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Effect of Captopril on Cyclic Nucleotide Concentrations During Long-Term NO Synthase Inhibition

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Summary

The aim of the present study was to determine the effect of angiotensin-converting enzyme inhibitor captopril on cGMP and cAMP concentration in the left ventricle and aorta after NO synthase inhibition by 4-week-lasting N^G-nitro-L-arginine-methyl ester (L-NAME) treatment. Five groups of rats were investigated: controls, L-NAME in the dose 20 mg/kg/day (L-NAME 20), L-NAME in the dose 40 mg/kg/day (L-NAME 40), captopril in the dose 100 mg/kg/day, L-NAME 40 mg/kg/day together with captopril 100 mg/kg/day. Captopril completely prevented L-NAME-induced hypertension and LV hypertrophy development. Compared to the controls, cGMP concentration in the L-NAME 20 and L-NAME 40 groups was decreased by 13 % and 22 %, respectively, in the left ventricle and by 27 % and 56 % in the aorta, respectively. Captopril did not influence this decrease of cGMP concentration. Cyclic AMP concentration in the aorta of L-NAME 20 group increased by 17 %. In the L-NAME 40 group, cAMP concentration increased by 17 % in the left ventricle and by 34 % in the aorta compared to controls. This increase was enhanced in rats given L-NAME together with captopril. Captopril alone had no effect on cAMP concentration. We conclude that captopril does not affect the concentration of cGMP, however, it has more than the additive effect on the cAMP concentration increase in the cardiovascular system during long-term NO synthase inhibition.

Key words

Nitric oxide • NO synthase • Cyclic nucleotides • L-NAME • ACE inhibitor • Hypertension

Introduction

Numerous interactions among NO, angiotensin II (Ang II), kinins and prostaglandins have been reported in the regulation of blood pressure and cardiac hypertrophy (Brunner 1993, Šimko and Šimko 2000). Recently, it has been shown that ACE inhibitors reduce blood pressure and cardiac hypertrophy not only in renin-dependent models of hypertension but also in the other forms, which are less clearly related to the renin action, such as the spontaneous hypertension in rats (Vapaatalo *et al.* 2000), essential hypertension in humans (Williams 1988) and NO-deficient hypertension (Pecháňová *et al.* 1997). The antihypertensive effect of ACE inhibitors was shown to be mediated *via* a reduced formation of Ang II in both plasma and tissues (Dzau 1990) and accumulation of bradykinin and related kinins, which in turn stimulate the synthesis of NO and PGI_2 in endothelial cells (Linz *et al.* 1995) and promote endothelium-dependent hyperpolarization (Mombouli *et al.* 1996). Additional mechanisms

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are supposed to be involved in the hypotensive and antiproliferative effect of ACE inhibitors, namely the equilibrium existing between the actions exerted by NO and Ang II (DeNicola *et al.* 1992). The vasodilator and antiproliferative effects of NO are mediated mainly *via* activation of soluble guanylate cyclase and generation of cGMP (Moncada 1992). The effects of bradykinin have been attributed to the increase of both cGMP and cAMP content, since NO as well as prostacyclin mediate the action of bradykinin (Wiemer *et al.* 1991, Yang *et al.* 1993). Elevation of cAMP as well as the cGMP is involved in the relaxation of vascular smooth muscle cells in response to a variety of vasodilators (Toyoshima *et al.* 1998a). Lincoln and Cornwell (1991) showed that cAMP might also be able to activate the cGMP-dependent protein kinase directly, thereby mediating the vasorelaxation. While both cyclic nucleotides and their related kinases decrease intracellular Ca^{2+} ($[Ca^{2+}_i]$) in vascular smooth muscle cells, cAMP-dependent protein kinase has a positive ionotropic effect in the cardiac muscle which results in an increase of $[Ca^{2+}_i]$ (Xiong and Sperelakis 1995).

The aim of the present study was to determine the effect of angiotensin-converting enzyme inhibitor captopril on cGMP and cAMP concentrations in the left ventricle and aorta after NO synthase inhibition by 4-week-lasting treatment with N^G-nitro-L-arginine-methyl ester (L-NAME).

