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Effect of Long-Term NO Synthase Inhibition on Cyclic Nucleotide Content in Rat Tissues

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Summary

The effect of 4 weeks' inhibition of NO synthase by nitro-L-arginine methyl ester (L-NAME) on haemodynamic parameters and cGMP and cAMP content was studied in rat tissues. L-NAME in both 20 mg/kg/day and 40 mg/kg/day doses significantly increased systolic blood pressure by 28 % and 30 % and decreased the heart rate by 14 % and 23 %, respectively, after the first week. These changes persisted during the following three weeks. Left ventricular weight/body weight (LVW/BW) ratio was significantly elevated in both L-NAME-treated groups by 19 % and 29 %, respectively. Radioimmunoassay was used to determine the cGMP and cAMP content. Cyclic GMP content in animals treated by L-NAME (20 mg/kg/day and 40 mg/kg/day) decreased significantly by 13 % and 22 % in the left ventricle, by 28 % and 62 % in the aorta, by 20 % and 34 % in the brain, and by 10 % and 15 % in the kidney, respectively. On the other hand, the cAMP content increased in both L-NAME treated groups by 8 % and 9 % in the left ventricle, by 28 % and 46 % in the aorta, and by 23 % and 32 % in the brain, respectively. There were no significant changes in kidney cAMP content as compared to control animals. The results suggest a simultaneous decrease of cGMP and increase of cAMP content in the majority of studied tissues during NO-deficient hypertension.

Key words

NO synthase – L-NAME – cGMP – cAMP – Hypertrophy

Introduction

It has recently been shown that long-term NO synthase inhibition by L-arginine analogues induced hypertension in normotensive rats and dogs (Chu *et al.* 1991, Ribeiro *et al.* 1992). Two of the inhibitors: nitro-monomethyl-L-arginine and nitro-L-arginine-methyl ester administered orally have been shown to be active in rats, in which they induced a sustained increase in blood pressure and reduction of heart rate (Gardiner *et al.* 1990). Moreover, Delacretaz *et al.* (1994) showed significant elevation of the LVW/BW ratio after L-NAME treatment in rats. However, the molecular mechanisms underlying the development of hypertension and followed by left ventricular hypertrophy after NO synthase inhibition are not clear. A number of reports demonstrated an involvement of cGMP decrease as guanylate cyclase can be directly affected by NO (Moncada 1992). It is clear now that many of the NO effects are related to cGMP and Ca^{2+}, but the relationship between NO and cAMP has been studied much less. In vascular smooth muscle cells, elevation of cAMP as well as cGMP have been involved in the relaxation of smooth muscle cells in response to a variety of vasodilators. Lincoln and Cornwell (1991) showed that cAMP might be able to activate the cGMP-dependent protein kinase directly, thereby inhibiting the Ca^{2+} channel activity. While in vascular smooth muscle cells both cyclic nucleotides and their related kinases decrease intracellular Ca^{2+} ($[Ca^{2+}{}_i]$), the cAMP-dependent protein kinase in the cardiac muscle has the positive inotropic effect which results in an increase of $[Ca^{2+}{}_i]$ (Xiong and Sperelakis 1995).

In this study, NO synthase activity was inhibited by L-NAME in the dose 20 and 40 mg/kg/day for 4 weeks with the aim assess (i) the development of NO-deficient hypertension, (ii) the hypertrophic process in the heart, (iii) the relationship between cGMP and cAMP content in the left ventricle, aorta, brain and kidney.

Materials and Methods

Animals

15-week-old male Wistar rats were divided into 3 groups (each n=6). The first group served as a control, the second group was given L-NAME (Sigma Chemical Co, Germany) in the dose 20 mg/kg/day and the third group was given L-NAME in the dose 40 mg/kg/day in the drinking water for 4 weeks. Systolic blood pressure and heart rate were measured by the noninvasive method – tail cuff plethysmography every day. After 4 weeks of L-NAME treatment, the animals were sacrificed, the left ventricle and body weight were assessed and LVW/BW ratio was calculated. The content of cyclic nucleotides was determined in the homogenates of rat tissues.

Cyclic nucleotide assay

Cyclic GMP and cAMP content was determined in crude homogenates of the left ventricle, aorta, brain and kidney using radioimmunoassay procedures with ^{125}I-labelled cGMP or cAMP (Immunotech, S.A., France). Briefly, cGMP radioimmunoassay used the competition between the succinylated cGMP of the sample and ^{125}I-cGMP for binding to polyclonal antibody coated onto tubes. Cyclic AMP radioimmunoassay was based on the same principle, however, without the succinylation. To determine the ^{125}I-cGMP or ^{125}I-cAMP content the vials were counted using a gamma counter. The cyclic nucleotide content in the samples was calculated from the standard curve.

