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# Captopril Prevents NO-Deficient Hypertension and Left Ventricular Hypertrophy Without Affecting Nitric Oxide Synthase Activity in Rats

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## Summary

**The aim of the study was to assess whether angiotensin converting enzyme (ACE) inhibition with captopril prevents the development of hypertension and myocardial hypertrophy and affects nitric oxide synthase (NOS) activity in rats. Animals were divided into five groups: control, two groups receiving $N^G$-nitro-L-arginine methyl ester (L-NAME) 20 or 40 mg/kg/day, a group receiving captopril 100 mg/kg/day and a group concomitantly treated with 40 mg/kg/day L-NAME plus 100 mg/kg/day captopril. After four weeks, systolic blood pressure (SBP) significantly increased in both L-NAME groups by 30 % and 34 %, respectively. In the captopril group, SBP significantly decreased by 30 % and in the captopril plus L-NAME group SBP was not changed as compared to the control. Although left ventricular weight/body weight (LVW/BW) ratio in both L-NAME groups was significantly elevated by 19 % and 29 %, respectively, no alterations in LVW/BW ratio were found in the captopril group and captopril plus L-NAME group. In both groups receiving L-NAME, NOS activity significantly decreased by 17 % and 69 % in the heart, by 14 % and 26 % in the aorta, by 60 % and 73 % in the brain and by 13 % and 30 % in the kidney, respectively. Captopril did not influence NO synthase activity in any of the studied tissues. We conclude that captopril prevents the development of hypertension and LV hypertrophy without affecting NO formation.**

**Key words**

Nitric oxide synthase – Captopril – L-NAME – Angiotensin II – Hypertrophy – Hypertension

## Introduction

The inhibition of NO synthesis by L-arginine analogues induces sustained hypertension in normotensive rats and dogs (Chu *et al.* 1991, Ribeiro *et al.* 1992). The mechanisms involved in the development of this type of hypertension are not quite clear. Besides the restriction of NO, activation of both the renin-angiotensin system (RAS) and the sympathetic nervous system are being considered (Rees *et al.* 1989, Jover *et al.* 1993, Sander *et al.* 1995).

The NO-deficient hypertension is associated with attenuation of vascular relaxation and with hypercontractility in different parts of the vascular tree. Of particular interest is the reduced relaxation in the renal artery (Holécyová *et al.* 1996). This might result in increased renin release and activation of the renin-angiotensin system.

Besides different vasomotor effects of angiotensin II (Ang II) and NO, available data indicate different effects of Ang II and NO on trophic processes. It was reported that Ang II stimulates collagen synthesis in cultured vascular smooth muscle cells (Kato *et al.* 1991) and cardiac hypertrophy in rats (Giacomelli *et al.* 1976). On the other hand, Garg and Hassid (1989) showed that NO-generating substances induce dose-dependent inhibition of smooth muscle cell mitogenesis and proliferation in culture. Also Kolpakov *et al.* (1995) demonstrated inhibitory effect of NO-generating compounds on total protein and

collagen synthesis in cultured vascular smooth muscle cells. Delacretaz *et al.* (1994) showed that long-term NO inhibition by L-NAME leads to LV hypertrophy in rats. ACE inhibitors were described as substances with an antihypertrophic effect in spontaneously hypertensive rats and in rats with aortic constriction (Linz *et al.* 1995). This effect of ACE inhibitors may be determined by a blockade of circulating and/or local Ang II (Dzau *et al.* 1988).

By ACE inhibition the level of bradykinin may increase, which in turn can result in increased production of prostaglandins and NO (Wilmer *et al.* 1991). This may be a crucial point in connection between RAS and NO system.

Despite this relation between RAS and NO system, there is a lack of information concerning influence of ACE inhibition on NO synthase activity in rats. The purpose of this study was to evaluate the effect of long-term oral administration of captopril on arterial blood pressure, LV hypertrophy and NOS activity in an experimental model of NO-deficient hypertension.

