Original Article

Antihypertensive Mechanisms of Chronic Captopril or *N*-Acetylcysteine Treatment in L-NAME Hypertensive Rats

Josef ZICHA[1)], Zdenka DOBEŠOVÁ[1)], and Jaroslav KUNEŠ[1)]

Hypertension due to chronic inhibition of NO synthase (NOS) by N^{ω}-nitro-L-arginine methyl ester (L-NAME) administration is characterized by both impaired NO-dependent vasodilation and enhanced sympathetic vasoconstriction. The aim of our study was to evaluate changes in the participation of major vasoactive systems in L-NAME–treated rats which were subjected to simultaneous antihypertensive (captopril) or antioxidant (*N*-acetylcysteine, NAC) treatment. Three-month-old Wistar males treated with L-NAME (60 mg/kg/day) for 5 weeks were compared to rats in which L-NAME treatment was combined with simultaneous chronic administration of captopril or NAC. Basal blood pressure (BP) and its acute responses to consecutive i.v. injections of captopril (10 mg/kg), pentolinium (5 mg/kg), L-NAME (30 mg/kg), tetraethylammonium (TEA, 16 mg/kg) and nitroprusside (NP, 20 μg/kg) were determined in conscious rats at the end of the study. The development of L-NAME hypertension was prevented by captopril treatment, whereas NAC treatment caused only a moderate BP reduction. Captopril treatment normalized the sympathetic BP component and significantly reduced residual BP (measured at full NP-induced vasodilation). In contrast, chronic NAC treatment did not modify the sympathetic BP component or residual BP, but significantly enhanced NO-dependent vasodilation. Neither captopril nor NAC treatment influenced the compensatory increase of TEA-sensitive vasodilation mediated by endothelium-derived hyperpolarizing factor in L-NAME–treated rats. Chronic captopril treatment prevented L-NAME hypertension by lowering of sympathetic tone, whereas chronic NAC treatment attenuated L-NAME hypertension by reduction in the vasodilator deficit due to enhanced NO-dependent vasodilation. (*Hypertens Res* 2006; 29: 1021–1027)

***Key Words*: NO-dependent vasodilation, sympathetic vasoconstriction, renin-angiotensin system, endothelium-derived hyperpolarizing factor**

Introduction

Blood pressure (BP) elevation in hypertension is caused by an imbalance of vasoconstrictor and vasodilator systems, which often act on structurally remodeled resistance vessels. The NO-deficient hypertension induced by chronic N^{ω}-nitro-L-arginine methyl ester (L-NAME) administration, which is initiated by a reduction of NO-dependent vasodilation, is maintained by involvement of the renin-angiotensin system (RAS) and sympathetic nervous system (SNS) (*1*, *2*). Chronic administration of angiotensin-converting enzyme (ACE) inhibitors (*3*, *4*) or angiotensin II type 1 (AT_1) receptor blockers (*5*, *6*) as well as sympathectomy (*2*, *7*) or pharmacological interventions on SNS (*8*, *9*) attenuate the development of this form of experimental hypertension. In contrast, the effects of chronic antioxidant treatment on the development of L-NAME hypertension are rather controversial (*10–12*). Our recent study (*12*) demonstrated considerable BP reduction in L-NAME–treated rats that were consuming high doses of *N*-

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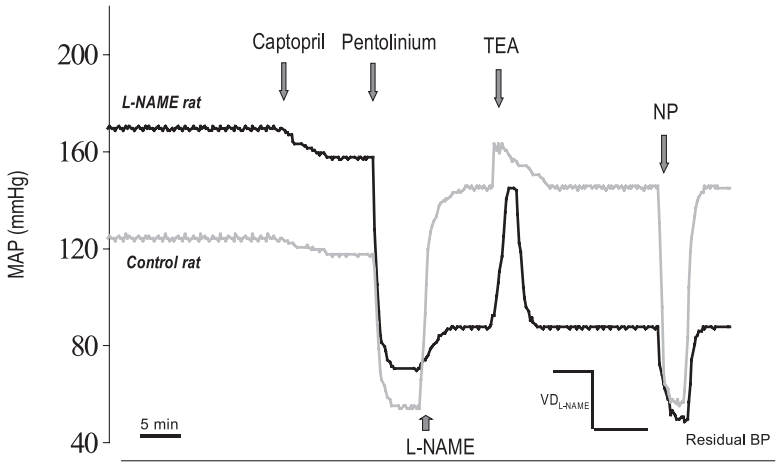

