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L-NAME-Induced Protein Remodeling and Fibrosis in the Rat Heart

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Summary

The aim of the present study was to determine whether NO deficiency itself or rather the elevation of systolic blood pressure is responsible for the protein and structural remodeling of the heart during hypertension induced by long-term treatment by nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). Three groups of rats were investigated. The first group served as control. In the second group L-NAME was given in the dose of 20 mg/kg/day in the drinking water and in the third group L-NAME was given in the dose of 40 mg/kg/day during 4 weeks. While L-NAME treatment in both doses caused essentially the same increase in systolic blood pressure (SBP), NO synthase activity and cGMP concentration in the left ventricle decreased by 17% and 13 %, respectively in the 20 mg/kg/day L-NAME group and by 69 % and 27 %, respectively in the 40 mg/kg/day L-NAME group. The protein profile of the left ventricle in both L-NAME groups was characterized by an increased concentration of metabolic proteins. Nevertheless, a significant increase in the concentration of pepsin-soluble collagenous proteins and the concentration of hydroxyproline in pepsin-insoluble collagenous proteins was found only in the group receiving 40 mg/kg/day L-NAME. The morphometric evaluation revealed a significant increase in myocardial fibrosis in both L-NAME groups. However, this was more pronounced in the 40 mg/kg/day L-NAME group. It is concluded that NO deficiency resulted in significant enhancement of fibrotic tissue growth in proportion to the administered L-NAME dose, while SBP was increased similarly in both L-NAME groups. Thus, NO deficiency rather than hemodynamic changes appears to be crucially involved in collagenous protein and fibrotic tissue changes of the left ventricle in hypertension induced by L-NAME.

Key words
L-NAME • NO synthase • Left ventricular hypertrophy • Collagenous proteins • Myocardial fibrosis

Introduction

It has been recently shown that chronic inhibition of nitric oxide (NO) synthase in rats resulted in a sustained increase in systolic blood pressure (SBP), a decrease in heart rate (Ribeiro *et al.* 1992, Xie *et al.* 1996), a reduction of cardiac output (Amrani *et al.* 1992) and changes in myocardial contractility (Lewis *et al.* 1990). Furthermore, Na^+ extrusion from cardiac cells (Vrbjar *et al.* 1998) was lowered and the heart weight/

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body weight ratio (Böger et al. 1994) or left ventricle weight/body weight (LVW/BW) ratio (Delacretaz et al. 1994, Pecháňová and Bernátová 1996) were elevated. Long-term administration of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) enhanced the contractility in different parts of the vascular tree (Török and Gerová 1996) and attenuated vascular relaxation (Holécyová et al. 1996). It was further reported that NO deficiency also resulted in structural alterations of the cardiovascular system. Myocardial alterations consisted of extensive areas of fibrosis and myocardial necrosis after 8 weeks of L-NAME administration (Moreno et al. 1995). An increase of collagenous proteins in the heart (Pecháňová et al. 1997) as well as focal myocardial fibrosis (Babál et al. 1997) were already described after 4 weeks of L-NAME administration. Vascular alterations were characterized by increased wall thickness/lumen ratio in coronary microvessels (Numaguchi et al. 1995), coronary and aortic vascular remodeling (Kristek et al. 1996, Bernátová et al. 1998), and arterial media hyperplasia (Babál et al. 1997). The question arose whether the structural alterations in the cardiovascular system were the result of increased blood pressure or of NO deficiency. In our previous work we demonstrated a dose-dependent increase of RNA and DNA concentrations and of [^{14}C]leucine incorporation into cardiac proteins in rats chronically administered L-NAME in doses of 20 and 40 mg/kg/day, while SBP was found to increase similarly in both L-NAME groups (Bernátová et al. 1996).

The aim of this study was to investigate whether the administration of two different doses of L-NAME producing a comparable increase of blood pressure would result in the development of similar structural changes in the heart. The findings were expected to decide whether the processes associated with NO insufficiency *per se* or the resulting hypertension were responsible for alterations in the protein profile and structural remodeling of the heart.