## Material and Methods

Male Wistar rats, 15 weeks old, were divided into five groups. The first group (n=8) served as the control, the second group (n=8) was given L-NAME in the dose 20 mg/kg/day and the third group (n=8) received 40 mg /kg/day of L-NAME. The fourth group (n=6) was treated with 100 mg/kg/day of captopril and the fifth group (n=8) was simultaneously treated with 40 mg/kg/day of L-NAME and 100 mg/kg/day of captopril. The substances were given in tap water for four weeks. Systolic blood pressure was measured by non-invasive tail-cuff plethysmography every day. The animals were sacrificed after four weeks. The heart, aorta, brain and kidneys were excised and the left ventricle weight/body weight ratio was calculated for each animal. A segment of the aorta, a section of the myocardium from the anterior wall of the left ventricle, a slice of the cerebral gray matter, and a sample of kidney tissue were used for estimating NO synthase activity and the protein content.

*Assay of NO synthase activity*

NO synthase activity was determined in crude homogenates of tissues by measuring the production of [$^3$H]-L-citrulline from [$^3$H]-L-arginine (Amersham, UK) as previously described by Bredt and Snyder (1990) with some modifications. Briefly, 50 $\mu$l of 10 % homogenates were incubated in the presence of 50 mmol/l Tris-HCl at pH 7.4, 10 $\mu$mol/l L-[$^3$H]arginine (specific activity 5 GBq/mmol, about 100 000 dpm), 30 nmol/l calmodulin, 1 mmol/l $\beta$-NADPH, 3 $\mu$mol/l $BH_4$ and 2 mmol/l $Ca^{2+}$ in a total volume of 100 $\mu$l. After 20 min incubation at 37 °C, the reaction was stopped by the addition of 1 ml of 20 mmol/l HEPES buffer pH 5.5, containing 2 mmol/l EDTA, 2 mmol/l EGTA and 1 mmol/l L-citrulline (L-Cit). Then the samples were applied to 1 ml Dowex 50WX-8 columns ($Na^+$ form). [$^3$H]-L-citrulline was eluted by 1 ml of water and measured by liquid scintillation counting.

The protein content was determined by Lowry's method modified according to Ramachandran and Fraenkel-Conrat (1958). All the chemicals used were from Sigma, Germany.

*Statistical analysis*

The results are presented as mean ± S.E.M. One-way ANOVA analysis (the Bonferroni test) was used and $p<0.05$ was taken as significant.

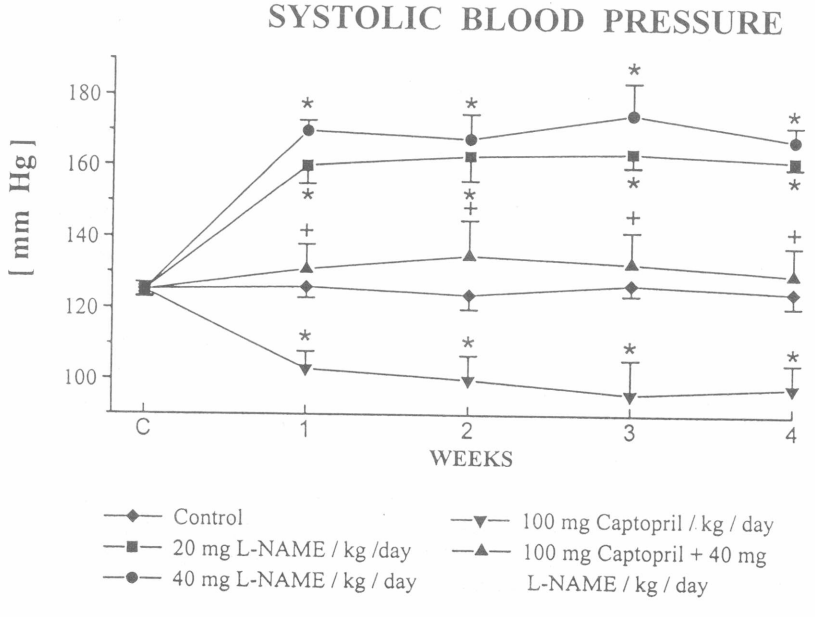


**Fig. 1**
*Effect of captopril and L-NAME on systolic blood pressure of rats. * $p<0.05$ as compared to control, + $p<0.05$ as compared to rats receiving 100 mg/kg/day of captopril.*