Fig. 1. *Schematic representation of mean arterial pressure (MAP) changes during the consecutive blockade of individual vasoactive systems in conscious control (gray line) or L-NAME hypertensive rats (black line). Arrows indicate the injections of captopril, pentolinium, tetraethylammonium (TEA), and sodium nitroprusside (NP). Residual pressure was recorded after NP injection at the end of the experiment. The vasodilator deficit in L-NAME hypertensive rats ($VD_{L\text{-}NAME}$) is indicated by the vertical bar, which is equal to the difference between the minimal BP value obtained after pentolinium injection and that obtained after NP injection.*

acetylcysteine (NAC; 1.5 g/kg/day). The BP reduction was associated with a significant lowering of superoxide production and decreased levels of conjugated dienes, indicating the antioxidant action of NAC (*12*). Concerning the antihypertensive effect of antioxidant NAC, it should be noted that NAC also protects the sulfhydryl groups of NO synthase (NOS) (*13*) and enables the formation of *S*-nitrosothiols that directly activate guanylate cyclase (*14*).

Our previous study (*15*) confirmed a major reduction of NO-dependent vasodilation in L-NAME hypertensive rats, in which high BP is maintained by enhanced sympathetic vasoconstriction, while the contribution of augmented angiotensin II–dependent vasoconstriction was only borderline. Subsequent experiments (*16*) revealed that endothelium-derived hyperpolarizing factor (EDHF) becomes the principal vasodilator in L-NAME hypertensive rats, whereas the compensatory up-regulation of inducible NOS makes only a modest contribution. Recently, we combined a consecutive blockade of particular vasoactive systems in conscious rats (*15*, *17*) with the subsequent application of sodium nitroprusside (NP). This approach enabled us to determine not only the contribution of major vasoactive systems (RAS, SNS, NO, EDHF) to BP maintenance but also to evaluate the magnitude of the vasodilator deficit and the level of residual BP, which reflects structural changes of resistance vessels.

The aims of our present study were 1) to further characterize hemodynamic alterations in L-NAME hypertensive rats, namely, EDHF-mediated vasodilation (tetraethylammonium [TEA]-sensitive BP changes), vasodilator deficit (the difference between actual vasodilation and the maximal vasodilation induced by an NO donor) and residual BP (recorded after a full NP-induced vasodilation), and 2) to determine the hemodynamic effects of chronic antihypertensive (captopril) or antioxidant (NAC) treatment in this model of experimental hypertension.

Methods

Animals

Male Wistar rats aged 3 months (Institute of Physiology AS CR, Prague, Czech Republic) were housed under standard laboratory conditions (temperature 23±1°C, 12-h light-dark cycle) and had free access to tap water and a pelleted ST1 diet containing 1% NaCl. They were randomly allocated to four experimental groups: controls (n=12), L-NAME–treated rats (n=10), L-NAME–treated rats drinking captopril solution (500 mg/l) (n=11) and L-NAME–treated rats drinking NAC solution (20 g/l) (n=12). L-NAME (60 mg/kg/day) was administered in the drinking fluid for 5 weeks to induce established hypertension. All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Physiology AS CR, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use.