Statistical analysis

The results are expressed as mean ± S.E.M. For analysis one-way ANOVA, Bonferroni test was used. Values were considered significant at $p<0.05$.

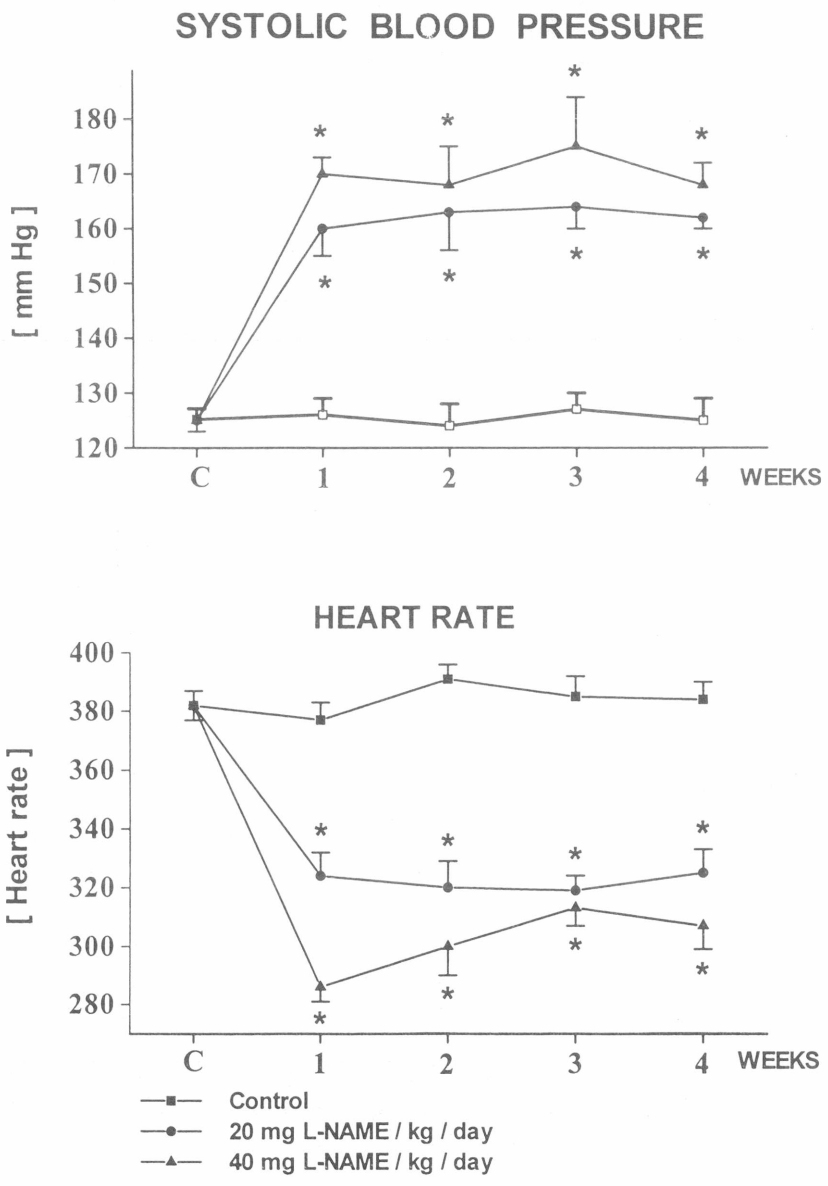

Fig. 1
*Systolic blood pressure and heart rate during 4 weeks of L-NAME administration. * $p<0.05$.*

Results

Cardiovascular parameters

At the end of the first week systolic blood pressure of the animals administered L-NAME (20 and 40 mg/kg/day) was higher by 28.4 % and 30.4 %, respectively ($p<0.05$) as compared to the controls. At the same time the heart rate decreased by 14.3 % and 23.2 % ($p<0.05$) in comparison with the control group. The changes of blood pressure and heart rate persisted during the following three weeks (Fig. 1). LVW/BW ratio increased by 19.2 % and 29.1 % after 4 weeks of L-NAME treatment with the dose of 20 and 40 mg/kg/day, respectively, as compared to the control group ($p<0.05$).

