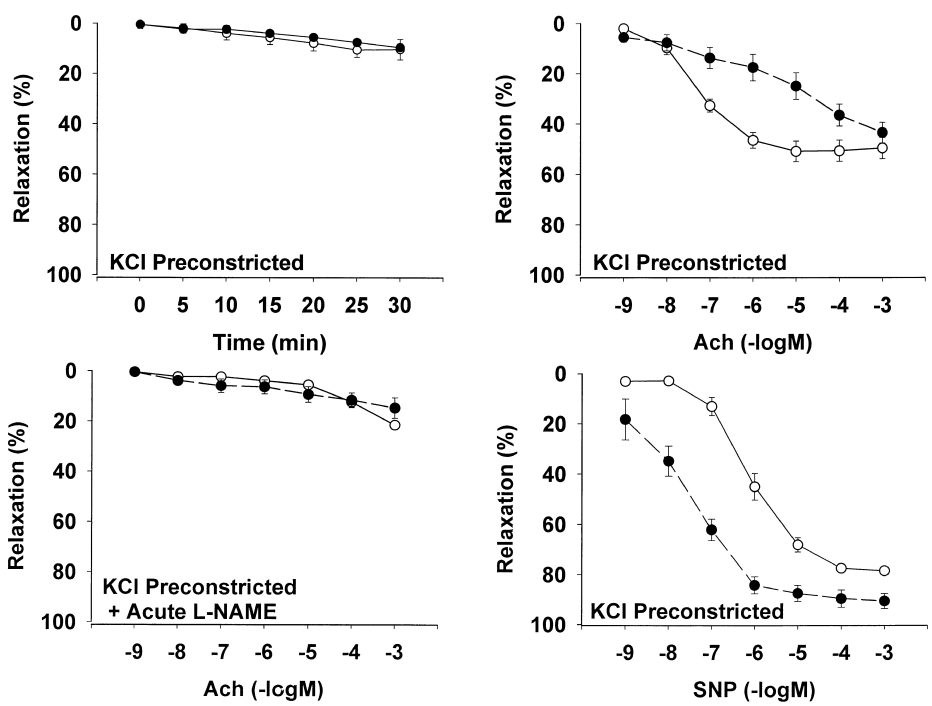

Fig. 2. Percentage relaxations in response to acetylcholine (Ach) in mesenteric arterial beds preconstricted with KCl (upper right panel, control, $n = 6$, open circles; L-NAME-treated, $n = 8$, closed circles) or KCl + L-NAME (lower left panel, $n = 3$ each group). Lower right panel: Percentage relaxations in response to sodium nitroprusside (SNP) in mesenteric arterial beds preconstricted with KCl ($n = 4$ each group). Upper left panel: time-control experiments ($n = 3$ in each group).

right panel). The responses to KCl were also significantly increased in the mesenteric vessels of the hypertensive rats and verapamil almost abolished those effects, except for the higher KCl doses, the responses to which were still greater in the mesenteric vessels of the L-NAME-treated rats (Fig. 1B, left panel). Pretreatment with prazosin and propranolol did not significantly modify the responses to KCl of the mesenteries of the hypertensive rats, although the maximum response was significantly lower, but it attenuated the responses of the control vessels. In this case, verapamil completely eliminated the changes in perfusion pressure elicited by KCl (Fig. 1B, right panel) (Table 1).

3.2. Vasodilator responses (Figs. 2 and 3, Table 2)

The results of the time-control experiments (Fig. 2, upper left panel and Fig. 3) showed that the KCl or methoxamine precontraction could be maintained more than 90% of the initial value during the time necessary to obtain the vasodilator curves. Responses to acetylcholine in KCl-preconstricted vessels (Fig. 2, upper right panel) were significantly reduced in the mesenteric vessels of the L-NAME-treated rats, but still showed marked relaxation. However, these vasodilator responses were almost completely eliminated and the differences between the experimental groups disappeared when NO was acutely inhibited (Fig. 2, lower left panel). Vasodilator responses to sodium nitroprusside (Fig. 2, lower right panel) were significantly enhanced in the mesenteric vessels of the L-NAME-treated rats as compared to those of the control rats (pD_2: 7.82 ± 0.29 vs. 6.12 ± 0.19, respectively). In methoxamine-preconstricted vessels (Fig. 3), relaxations to acetylcholine were similar in both control and L-NAME-treated vascular beds. After acute NO and prostaglandins synthesis inhibition, the vasodilator responses to acetylcholine were lower in the control mesenteries than those of the hypertensive animals (Table 2).