Experimental Protocol

One day before BP measurement, polyethylene catheters were inserted into the carotid artery and jugular vein and exteriorized in the interscapular region. BP was recorded in conscious animals using a PowerLab system (ADInstruments,

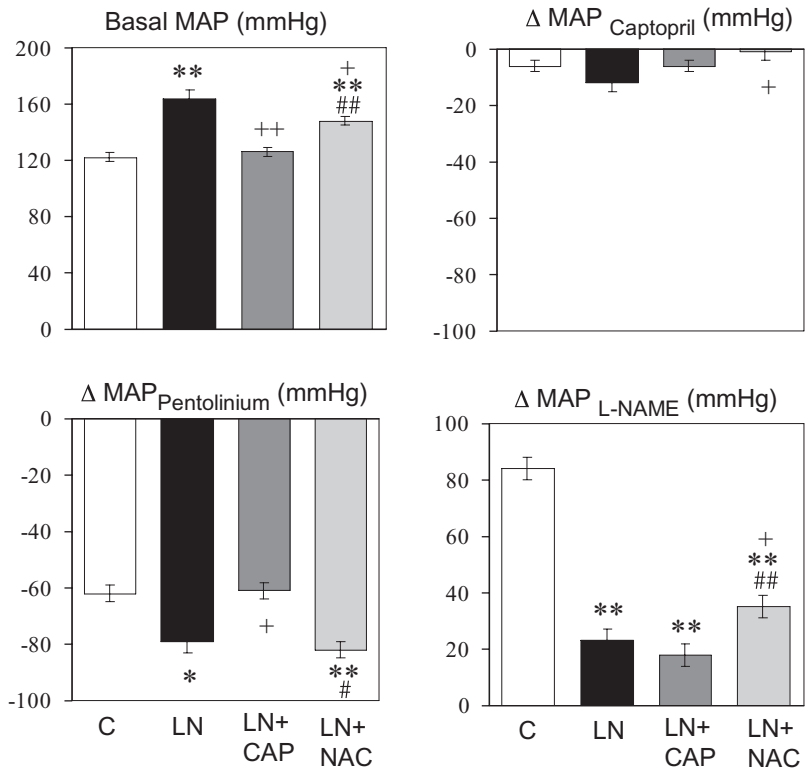

Fig. 2. *Basal MAP (upper left) and MAP changes elicited by acute captopril (upper right), pentolinium (lower left) and L-NAME injection (lower right) in controls (C), L-NAME hypertensive rats (LN), and L-NAME–treated rats simultaneously administered captopril (LN+CAP) or* N*-acetylcysteine (LN+NAC). Significantly different (*$p < 0.05$, $p < 0.01$*) from: *, ** control rats, $^{+}$, $^{++}$ LN animals, $^{\#}$, $^{\#\#}$ LN+CAP rats.*

Colorado Springs, USA) between 8 and 12 AM. Four animals (chosen in random order) were monitored simultaneously.

Baseline BP values were monitored in conscious animals for 30 min prior to a consecutive blockade of RAS, SNS, NOS and EDHF, which was performed according to the protocol of Minami *et al*. (*17*) with some modifications (Fig. 1). Initially, captopril (10 mg/kg body weight [bw]; Sigma, St. Louis, USA) was injected intravenously to block RAS through ACE inhibition. Fifteen minutes later, SNS blockade was induced by ganglion blocker pentolinium (5 mg/kg bw; Sigma) which was followed by a rapid BP fall. When BP reached its minimum and was temporarily stabilized for about 5 min, NOS inhibitor L-NAME (30 mg/kg bw; Sigma) was given and BP restoration was monitored for the next 10 min. Thereafter TEA (16.5 mg/kg bw; Sigma) was injected to preferentially block Ca^{2+}-activated K^{+} channels targeted by EDHF (the TEA concentration in extracellular fluid did not surpass 0.5 mmol/l). At the end of the experiment, NP (20 μg/kg bw; Sigma) was injected into animals in which all of the above-mentioned vasoactive systems had been blocked in order to determine the level of residual BP that reflects structural changes of resistance vessels. All drugs were given as an intravenous bolus in a volume of 1 ml/kg bw.