Material and Methods

Animals

Male 12-week-old Wistar rats, were randomly divided into five groups (n=8 in each group). The first group served as the control. In the second group, L-NAME (Sigma Chemical Co, Diesenhofen, Germany) was given in the dose of 20 mg/kg/day (L-NAME 20 group), whereas in the third group L-NAME was given in the dose of 40 mg/kg/day (L-NAME 40 group). The fourth group received captopril (Egis Pharmaceutical Ltd., Budapest, Hungary) in the dose of 100 mg/kg/day (captopril group). The fifth group received simultaneously L-NAME in the dose of 40 mg/kg/day and captopril in the dose of 100 mg/kg/day (L-NAME + captopril group). The substances were given in tap water for four weeks. Systolic blood pressure (SBP) was measured by the non-invasive method of tail-cuff plethysmography every day. The animals were sacrificed after 4 weeks, the body weight (BW), heart weight (HW) and left ventricle weight (LVW) were determined and the LVW/BW ratio was calculated.

In each group, fresh samples of the left ventricle and thoracic aorta were used for determination of NO synthase activity. The samples for assay of cGMP and cAMP were frozen in liquid nitrogen and kept at –80 °C.

Assay of NO synthase activity

NO synthase activity was determined in crude homogenates of the left ventricle and aorta by measuring the formation of [^{3}H]-L-citrulline from [^{3}H]-L-arginine (Amersham, Little Chalfont, UK) as previously described by Bredt and Snyder (1990) with some modifications (Pecháňová et al. 1997, 1999c). Briefly, 50 mg of wet tissue were homogenized (2 min, Ultra-Turrax homogenizer) in 500 µl of ice-cold 50 mmol/l Tris-HCl buffer, pH 7.4, containing 2 µmol/l leupeptin, 0.5 mmol/l dithiothreitol, 1 µmol/l pepstatin A and 1 mmol/l phenylmethyl-sulphonyl fluoride. Then 50 µl of the crude homogenate were incubated in the presence of 50 mmol/l Tris-HCl, pH 7.4, containing 10 µmol/l L-[^{3}H]arginine (specific activity 5 GBq/mmol, about 100 000 dpm), 30 nmol/l calmodulin, 1 mmol/l β-NADPH, 3 µmol/l tetrahydro-biopterin, and 2 mmol/l Ca^{2+}, in a total volume of 100 µl. 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-citrullin (L-Cit). The samples were centrifuged at 10 000 x*g* for 1 min at 4 °C and then applied to 1 ml Dowex 50WX-8 columns (Na^+ form). [^{3}H]-L-citrulline (L-Cit) was eluted by 1 ml of water and measured by liquid scintillation counting. NO synthase activity was expressed as pmol L-Cit/mg protein/min.

Cyclic nucleotide assay

Cyclic GMP and cAMP concentration was determined in crude homogenates of the left ventricle and aorta using radioimmunoassay. Kit No. 1118 for cGMP and kit No. 1117 for cAMP were purchased from Immunotech, S.A. (Marseille, France). Briefly, cGMP radio-immunoassay was based on the competition between the succinylated cGMP of the sample and ^{125}I-labeled tracer for binding to polyclonal antibody coated onto tubes. Cyclic AMP radioimmunoassay used the same principle, however, without succinylation. To determine the cGMP or cAMP concentration, the vials were assessed using a gamma counter. The cyclic nucleotide concentrations in the samples were calculated

from the standard curve and expressed as pmol/g wet tissue. Finally, the cAMP/cGMP ratio was calculated for each sample.

Statistical analysis

Results are expressed as mean ± SEM. One-way ANOVA and Bonferroni test were used for analysis. Values were considered significant at $p<0.05$.

Results

Cardiovascular parameters

After the first week of the experiment, SBP was 126±5 mm Hg in the control group, whereas in L-NAME 20 and L-NAME 40 groups, SBP increased by 31 and 37 %, respectively ($p<0.05$). In the captopril group SBP was decreased by 17 % ($p<0.05$) and in the L-NAME + captopril group SBP did not change compared to controls. SBP changes persisted during the following three weeks. After the fourth week of the experiment, the LVW/BW ratio was 1.21±0.02 in the control group. In the L-NAME 20 and L-NAME 40 groups, the ratio increased by 20 and 26 %, respectively ($p<0.05$), compared to controls. In the captopril as well as in the L-NAME + captopril groups, the LVW/BW ratio did not change significantly. However, in the latter group the ratio decreased significantly by 28 % compared to the L-NAME 40 group (Table 1).