Table 2
Maximum relaxations in response to acetylcholine (MAX, percentage of constriction) and ED_{50} ($-\log M$) in the protocol of vasodilator responses

Preconstriction with	*n*	MAX	ED_{50}
KCl			
Control	6	51.3 ± 4.2	7.07 ± 0.15
L-NAME hypertension	8	43.4 ± 4.1	5.87 ± 0.35[a]
KCl + Acute L-NAME			
Control	3	18.6 ± 3.8	5.60 ± 0.37
L-NAME hypertension	3	16.3 ± 4.3	5.78 ± 0.60
MTX			
Control	4	86.8 ± 2.6	7.10 ± 0.14
L-NAME hypertension	4	90.6 ± 2.2	7.06 ± 0.04
MTX + L-NAME + INDO			
Control	6	70.3 ± 2.8[b]	6.71 ± 0.22
L-NAME hypertension	6	83.5 ± 4.1[a]	7.22 ± 0.16[a]

Data are means ± E.E. INDO, indomethacin.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.05$ vs. respective group under untreated conditions.

4. Discussion

The present results showed that chronic inhibition of NO synthesis produces an increased vascular responsiveness in the rat isolated and perfused mesenteric bed to an α-agonist such as methoxamine and to receptor-independent vasoconstrictor, KCl. Previous studies evaluating vascular reactivity in this model of arterial hypertension have shown divergent results. Generally, depressed pressor responses in conduit vessels such as the aorta with a variety of drugs (Kung et al., 1995; Henrion et al., 1996). This has been thought to be due to down-regulation of the smooth muscle cell's contractile machinery as a consequence of the elevation in blood pressure. In contrast, in resistance vessels, such as those of the mesenteric bed (Deng et al., 1993; Li and Schiffrin, 1994) and kidney (Bryant et al., 1995; Vargas et al., 1996), increased pressor responses to several vasoconstrictors have been described. Thus, the present results agree with data obtained by Navarro-Cid et al. (1996) who, using a single dose of phenylephrine and angiotensin II, found higher pressor responses in the mesenteric bed of chronically L-NAME-treated rats. In our experiments, however, whole dose–response curves were performed and their analysis indicated that chronic NO inhibition increases both the maximum pressor responses and the sensitivity of the preparation to methoxamine and to KCl. It is also noteworthy that, in mesenteric rings of second and third order branches of the mesenteric artery, both increased (Deng et al., 1993; Li and Schiffrin, 1994) and decreased (Dowell et al., 1996) pressor responses have been found, and the explanations for these divergent results could be related to the different preparations used, e.g., ring or cannulated preparations (Buus et al., 1994).

The present results also give some insight into the cellular mechanisms mediating this increased vascular responsiveness to vasoconstrictors in the L-NAME-treated rats. It is known that KCl produces constriction of the smooth muscle because of the depolarization induced by the increase in extracellular K^+ which opens voltage-activated Ca^{2+} channels. Thus, we hypothesized that the smooth muscle cell of the L-NAME-treated rat could be more sensitive to Ca^{2+} than the control cell. Two different experiments support this idea. First, verapamil, a Ca^{2+} channel blocker, almost completely blocked the KCl-induced increase in perfusion pressure in both groups, although perfusion pressure of the L-NAME-treated rats was still slightly but significantly higher, specially at high-KCl doses. Second, the re-addition of Ca^{2+} to mesenteric beds perfused with Ca^{2+}-free Krebs and containing high extracellular K^+ and, therefore with the voltage-sensitive Ca^{2+} channels open, showed an important increase in pressure,

significantly greater in the vessels from the hypertensive animals. Therefore, the increased vascular response to KCl of the mesenteric bed of the chronically NO-deficient hypertensive rats is due to Ca^{2+} channels. However, it is also probable that the smooth muscle contractile machinery of the hypertensive rats is more sensitive to Ca^{2+} since it has been demonstrated that the L-NAME-treated rats show an increased media/lumen ratio (Deng et al., 1993; Li and Schiffrin, 1994; Dowell et al., 1996) which may amplify the effect of any hypertensive stimulus.

A second mechanism whereby the KCl-induced depolarization could participate in the elevated vascular reactivity of the hypertensive mesenteric vascular beds is through the entry of Ca^{2+} into the autonomic nerve terminals and the subsequent release of catecholamines (Vanhoutte and Verbeuren, 1976). In fact, previous data have shown that transmural electrical field stimulation of mesenteric resistance arterial rings results in greater increases in tension in L-NAME-treated vessels than in controls (Li and Schiffrin, 1994). Our results show that the response to KCl of the control mesenteric vessels was greatly diminished when this mechanism was inhibited by the blockade of α- and β-adrenoceptors with prazosin and propranolol. Thus, the release of catecholamines from mesenteric nerve endings is an important mechanism mediating the pressor response to KCl in normal mesenteries. However, our data also indicated that this mechanism is less important in the mesenteric beds from the hypertensive rats. The responses in the absence and presence of prazosin and propranolol were almost identical, and the sensitivity to KCl was not modified by the addition of the adrenoceptor antagonists. However, there was a small residual pressor effect of KCl in the mesenteric beds pretreated with verapamil which was greater in the L-NAME-treated rats and this residual effect disappeared completely with α- and β-blockade. This indicates that a portion of the enhanced vasoconstrictor effect of KCl in the mesenteric vascular beds of the L-NAME-treated rats was due to the release of catecholamines from the mesenteric nerve endings.