The contribution of individual vasoactive systems (RAS, SNS, NOS and EDHF) to BP maintenance was calculated from the absolute changes of mean arterial pressure (MAP) induced by captopril, pentolinium, L-NAME and TEA, respectively. Vasodilator deficit was estimated from the difference between the BP level achieved after the blockade of vasoconstrictors (RAS, SNS) and the residual BP, which was recorded as the minimum BP reached after the injection of NP (Fig. 1). The contribution of particular vasoactive systems to the BP elevation found in L-NAME hypertensive animals was calculated so that the difference between normotensive and hypertensive rats in the MAP response to the acute blockade of a given vasoactive system could be expressed as a percentage of the difference in the basal level of MAP between normotensive and hypertensive rats. Based on this calculation, the sympathetic nervous system made a 40% contribution to the BP difference between the L-NAME hypertensive animals and controls.

Statistical Analysis

Results were expressed as the means±SEM. The statistical significance of differences was evaluated by one-way ANOVA followed by post-hoc least significant difference test.

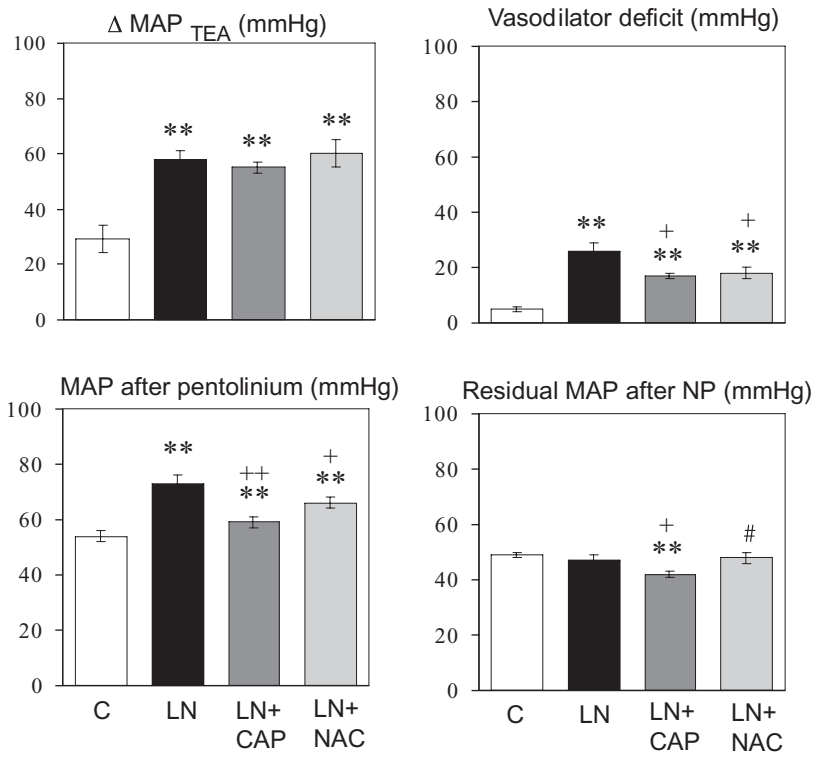

Fig. 3. *MAP changes elicited by TEA injection in animals subjected to a consecutive RAS, SNS and NOS blockade (upper left), vasodilator deficit (upper right), MAP recorded after RAS and SNS blockade (lower left), and residual MAP recorded after NP injection (lower right) in controls (C), L-NAME hypertensive rats (LN), and L-NAME–treated rats simultaneously administered captopril (LN+CAP) or* N*-acetylcysteine (LN+NAC). Significantly different (*$p < 0.05$, $p < 0.01$*) from: *,** control rats,* $^{+}$, $^{++}$ *LN animals,* $^{\#}$, $^{\#\#}$ *LN+CAP rats.*