Table 1. Effect of 4-week lasting treatment with L-NAME (20 and 40 mg/kg/day), captopril (100 mg/kg/day), and L-NAME (40 mg/kg/day) + captopril (100 mg/kg/day) on systolic blood pressure (SBP), left ventricle weight/body weight (LVW/BW) ratio and cAMP/cGMP ratio in the left ventricle and aorta.

	Controls	**L-NAME 20**	**L-NAME 40**	**Captopril**	**L-NAME + Captopril**
SBP (mm Hg)	126 ± 5	165 ± 7*	173 ± 8*	104 ± 7*	129 ± 6$^{\#}$
LVW/BW (mg/g)	1.21 ± 0.02	1.45 ± 0.04*	1.53 ± 0.04*	1.17 ± 0.05	1.10 ± 0.02$^{\#}$
cAMP/cGMP Left ventricle	232 ± 9	290 ± 9*	346 ± 15*	191 ± 13	462 ± 21*$^{+}$
cAMP/cGMP Aorta	74 ± 6	130 ± 10*	247 ± 14*	69 ± 5	308 ± 17*$^{+}$

** $p<0.05$ as compared to the control value, $^{+}$ $p<0.05$ as compared to the captopril group, $^{\#}$ $p<0.05$ as compared to the L-NAME (40 mg/kg/day) group.*

NO synthase activity

NO synthase activity was 10.08±0.75 pmol L-Cit/mg protein/min in the left ventricle and 20.14±0.68 pmol L-Cit/mg protein/min in the aorta of the control group. In the L-NAME 20 and L-NAME 40 groups, NO synthase activity was inhibited by 17 and 69 %, respectively ($p<0.05$), in the left ventricle and by 14 and 26 %, respectively ($p<0.05$) in the aorta compared to the control values. In the captopril group, NO synthase activity did not differ from the control group, whereas in the L-NAME + captopril group it was comparable to that in the L-NAME 40 group in both left ventricle and aorta (Fig. 1).

Cyclic GMP concentration

Cyclic GMP concentration was 1.46±0.01 pmol/g tissue in the left ventricle and 2.73±0.05 pmol/g tissue in the aorta of the control group. In the L-NAME 20 and L-NAME 40 groups, cGMP concentration decreased by 13 and 22 %, respectively ($p<0.05$), in the left ventricle and by 27 and 56 %, respectively ($p<0.05$), in the aorta compared to controls. In the captopril group,

the cGMP concentration did not differ from the control group and in the L-NAME + captopril group it was comparable to that in the L-NAME 20 and L-NAME 40 groups in the left ventricle as well as in the aorta (Fig. 2).