The results obtained with methoxamine also suggest an increased role of Ca^{2+} as a mechanism contributing to the enhanced response to the α-adrenoceptor agonist in the L-NAME-treated rats. Thus, verapamil reduced the response to methoxamine in both groups, but that of the hypertensive rats was still increased with respect to that of the controls. This result suggests an important role for Ca^{2+} entry through verapamil-sensitive channels. The residual, enhanced, pressor effects may be due to Ca^{2+} entry through verapamil-insensitive Ca^{2+} channels and also possibly to the intracellular release of Ca^{2+}.

Another objective of the present study was the evaluation of a possible role for EDHF as a compensatory vasodilator mechanism which could be operative in the absence of NO. Our results support this possibility since we observed enhanced vasodilatory responses to acetylcholine in the methoxamine-preconstricted mesenteric bed, in the presence of acute inhibition of NO and prostaglandins synthesis. It is known that the relative importance of NO and EDHF varies depending on the type of vessel studied (Nagao et al., 1992; Nagao and Vanhoutte, 1993). In large conduit arteries such as the aorta and the pulmonary artery of rats, the endothelium-dependent relaxations to acetylcholine are mediated mainly by NO. Endothelium-dependent relaxations of more peripheral arteries, such as the mesenteric, depend not only on NO but also on other mechanisms that are resistant to inhibitors of NO synthases (McCulloch et al., 1997). The component that is resistant to these inhibitors is probably mediated by endothelium-dependent hyperpolarization of the vascular smooth muscle (Nagao et al., 1992; Nagao and Vanhoutte, 1993; McCulloch et al., 1997), a phenomenon induced by an unknown endothelium-derived substance that has been named EDHF, which acts by increasing membrane K^+ conductance, thus inducing hyperpolarization and relaxation. Moreover, there is evidence that, in the rat mesenteric bed, there is an interaction or "cross-talk" between NO and EDHF. Thus, the activity of EDHF is increased and this may compensate completely or in part for the loss of NO activity (McCulloch et al., 1997). Previous data from Vargas et al. (1996) support this idea of a compensatory elevation of EDHF in the kidney of chronic L-NAME hypertensive rats. Our data extend these observations also to the mesenteric bed also. Moreover, the present results suggest that this elevated EDHF activity in the mesenteric bed of the L-NAME hypertensive rat may be of physiological importance since the acetylcholine vasodilator response in basal conditions was similar in the control and in the hypertensive mesenteric beds. It is important to note that other authors have reported that mesenteric arterial rings from L-NAME-treated hypertensive rats show a reduced vasodilator response to acetylcholine (Kung et al., 1995; Dowell et al., 1996). The reason for these discrepancies is not apparent but it may be related to methodological differences and also to differences in the release of EDHF or NO in rings or in perfused vascular preparations. Therefore, our results suggest that an enhanced activity of EDHF may play a role as a physiological mechanism counteracting the arterial hypertension of this experimental model.

The present results also suggested that chronic NO inhibition does not result in the complete inhibition of the acetylcholine-mediated vasodilation from the mesenteric vessels. This was found in KCl-preconstricted vessels, therefore eliminating the possibility of acetylcholine-induced EDHF release (Adeagbo and Triggle, 1993). Thus, acetylcholine substantially relaxed the mesenteric bed of the hypertensive rats, although clearly much less than in the controls, suggesting a reduced release of NO. Moreover, when NO was acutely inhibited in the presence of high extracellular potassium, the differences in acetylcholine relaxation between groups were abolished and relaxations in response to acetylcholine were markedly impaired. However, in spite of the significant amount of

NO available, the reduction was enough to evoke the well-known phenomenon of supersensitivity to exogenous NO, as observed after the administration of sodium nitroprusside (Moncada et al., 1991; Dowell et al., 1996).

Therefore, the present data indicate that the enhanced vascular reactivity to vasopressors in the isolated and perfused mesenteric bed of L-NAME hypertensive rats is related to a higher response to Ca^{2+} entry. An enhanced role of EDHF is also operative in the vessels of the L-NAME-treated rats, and it may play a compensatory role to maintain local responses in this and other vascular beds.

Acknowledgements

This work has been performed with grants from the Comisión Interministerial de Investigación Científica y Técnica of Spain (SAF97-0176) and Fundación Séneca de la Comunidad Autónoma de Murcia (PB/9/FS/99). M.C.O. (FP94-29000129), L.A.F. (PN95-42849602) and F.J.A.N. (AP98-22998855) are recipients of a predoctoral fellowship from the Ministerio de Educación y Cultura of Spain.

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