Results

L-NAME hypertensive rats showed a major reduction of NO-dependent vasodilation ($\Delta MAP_{L\text{-}NAME}$) (Fig. 2), which was partially compensated by an increase of EDHF-dependent vasodilation (ΔMAP_{TEA}) (Fig. 3). Hypertension was associated with a pronounced enhancement of sympathetic vasoconstriction ($\Delta MAP_{Pentolinium}$), whereas there was only a mild augmentation of angiotensin II–dependent vasoconstriction ($\Delta MAP_{Captopril}$) (Fig. 2). Thus the imbalance between augmented vasoconstriction and attenuated vasodilation resulted in a considerable vasodilator deficit in L-NAME hypertensive rats (Fig. 3). The failure to disclose any significant changes in residual BP indicated the absence of major remodeling of resistance vessels in L-NAME hypertension.

Chronic captopril treatment completely prevented the development of L-NAME hypertension, and this was mainly due to the normalization of sympathetic vasoconstriction ($\Delta MAP_{Pentolinium}$) (Fig. 2). Although chronic captopril administration did not modify NO- or EDHF-dependent vasodilation, it slightly reduced the existing vasodilator deficit. Surprisingly, chronic captopril treatment lowered residual BP to the level below that found in untreated control rats (Fig. 3).

The effects of chronic NAC treatment were completely different from those exerted by chronic captopril administration. NAC treatment attenuated the development of L-NAME hypertension, but BP remained significantly higher in NAC-treated rats compared to the controls (Fig. 2). It had no effect on sympathetic vasoconstriction, but it significantly lowered angiotensin II–dependent vasoconstriction ($\Delta MAP_{Captopril}$). The major antihypertensive effect of NAC administration in L-NAME–treated rats was due to the augmentation of NO-dependent vasodilation ($\Delta MAP_{L\text{-}NAME}$) (Fig. 2) and the reduction of vasodilator deficit, although there was no significant influence on the compensatory increase of EDHF-dependent vasodilation (Fig. 3). Chronic NAC treatment did not modify the residual BP of L-NAME–treated animals.

Figure 4 shows the relationship of basal MAP to the participation of various vasoactive systems in BP maintenance. Basal MAP values correlated closely with both sympathetic vasoconstriction ($\Delta MAP_{Pentolinium}$) and the vasodilator deficit (the difference between the actual vasodilation and the maximal vasodilation induced by the NO donor). In contrast, there was only a borderline correlation with angiotensin II–dependent vasoconstriction ($\Delta MAP_{Captopril}$) but no significant rela-