**Table 1**
Effect of L-NAME and captopril on heart weight/body weight (HW/BW) ratio and left ventricle weight/body weight (LVW/BW) ratio

| | Control<br>n=8 | L-NAME 20 mg/ /kg/day<br>n=8 | L-NAME 40 mg/ kg/day<br>n=8 | Captopril 100 mg/ /kg/day<br>n=6 | 100 mg Captopril + 40 mg L-NAME /kg/day<br>n=8 |
|---|---|---|---|---|---|
| HW/BW mg/kg | 2.44±0.066 | 2.58±0.032 | 2.63±0.068 | 2.23±0.029 | 2.24±0.028$^{+}$ |
| LVW/BW mg/g | 1.21±0.024 | 1.43±0.014* | 1.54±0.047* | 1.18±0.051 | 1.10±0.022$^{+}$ |

** $p<0.05$ as compared to control, $^{+}$ $p<0.05$ as compared to rats receiving L-NAME 40 mg/kg/day.*

## Results

After the first week of the experiment, SBP was 160±5 mm Hg in the group of rats treated with 20 mg/kg/day of L-NAME versus 125±2 mm Hg in the control group (Fig. 1). Increased SBP persisted during the next three weeks and after the fourth week of L-NAME treatment SBP increased by 30 % as compared to the control. A similar situation was found in the group of animals receiving 40 mg/kg/day of L-NAME. In this group, SBP increased by 34 % after four weeks of treatment. In the rats treated with 100 mg/kg/day of captopril, SBP decreased significantly. After the first week, SBP was 103±5 mm Hg, and after the fourth week, SBP decreased by 27 % as compared to the control group. A different situation was in the group of rats simultaneously treated with 40 mg/kg/day of L-NAME plus 100 mg/kg/day of captopril where SBP was 131±7 mm Hg after the first week and similar value of SBP persisted during the next three weeks (Fig. 1).

In our experiment HW/BW ratio was not changed either in the L-NAME or captopril group (Table 1). A significant decrease of HW/BW ratio was found in the rats treated with L-NAME plus captopril as compared to rats treated only with 40 mg/kg/day of L-NAME. However, there was significant elevation of LVW/BW ratio in both L-NAME treated groups by 19 % and 29 %, respectively. On the other hand, in groups treated with captopril or captopril plus L-NAME the LVW/BW ratio was not changed as compared to the controls. Similarly as the HW/BW ratio, the LVW/BW ratio in the L-NAME plus captopril group was also significantly smaller than in the group treated with 40 mg/kg/day L-NAME.