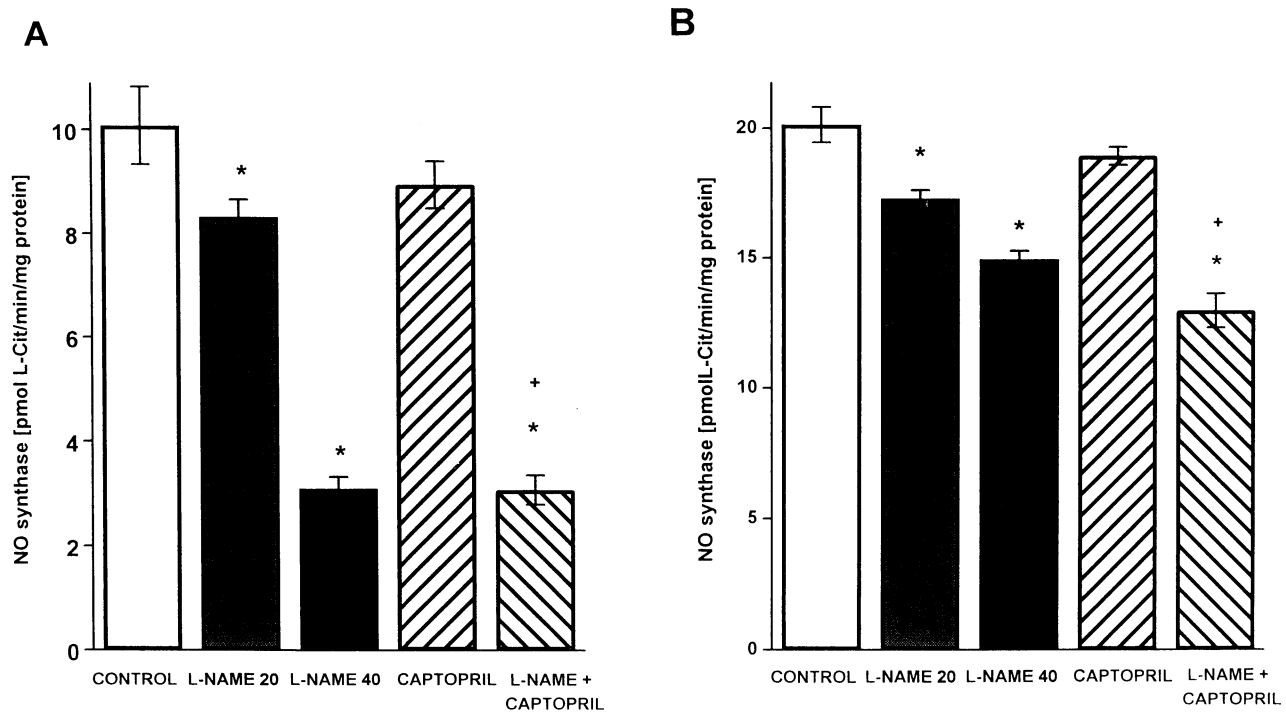

Fig. 1. *Effect of 4-week lasting treatment with L-NAME (20 and 40 mg/kg/day), captopril (100 mg/kg/day), and L-NAME (40 mg/kg/day) + captopril (100 mg/kg/day) on NO synthase activity in the left ventricle* ***[A]*** *and aorta* ***[B]***. * $p<0.05$ *as compared to the control value,* + $p<0.05$ *as compared to the captopril group.*