Material and Methods

Animals and treatment

Male Wistar rats, 15 weeks old, were randomly divided into three groups (n=16 in each group). The first group served as controls. In the second group, L-NAME (Sigma Chemical Co, Germany) was given in the dose of 20 mg/kg/day and the third group received L-NAME in the dose of 40 mg/kg/day. The substance was given in drinking water for 4 weeks. Systolic blood pressure and heart rate (HR) were measured by non-invasive tail-cuff plethysmography every day. After 4 weeks the animals were sacrificed, their body weight, weight of the heart (HW), left ventricle weight and right ventricle weight (RVW) were determined, and the LVW/BW as well as RVW/BW ratio were calculated. Left ventricles from 8 animals in each group were used for the determination of NO synthase activity, cGMP concentration and of the protein profile. The remaining 8 animals of each group were used for histochemical, morphometric and immunohistochemical observations.

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 8523, revised 1985).

Assay of NO synthase activity

NO synthase activity was determined in crude homogenates of fresh tissues by measuring the formation of [^{3}H]-L-citrulline from [^{3}H]-L-arginine (Amersham International plc, UK), as described previously (Bredt and Snyder 1990) with some modifications. Briefly, 50 μl of 10% homogenates of the left ventricle were incubated in the presence of 50 mmol/l Tris-HCl, pH 7.4, 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 BH_4 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-citrulline. The samples were then 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.

Determination of cGMP concentration

The tissue samples for cGMP assay were frozen in liquid nitrogen and kept at –80 °C. Before determination, one volume of each tissue sample was homogenized with 10 volumes of 1.07 mol/l perchloric acid. The samples were then centrifuged for 1 min at 10 000 x *g*. The cyclic GMP concentration was determined in the supernatant using radioimmunoassay (kit No. 1118) purchased from Immunotech, S.A., France. Briefly, cGMP radioimmunoassay was based on the competition between the succinylated cGMP of the sample and ^{125}I-labelled tracer for binding to polyclonal antibody coated onto tubes. To determine the cGMP concentration, the vials were assessed using a gamma

counter. The cGMP concentrations in the samples were calculated from the standard curve.

Determination of left ventricle protein profile

Pieces of LV were rapidly weighed, transferred into precooled homogenization test tubes and frozen subsequently to –50 °C. Tissue samples were later thawed, a 40fold volume was achieved by adding 50 mmol/l sodium-potassium-phosphate buffer, pH 7.4, containing 10 mmol/l EDTA and 1 % Triton X100, homogenized and centrifuged at 15000 x *g*; the supernatant was used for the determination of metabolic proteins (MP). The pellet was resuspended and fractions of contractile and collagenous proteins were obtained in a stepwise manner by extracting contractile proteins into a supernatant with phosphate buffer (0.1 mol/l, pH 7.4, containing 1.1 mmol/l KCl). Thereafter, the pellet was shortly washed with 0.5 mol acetic acid and extracted with 0.5 mol/l CH_3COOH-pepsin (pH 1.45); pepsin concentration was kept in the range 1:100 – 1:50. After 24 h at 4 °C the extracts were centrifuged. The supernatant contained the fraction of pepsin-soluble collagenous proteins (PSC). The pellet was further suspended in 1.1 mol/l NaOH and left for 45 min at 105 °C. This fraction contained pepsin-insoluble collagenous proteins (PIC). The protein profile procedure yielded 3 basic fractions: a) metabolic proteins (containing predominantly enzyme systems for aerobic and anaerobic substrate utilization) (see Bass *et al.* 1988), b) contractile proteins (CP, complex of contractile, regulatory and modulatory proteins of myofibrils (see Pelouch 1995), c) structural collagenous proteins (the fraction included collagens, elastins, proteoglycans and glycoproteins), which were divided into two fractions: 1) PSC (represented mainly by collagen I and III) and 2) PIC (collagen aggregates, elastins and other extracellular matrix proteins). This methodological approach was described in detail elsewhere (Pelouch *et al.* 1993, 1996). The protein concentration in individual fractions was determined according to Lowry *et al.* (1951) and expressed in mg per gram of tissue wet weight; the protein content was expressed in mg per total LV wet weight. Hydroxyproline concentration was estimated in pepsin-insoluble collagenous proteins (Pelouch *et al.* 1993).