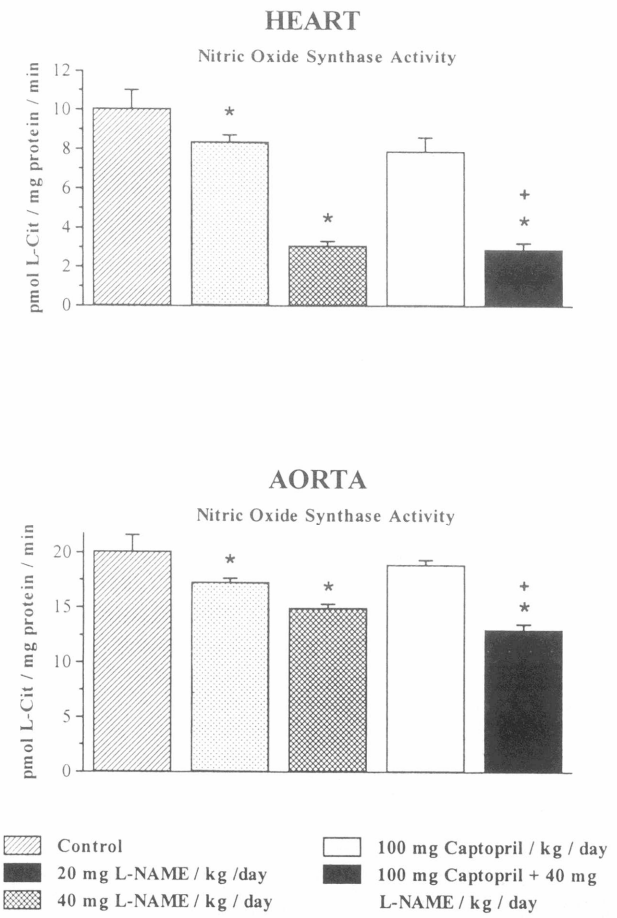


**Fig. 2**
*NOS activity in heart and aorta of rats treated with L-NAME and captopril. * $p<0.05$ as compared to control, + $p<0.05$ as compared to rats receiving 100 mg/kg/day of captopril.*

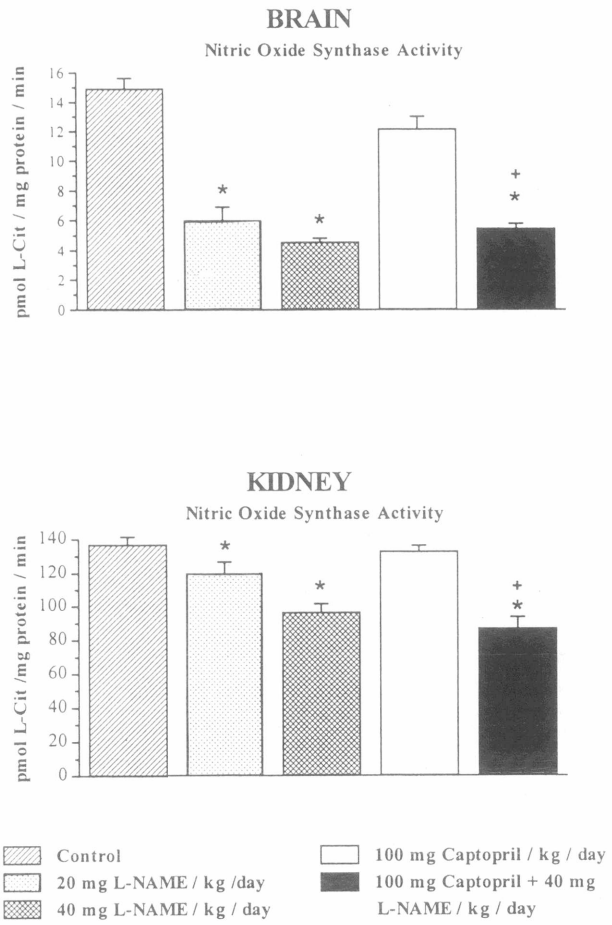


**Fig. 3**
*NOS activity in brain and kidney of rats treated with L-NAME and captopril. * $p<0.05$ as compared to control, + $p<0.05$ as compared to rats receiving 100 mg/kg/day of captopril.*