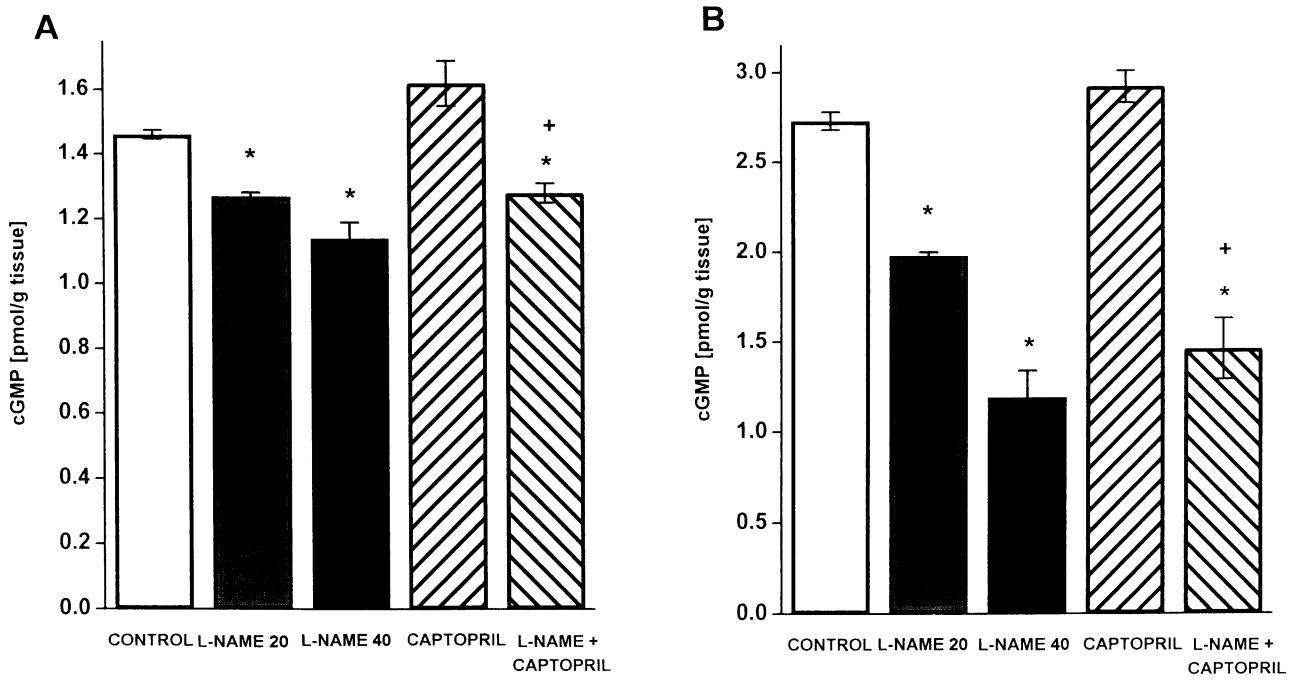

Fig. 2. *Effect of 4-week lasting treatment with L-NAME (20 and 40 mg/kg/day), captopril (100 mg/kg/day), and L-NAME (40 mg/kg/day) + captopril (100 mg/kg/day on cGMP concentration in the left ventricle* ***[A]*** *and aorta* ***[B]***. * $p<0.05$ *as compared to the control value,* + $p<0.05$ *as compared to the captopril group.*

Cyclic AMP concentration

Cyclic AMP concentration was 338±7 pmol/g tissue in the left ventricle and 202±11 pmol/g tissue in the aorta of the control group. In the L-NAME 40 group, cAMP concentration increased by 17 % in the left ventricle, and in the aorta it increased by 29 and 46 % in the L-NAME 20 and L-NAME 40 groups, respectively ($p<0.05$). In the captopril group, the cAMP concentration did not differ from the control group but it increased by 75 % and 166 %, respectively ($p<0.05$), in the L-NAME + captopril group in the left ventricle and aorta compared to the control value (Fig. 3).

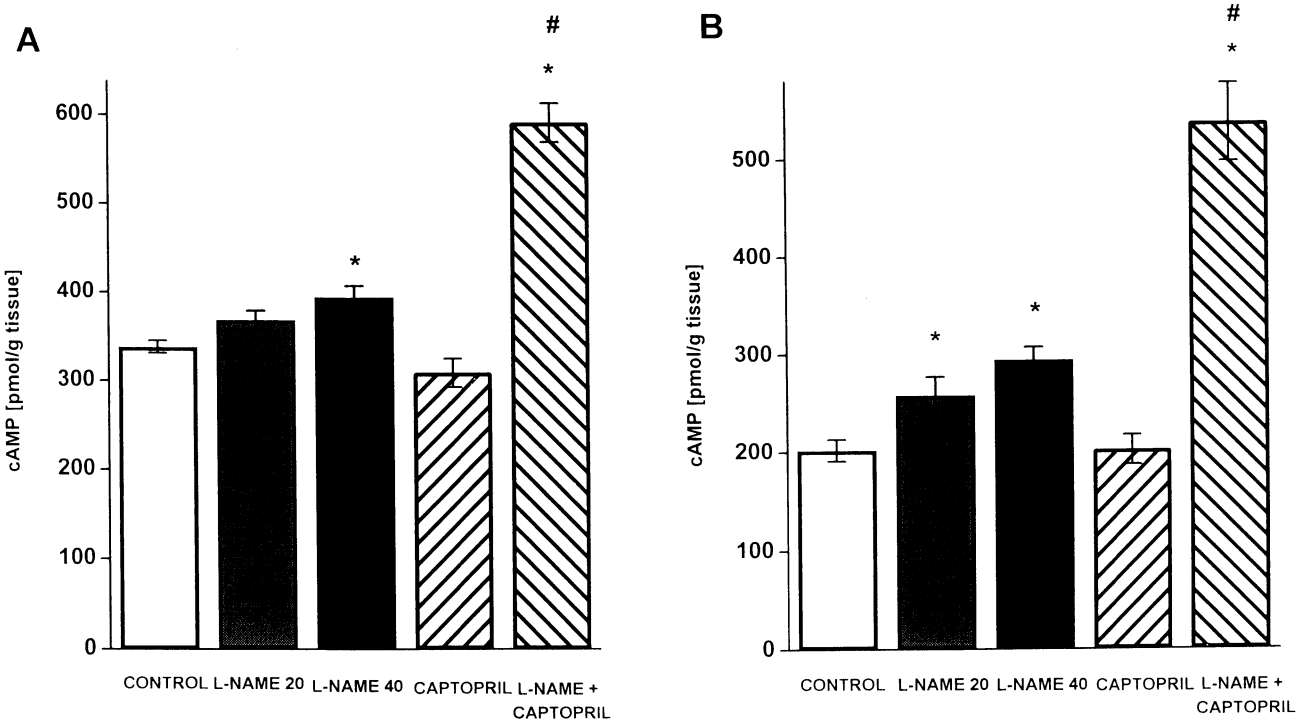

Fig. 3. *Effect of 4-week lasting treatment with L-NAME (20 and 40 mg/kg/day), captopril (100 mg/kg/day), and L-NAME (40 mg/kg/day) + captopril (100 mg/kg/day) on cAMP concentration in the left ventricle* ***[A]*** *and aorta* ***[B]****.* * *$p<0.05$ as compared to the control value,* # *$p<0.05$ as compared to the L-NAME (40 mg/kg/day) group.*