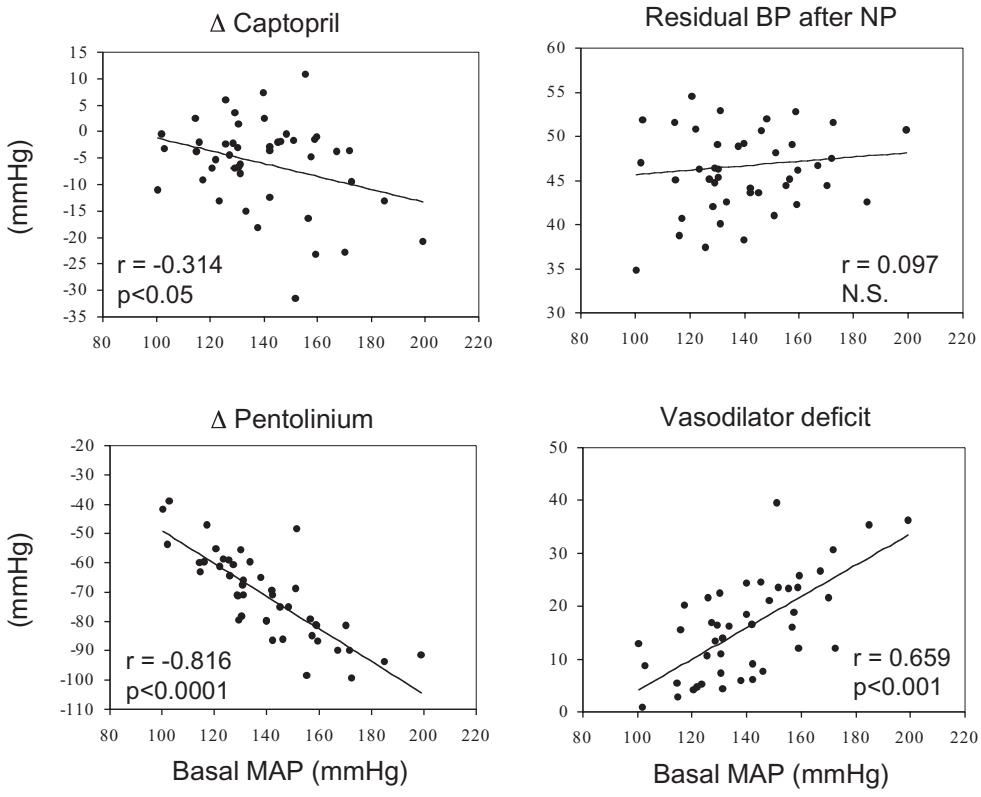

Fig. 4. *The relationships between basal MAP and captopril-induced MAP changes (upper left), residual MAP (upper right), pentolinium-induced MAP changes (lower left) or vasodilator deficit (lower right) in the complete group of all rats studied (*n*=45).*

tionship to residual BP. Similar relationships were also disclosed if the correlation analysis was performed only in L-NAME–treated rats (basal MAP *vs*. sympathetic vasoconstriction: $r=-0.793$, $n=33$, $p<0.0001$; basal MAP *vs*. vasodilator deficit: $r=0.531$, $n=33$, $p<0.002$). In the whole set of experimental animals there was an inverse relationship between BP changes induced by acute L-NAME and TEA injection ($r=-0.798$, $n=45$, $p<0.0001$) but this was not true within the three groups of L-NAME–treated rats ($r=-0.299$, $n=33$, n.s.).

This correlation analysis suggests that sympathetic vasoconstriction and vasodilator deficit played a major role in the BP variation seen in our experiments. It also supports our estimate that enhanced sympathetic vasoconstriction ($\Delta MAP_{Pentolinium}$ greater by 17 mmHg in L-NAME rats compared to normotensive controls), more pronounced vasodilator deficit (greater by 16 mmHg) and moderately increased angiotensin II–dependent vasoconstriction (greater by 6 mmHg) were responsible for 40%, 40% and 15% of the difference in MAP between the L-NAME hypertensive rats and normotensive controls, which was about 40 mmHg.

Discussion

Our study indicated that both augmented vasoconstriction (mainly sympathetic) and attenuated vasodilation (insufficient compensation of missing NO by EDHF) participate in the maintenance of elevated BP in L-NAME hypertension. Simultaneous administration of antihypertensive (captopril) or antioxidant (NAC) drugs to L-NAME–treated rats suggested that the increased vasoconstriction plays a more important role than the decreased vasodilation. Captopril treatment prevented the development of L-NAME hypertension by abolishing enhanced sympathetic vasoconstriction, whereas antioxidant NAC, which did not modify sympathetic vasoconstriction, had less pronounced antihypertensive effects, although it augmented NO-dependent vasodilation. This supports the concept of neural mechanisms in the pathogenesis of NO-deficient hypertension (*2*).

Chronic captopril treatment is often used to reduce the involvement of RAS in the pathogenesis of particular forms of experimental hypertension. In our study, chronic captopril administration to L-NAME–treated rats had only a marginal effect on peripheral angiotensin II–dependent vasoconstriction, but it considerably lowered BP through the reduction of

sympathetic vasoconstriction, indicating the crucial role of enhanced sympathetic tone in L-NAME hypertension. Similar reduction of sympathetic tone was also demonstrated in captopril-treated spontaneously hypertensive rats (SHR) (*18*, *19*). This can be explained either by attenuation of angiotensin II–induced facilitation of peripheral sympathetic neurotransmission (*20*) or by lowering of the increased activity of brain RAS that drives the enhanced sympathetic outflow (*18*, *21*, *22*). However, in our experiments the differences in sympathetic vasoconstriction were demonstrated in animals which were acutely pretreated with captopril for 15 min. This is a sufficient time to eliminate the angiotensin II–induced decrease of norepinephrine reuptake to nerve endings leading to enhanced sympathetic vasoconstriction (*20*).