NOS activity in control hearts was 10.07±0.94 pmol L-Cit/mg protein/min. In the hearts of both L-NAME-treated groups, NOS activity was inhibited by 17 % and 69 %, respectively (Fig. 2). In captopril group, NOS activity did not differ from the control. NOS activity in the hearts of the L-NAME plus captopril group was inhibited by 71 % as compared to control. Control NOS activity in the aorta was 20.14±2.41 pmol L-Cit/mg protein/min and was also inhibited in both L-NAME-treated groups by 14 % and 26 %, respectively (Fig. 2). In the captopril group, NOS activity was not altered, and in L-NAME plus captopril group, NOS activity was decreased by 35 % as compared to the control. No differences were found in NOS activity between the groups treated with 40 mg/kg/day of L-NAME and L-NAME plus captopril. A similar effect of L-NAME on NOS activity was found in the brain and kidney (Fig. 3), where control NOS activity was 14.94±0.71 pmol L-Cit/mg protein/min and 137.21±4.91 pmol L-Cit/mg protein/min, respectively. In rats receiving 20 and 40 mg /kg/day of L-NAME, NOS activity decreased by 60 % and 73 % in the brain and by 13 % and 30 % in the kidney. Furthermore, captopril alone or administrated simultaneously with 40 mg/kg/day of L-NAME did not affect NOS activity in these tissues. In all tissues of cotreated animals, NOS activity was similar as compared to rats receiving 40 mg/kg/day of L-NAME.

## Discussion

It has been shown that long-term NOS inhibition by L-NAME results in NO-deficient hypertension (Arnal *et al.* 1992, Ribeiro *et al.* 1992). Our results support this finding. However, the data concerning myocardial hypertrophy after L-NAME treatment are still controversial.

In our study, the HW/BW ratio in both L-NAME-treated groups was not significantly elevated, while the LVW/BW ratio significantly increased. The same effect of L-NAME on the LVW/BW ratio was shown by Delacretaz *et al.* (1994). On the other hand, Kristek *et al.* (1995), reported significant elevation of HW/BW after six weeks of L-NAME treatment in rats. In contrast, several authors did not find any hypertrophy of the whole heart (Arnal *et al.* 1992), or of the left ventricle (Arnal *et al.* 1993), after oral L-NAME treatment of rats for four or eight weeks, respectively.

Treatment with captopril prevented both NO-deficient hypertension and LV hypertrophy. The results suggest a significant role of renin-angiotensin system in this type of hypertension. The literature provides enough evidence that ACE inhibitors may prevent LV hypertrophy or cause regression of hypertrophy in pressure- and volume-overloaded hearts (Šimko 1994, Weinberg *et al.* 1994, Šimko 1996). Similarly, in L-NAME treated rats, the ACE inhibitor ramipril prevented SBP elevation and myocardial hypertrophy (Hropot *et al.* 1994, Linz *et al.* 1995).

NO synthase activity was decreased in the heart, aorta, brain and kidney in both L-NAME treated groups. This is the first evidence that captopril alone or with 40 mg/kg/day of L-NAME did not cause significant activation of NOS in any of the investigated organs *in vivo*. Similarly, *in vitro*, Ikeda and Shimada (1994) showed that ACE inhibitors did not affect NO synthesis in vascular smooth muscle cells.

The molecular mechanisms of the beneficial effect of ACE inhibitors are not yet quite clear. As the ACE is the enzyme identical with kinase II in the kallikrein-kinin system, it is possible that accumulation of bradykinin may mediate cardioprotection of ACE inhibitors. However, the beneficial effect of endo- and exogenous bradykinin was shown to be NO-dependent and was completely abolished by simultaneous application of NOS inhibitors (Massoudy *et al.* 1995).

On the other hand, the elevation of bradykinin could enhance the content of prostaglandins (Greenwald and Becker 1994). One of them, $PGI_2$, can activate adenylate cyclase which results in the elevation of cAMP – a potent vasodilator. Additionally, Swarz and Williams (1982) showed that indomethacin, a potent cyclooxygenase inhibitor, can attenuate the antihypertensive effect of captopril.

We conclude that captopril prevents NO-deficient hypertension and concomitant hypertrophy of the left ventricle. NOS activity in the heart, aorta, brain and kidney was not affected in either captopril-treated or captopril plus L-NAME-cotreated rats. The results suggest that the protective effect of captopril in NO-deficient hypertension does not involve alterations in NOS activity. If the protective effect of captopril is caused by inhibition of circulating or local RAS, or by elevation of prostaglandins, remains to be elucidated.

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