Histology

The hearts were cut perpendicularly in the middle between the apex and sulcus coronarius. The apical halves of the heart were fixed for 24 h in 10 % phosphate buffered formalin and routinely processed in paraffin. Serial 5 μm thick sections were stained with hematoxylin and eosin and by Van Gieson's staining for collagen.

Morphometry

Morphometric evaluation was performed under an Olympus light microscope equipped with a two-dimensional image analyzer AlphaImager 2000. Van Gieson's histochemical staining was applied to enhance the red colour contrast of collagen. The fibrous tissue was expressed as percentage of the total measured area of the heart slice.

Immunohistochemistry

Deparaffinized slides were treated with methanol containing 0.5 % H_2O_2 for 10 min and incubated in an antigen-retrieving solution (Innovex Biosciences, Richmond, CA) in boiling water bath for 10 min. Primary antibodies against proliferative cell nuclear antigen (PCNA) (DAKO Corp., Carpintera, CA) were applied diluted 1:200 in phosphate buffered saline at pH 7.3 (PBS) for 60 min. The immune reaction was performed with DAKO LSAB 2 Peroxidase Kit: 20 min incubation with biotinylated anti-mouse IgG antibodies followed by 10 min incubation with avidin-horseradish-peroxidase complex. The peroxidase reaction was developed with Diaminobenzidine Chromogen Kit (Biomeda Corp., Foster City, CA). All washes were done in PBS for 10 min, incubations at room temperature. Slides were counterstained with hematoxylin or 1% methyl green water solution.

Statistical analysis

The results are expressed as means ± S.E.M. Values were considered to differ significantly if the p value was less than 0.05. One-way ANOVA and the Bonferroni test were used for analysis.

Results

Cardiovascular parameters

After the first week of the experiment, SBP was 128±5 mm Hg and HR was 385±6 beats/min in the control group. In the group receiving 20 mg/kg/day L-NAME, SBP increased by 30 % ($p<0.05$) and HR decreased by 17 % ($p<0.05$) compared to the control group. In the group receiving 40 mg/kg/day L-NAME, SBP increased by 36 % ($p<0.05$) and HR decreased by 25 % ($p<0.05$) vs. control group. The changes in SBP and HR persisted during the following three weeks. After 4 weeks of experiment, the LVW/BW ratio was

1.21±0.02 in the control group. In the group with 20 mg/kg/day L-NAME, the ratio increased by 20 % ($p<0.05$) and in the group with 40 mg/kg/day by 27 % ($p<0.05$). There were no significant differences in SBP, HR and the LVW/BW ratio between the groups treated with 20 mg/kg/day and 40 mg/kg/day of L-NAME. The body weight and the RVW/BW ratio was not affected in any group (Table 1).

Table 1. Effect of 4-week L-NAME treatment (20 and 40 mg/kg/day) on systolic blood pressure (SBP), heart rate, body weight, left ventricle weight/ body weight ratio (LVW/BW), right ventricle weight/body weight ratio (RVW/BW), NO synthase activity, cGMP concentration and myocardial fibrosis.

	Control	20 mg/kg/day L-NAME	40 mg/kg/day L-NAME
SBP (mm Hg)	128±5	166±7*	174±8*
Heart rate (beats/min)	385±6	319±7*	290±9*
Body weight (g)	443±19	439±15	425±18
LVW/BW (mg/g)	1.21±0.02	1.45±0.03*	1.54±0.05*
RVW/BW (mg/g)	0.45±0.01	0.43±0.02	0.42±0.02
NO-synthase (pmol L-Cit/min/mg)	10.11±0.96	8.36±0.68*	3.12±0.25*⁺
cGMP concentration (pmol/g tissue)	1.47±0.06	1.29±0.05*	1.09 0±05*⁺
Myocardial fibrosis (%)	1.30±0.07	4.70±0.31*	10.54±0.46*⁺

$p<0.05$ as compared to control, $^{+}p<0.05$ as compared to 20 mg/kg/day L-NAME group