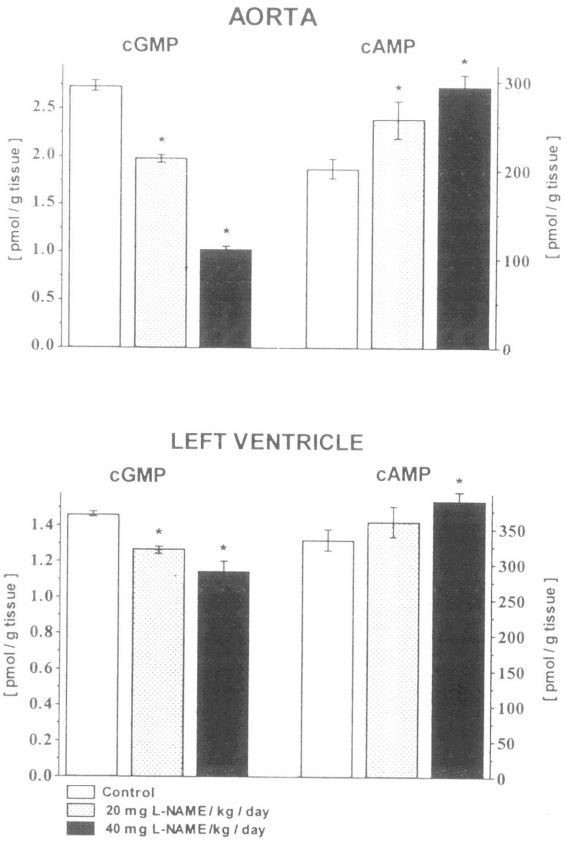

Fig. 2
*Cyclic GMP and cyclic AMP content in aorta and left ventricle after 4-week L-NAME administration, * $p<0.05$.*

Cyclic nucleotide content

The cyclic GMP content decreased significantly by 27.5 % and 62.3 % in the aorta after L-NAME treatment with the dose 20 mg/kg/day and 40 mg/kg/day, respectively, as compared to the control group ($p<0.05$). On the other hand, the cAMP content in the aorta of the same animals increased by 27.8 % and 45.9 %, respectively, as compared to the controls ($p<0.05$). In the left ventricle, the cGMP content decreased significantly by 13.0 % and 21.5 % ($p<0.05$) after L-NAME treatment with the two doses, respectively. However, the cAMP content increased significantly by 8.8 % ($p<0.05$) only after L-NAME treatment with the dose 40 mg/kg/day as compared to the control group (Fig. 2). In the brain, the cGMP content decreased significantly by 20.3 % and 33.6 % ($p<0.05$) and cAMP content increased significantly by 23.4 % and 31.5 % ($p<0.05$) after L-NAME treatment with the dose 20 and 40 mg/kg/day, respectively. In the kidney, cGMP content decreased significantly by 9.5 % and 15.3 % after L-NAME treatment with the two doses, respectively ($p<0.05$). There were no significant changes in the kidney cAMP content as compared to control animals (Fig. 3).

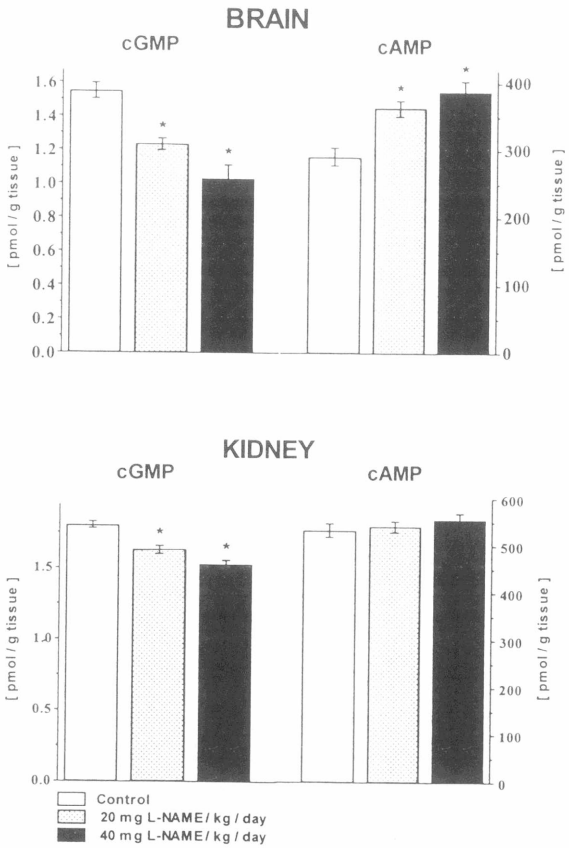

Fig. 3
*Cyclic GMP and cyclic AMP content in brain and kidney after 4-week L-NAME administration, * $p<0.05$.*