On the other hand, it is well known that the modulation of RAS activity or NO synthesis in the brain has reciprocal effects on sympathetic tone, indicating a central counterbalance of angiotensin II and NO in regulation of sympathetic output (*23–26*). It should be noted that chronic L-NAME administration inhibits NOS activity not only in peripheral organs (including the cardiovascular system) but also in the central nervous system (CNS) (*4*). Brain NOS activity is inhibited by 40–60% in L-NAME hypertensive animals, and this NOS inhibition is not affected by chronic captopril treatment (*4*). It seems that reduced NO formation in the brain of L-NAME–treated rats might attenuate the control of sympathetic tone, which is driven by unaltered levels of brain angiotensin II. This would be compatible with the neural concept of L-NAME hypertension proposed by Sander and Victor (*2*). In fact, intracerebroventricular (i.c.v.) administration of L-arginine lowers blood pressure and sympathetic activity in Wistar rats (*27*), whereas i.c.v. injection of L-NMMA has just the opposite effects (*28*). In addition, the absence of NO in L-NAME hypertensive rats increases the pressor effects of acute i.c.v. administration of angiotensin II (*29*), and chronic i.c.v. infusion of losartan attenuates the development of L-NAME hypertension (*30*).

In the present study we used NAC to reduce oxygen free radical formation and to increase NO bioavailability. Chronic NAC administration partially attenuated the development of L-NAME hypertension due to moderate augmentation of NO-dependent vasodilation and partial reduction of the vasodilator deficit. This is in agreement with our previous studies in which chronic NAC treatment reduced oxidative stress and augmented the NOS activity not only in L-NAME–treated rats (*12*) but also in SHR (*31*).

The profound NO deficiency in L-NAME–treated rats was largely compensated by increased EDHF-dependent vasodilation, as in an earlier study using NO-deficient rats (*32*). However, this EDHF up-regulation was not able to compensate for the entire vasodilator deficit resulting from chronic NOS inhibition in L-NAME–treated rats, indicating that the fine tuning of vasodilatory mechanisms by the reciprocal NO-EDHF relationship (*33*) is altered in hypertension.

The present study did not reveal any functional signs of the resistance vessel remodeling, because residual BP (recorded after NP injection to animals subjected to a combined blockade of RAS, SNS and NOS) was the same in L-NAME hypertensive rats as in normotensive controls. This is in contrast with our previous study (*15*) in which L-NAME hypertensive rats were characterized by a 20–40% elevation of "residual" BP values (recorded just after a consecutive RAS and SNS blockade) compared to normotensive controls. This apparent contradiction can be easily explained as shown in Fig. 3 of the present study, which indicates that the elevation of BP measured after pentolinium injection in L-NAME hypertensive rats was due to an augmented vasodilator deficit but not due to structural changes of the resistance vessels, as we believed earlier.

The observed reduction of residual BP in captopril-treated NO-deficient rats was rather surprising, but we have observed similar captopril-induced lowering of residual BP by chronic captopril treatment not only in SHR but also in normotensive Wistar-Kyoto rats (Zicha *et al*., unpublished data). It was recently reported that ACE inhibitors or AT_1 receptor blockers increase blood levels of NO, the antiproliferative effects of which can combine with the absence of proliferative effects of angiotensin II *via* AT_1 receptors (*34*).

In conclusion, chronic captopril treatment prevented L-NAME hypertension by lowering of the sympathetic tone, whereas chronic NAC treatment attenuated L-NAME hypertension by reduction in vasodilator deficit due to enhanced NO-dependent vasodilation.

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