Cyclic AMP/cGMP ratio

The cAMP/cGMP ratio was 232±9 in the left ventricle and 74±6 in the aorta of the control group. In the L-NAME 20 and L-NAME 40 groups, cAMP/cGMP ratio increased by 25 and 49 %, respectively ($p<0.05$), in the left ventricle and by 76 and 234 %, respectively ($p<0.05$), in the aorta compared to controls. In the captopril group, the cAMP/cGMP ratio did not differ from the control group, but it increased by 99 % and 316 %, respectively ($p<0.05$), in the L-NAME + captopril group in the left ventricle and aorta compared to the control values (Table 1).

Discussion

The present study showed that captopril had no effect on left ventricular and aortic cGMP concentrations in the control as well as in L-NAME treated animals, although it completely prevented NO-deficient hypertension and LV hypertrophy development. Captopril alone did not affect cAMP concentration, however, it exerted a more than additive effect on the cAMP concentration increase in the left ventricle and aorta of L-NAME + captopril-treated animals.

NO synthase activity and cGMP concentration were also found to be decreased in the brain and kidneys of L-NAME treated animals from our previous studies using the same experimental model (Pecháňová and Bernátová 1996, Bernátová *et al.* 1999a). This decrease was linked with an increase in blood pressure and elevation of LVW/BW ratio, as has also been confirmed in the present study. The blood pressure increase and changes in left ventricle weight are in agreement with the data published by others (Gardiner *et al.* 1990, Delacretaz *et al.* 1994, Gomes Pessanha *et al.* 1999). Captopril protected against the blood pressure increase and LV

hypertrophy development. ACE inhibitors were shown to prevent or reverse hypertension and LV hypertrophy in other modifications of L-NAME-induced hypertension (Linz *et al.* 1995, Toyoshima *et al.* 1998b, Bernátová *et al.* 1999b). Besides lowering Ang II production, the protective mechanisms are supposed to involve kinin signal transduction pathways which generate cGMP *via* an increase in NO, and cAMP *via* an increase in PGI_2 (Linz *et al.* 1995, Šimko and Šimko 1999). In our study, L-NAME in both doses decreased significantly NO synthase activity and cGMP concentration in the left ventricle as well as in the aorta. In the animals treated with the higher dose of L-NAME, the remaining NO synthase activity represented 31 % in the left ventricle and 74 % in the aorta. Captopril did not have any protective and/or activating effect on NO synthase. Similarly, captopril had no effect on cGMP concentration. Hence, the possible beneficial effect of kinins in our experiments was not mediated *via* cGMP. The same results were obtained by Takase *et al.* (1996) who demonstrated that trandolapril treatment for 6 weeks prevented the increase in systolic blood pressure and deteriorated acetylcholine-induced relaxation of the mesenteric artery observed in L-NAME induced hypertension, without improving NO synthase activity. In accordance, Bao *et al.* (1988) and Cachofeiro *et al.* (1995) reported that long-term administration of bradykinin B_2 receptor antagonist Hoe 140 did not modify the long-term hypotensive action of ramipril in adult SHR. This hypotensive effect of ramipril was, however, attenuated by co-treatment with L-NAME or indomethacin. Furthermore, the depressor effect of ramipril was abolished when L-NAME and indomethacin were administered together (Cachofeiro *et al.* 1995), indicating a contribution of prostaglandins to the blood-pressure lowering effect of ACE inhibitors. This correlates well with our observations of increased cAMP concentration after L-NAME and captopril co-treatment. However, in our model of NO-deficient hypertension, bradykinin can also exert its effect *via* PGI_2. Thus the action of bradykinin should not be excluded from the beneficial mechanisms that prevented hypertension and hypertrophy in this particular model. A contribution of prostaglandins to the antihypertensive effect of ACE inhibitors has been documented by various investigators. Moore *et al.* (1981) demonstrated that the short-term antihypertensive effect of captopril was reduced by concomitant treatment with aspirin or indomethacin in hypertensive patients. Furthermore, ACE inhibitors have been shown to promote prostaglandin synthesis (Wiemer *et al.* 1991). We did not observed the cAMP increase when captopril was administered alone. Captopril, however, potentiated the increase of cAMP concentration in L-NAME-treated animals. The increase in cAMP levels during long-term L-NAME treatment may involve decreased cGMP-mediated inhibition of cAMP phosphodiesterase class II (Schmidt *et al.* 1993), elevation of catecholamines (Cohen and Weisbrod 1988) and release of adenosine (Jakovljevic *et al.* 1999).