Discussion

The significant increase of blood pressure, and the decrease of heart rate in the animals receiving L-NAME in our experiments are in agreement with the data recently published by others (Gardiner *et al.* 1990, Ribeiro *et al.* 1992). Although it has been shown that long-term NO synthase inhibition by L-NAME results in NO-deficient hypertension (Arnal *et al.* 1992,

Bernátová and Pecháňová 1994), the data concerning LVW/BW ratio are still controversial (Arnal *et al.* 1993, Delacretaz *et al.* 1994, McDonald *et al.* 1992). The significant increase of LVW/BW ratio in L-NAME treated animals in our experiments was in good agreement with data published by Delacretaz *et al.* (1994) and Kristek *et al.* (1995). It seems that the concentration and duration of NO synthase inhibitor administration play a critical role in the development of myocardial hypertrophy.

The inhibition of NO synthase followed by a decrease in the cGMP content results in NO-deficient hypertension (Moncada 1992). In our study, L-NAME in both doses decreased significantly the cGMP content in the studied tissues. The cyclic GMP content appears to be responsible for the $[Ca^{2+}_i]$ mainly in smooth muscle cells. Lowering of $[Ca^{2+}_i]$ content could be achieved by a direct effect of cGMP-protein kinase on Ca^{2+}-ATPase activity, by inhibition of L-type Ca^{2+} channel current (Francis and Corbin 1994), and by lowering of inositol phosphates in response to the G-binding protein (Hirata *et al.* 1990). All these factors contribute to the vasodilatation. Since after L-NAME treatment the content of cGMP decreased in the aorta and/or smooth muscle cells an increase in $[Ca^{2+}_i]$ occurred, resulting in contraction of smooth muscle cells. It seems justifiable to suppose that a similar process takes place in the smooth muscle of various vascular beds leading to contraction with a subsequent increase in blood pressure.

However, in many other cells and tissues the other second messenger, cAMP, seems to play a role in $[Ca^{2+}_i]$. In our experiments, in contrast to cGMP, the cAMP content increased significantly in all the studied tissues except of the kidney. In vascular smooth muscle cells, the role of cGMP can be partially compensated by cAMP as both these cyclic nucleotides inhibit $[Ca^{2+}i]$, which can lead to vasorelaxation (Sperelakis and Ohya 1990). However, decreased vasorelaxation to acetylcholine in the aorta was observed in this model of hypertension. On the other hand, unchanged relaxation to isoprenaline was found in the same experiments (Holécyová *et al.* 1995). This could be explained by an increase in cAMP as Gray and Marshall (1992) found diminished relaxation to isoprenaline after acute inhibition of NO synthase. Lincoln *et al.* (1990) hypothesized that cGMP-dependent protein kinase can be activated by cAMP. According to this idea, cAMP can produce both an increase and a decrease in $[Ca^{2+}_i]$ depending on the absence or presence of cGMP-dependent protein kinase in the cells. If cAMP activated cGMP-dependent protein kinase to reduce $[Ca^{2+}_i]$ in smooth muscle cells, it can be asked whether or not this represents a general action of cAMP in other cell types and tissues. However, it has been shown that in most cells and tissues cAMP does not reduce $[Ca^{2+}_i]$ but rather increases it. In cardiac myocytes, cAMP-dependent protein kinase has a stimulating effect on L-type Ca^{2+} channel current, however, phosphorylation of myosin light chain kinase by cAMP-dependent protein kinase can also lead to relaxation (Xiong and Sperelakis 1995). In the brain, the increase of cAMP as well as cGMP content can be mediated by Ca^{2+}, acting *via* calmodulin, to stimulate adenylyl cyclase (Cooper *et al.* 1994) and/or NO synthase (Bredt and Snyder 1990). However, the question still remains of how does NO and/or NO deficiency affect the tissue of individual organ itself.

Cyclic GMP may also exert its effects by activating class II and/or inhibiting class III cAMP phosphodiesterases (Schmidt *et al.* 1993). It is possible that a decrease of cGMP levels could affect cAMP phosphodiesterase class II, which can result in an cAMP increase. But there are also other possible ways of the cAMP content increase after NO synthase inhibition. One of them can involve the release of catecholamines (Cohen and Weisbrod 1988). Taken together, the increase in cAMP content in the aorta after NO synthase inhibition can partially compensate the decrease in cGMP content probably *via* $[Ca^{2+}_i]$ reduction. However, this compensation is insufficient for improving endothelium-dependent relaxation. On the other hand, the simultaneous decrease in cGMP and increase in cAMP content may elevate $[Ca^{2+}_i]$ in heart. These processes could participate in the development of hypertension and LV hypertrophy. Probably, several mechanisms are involved in the increase of cAMP and more experiments are needed to evaluate them.

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