Cyclic nucleotides are known to play a central role in modulating the responsiveness of vascular smooth muscles to vasoconstrictor agonists (Toyoshima *et al.* 1998a). Although cAMP may exert a direct inhibitory effect on the contractile proteins, mainly by phosphorylation of myosin light chain kinase, numerous findings suggest that cyclic nucleotides act primarily *via* a decrease of intracellular Ca^{2+} concentration. Lowering of $[Ca^{2+}_i]$ can be achieved by activation of calcium pumps located on the sarcolemma and sarcoplasmic reticulum (Furukawa *et al.* 1988), by inhibition of receptor-mediated activation of phospholipase C, probably *via* phosphorylation of G protein which couples the receptors to phospholipase C (McAtee and Dawson 1990) and possibly by inhibition of the IP_3-mediated release of calcium from intracellular stores (Supattapone *et al.* 1988). In vascular smooth muscle cells, the role of cGMP can be partially substituted by cAMP since both cyclic nucleotides decrease $[Ca^{2+}_i]$, which leads to vasorelaxation (Taguchi *et al.* 1997). Inhibition of NO synthesis in this model of hypertension attenuated cGMP-mediated relaxation to acetylcholine, but did not affect relaxation to isoprenaline mediated by cAMP (Holécyová *et al.* 1996). Lincoln and Cornwell (1991) hypothesized that cGMP-dependent protein kinase can be activated by cAMP. According to this idea, cAMP can decrease $[Ca^{2+}_i]$ depending on the presence of cGMP-dependent protein kinase in the cells. Despite several reports suggesting an activation of the voltage-dependent Ca^{2+} current in smooth muscle cells by cAMP (Fukumitsu *et al.* 1990, Ishikawa *et al.* 1993), the possible physiological significance of cAMP in this activation is not clear because a rise of intracellular cAMP in vascular smooth muscle cells results in vasodilatation. In cardiac muscles, the phosphorylation of L-type Ca^{2+} channels by cAMP-dependent protein kinase is thought to be the most important factor in determining the force of contraction induced by β-adrenergic stimulation (Trautwein and Hescheler 1990). However, phosphorylation of myosin

light chain kinase by cAMP-dependent protein kinase can also exert an inhibitory effect on the contractile proteins (Xiong and Sperelakis 1995).

Taken together, the increase in cAMP concentration in the aorta after NO synthase inhibition can partially compensate the decrease in cGMP content probably by decreasing $[Ca^{2+}_i]$. However, this compensation was insufficient for improving endothelium-dependent relaxation as was shown previously by Holécyová *et al.* (1996). On the other hand, a simultaneous decrease in cGMP and an increase in cAMP content may elevate $[Ca^{2+}_i]$ in the left ventricle which could participate in the development of LV hypertrophy. Captopril potentiated the increase of cAMP concentration in both left ventricle (by 57 %) and aorta (by 120 %) of L-NAME treated animals. A twofold increase in the cAMP/cGMP ratio was found during L-NAME and captopril co-treatment in the left ventricle and a fourfold increase in the aorta compared to the control values. The increase in cAMP/cGMP ratio indicate that the studied systems become more sensitive to alterations of cGMP levels. The marked potentiation of increased cAMP levels in the aorta may lead to a further improvement of endothelium-dependent relaxation which could represent one of the beneficial effects of captopril in the prevention of hypertension. The potentiation of increased cAMP levels in the left ventricle was not as high as in the aorta. Whether this potentiation may affect the dilatation remains to be elucidated. As far as the captopril effect in prevention of myocardial hypertrophy is concerned, a direct inhibitory action of captopril on angiotensin II production (resulting in a reduction of trophic processes) is plausible.

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