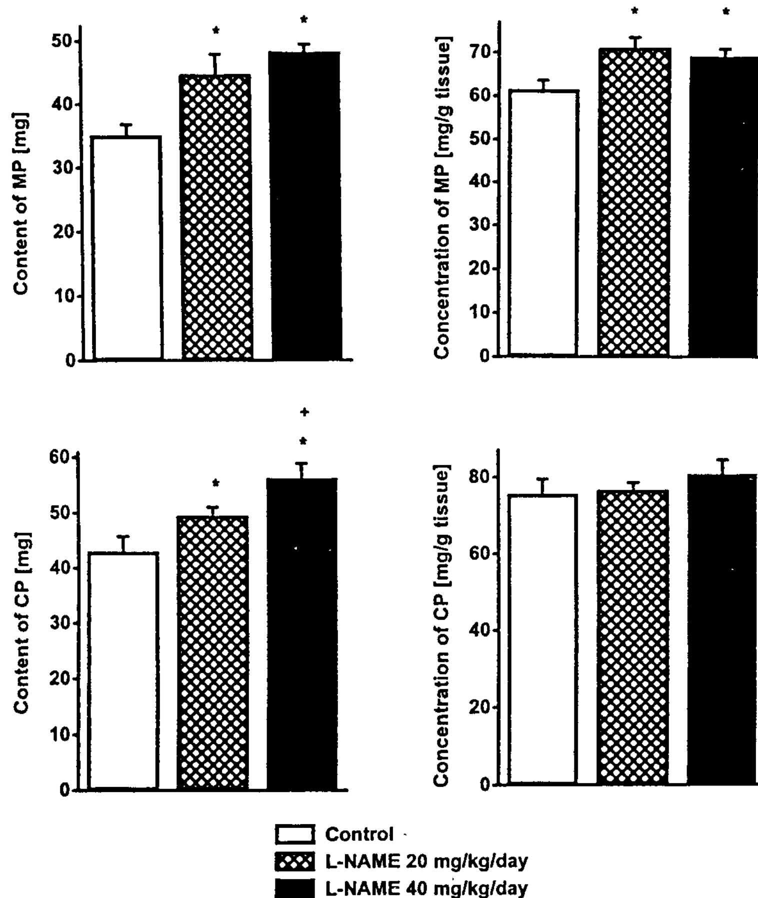

Fig. 1. *Effect of 4-week L-NAME treatment on the content and concentration of non-collagenous proteins in the left ventricle. Metabolic proteins (MP) (top panel); Contractile proteins (CP) (bottom panel). * $p<0.05$ as compared to control, $^{+}p<0.05$ as compared to 20 mg/kg/day L-NAME group.*

NO synthase activity

NO synthase activity was 10.11±0.96 pmol L-Cit/min/mg protein in the left ventricle of the control group. In the L-NAME groups receiving the dose of 20 or 40 mg/kg/day, NO synthase activity was significantly inhibited ($p<0.05$) by 17 % and 69% compared to controls, respectively. The decrease in NO synthase activity caused by L-NAME was significantly greater after a higher dose (Table 1).

Cyclic GMP concentration

Cyclic GMP concentration was 1.47±0.06 pmol/g tissue in the left ventricle of the control group. In the L-NAME groups receiving 20 or 40 mg/kg/day, cGMP concentration was significantly inhibited ($p<0.05$) by 13 % and 27 % vs. controls, respectively. The decrease in cGMP concentration caused by the two doses of L-NAME was more pronounced after a higher dose (Table 1).

Protein profile

The content of metabolic proteins (MP) was 34.03±1.67 mg and the concentration of MP was 62.74±2.02 mg/g tissue in the left ventricle of the control group. In the L-NAME group receiving 20 mg/kg/day, the content of MP increased by 28 % ($p<0.05$) and the concentration increased by 16 % ($p<0.05$) vs. controls. In the L-NAME group receiving 40 mg/kg/day, MP content increased by 38 % ($p<0.05$) and MP concentration increased by 12 % ($p<0.05$) vs. control. (Fig. 1, top panel). There were no significant differences in the content and concentration of MP between the groups treated with 20 or 40 mg/kg/day of L-NAME.

The content of contractile proteins (CP) was 42.01±2.68 mg and the concentration of CP was 74.65±3.92 mg/g tissue in the left ventricle of the control group. In the L-NAME groups receiving 20 and 40 mg/kg/day, the content of CP increased by 15 % ($p<0.05$) and by 31 % ($p<0.05$) vs. controls, respectively. The CP concentration, however, did not differ in either group (Fig. 1, bottom panel).

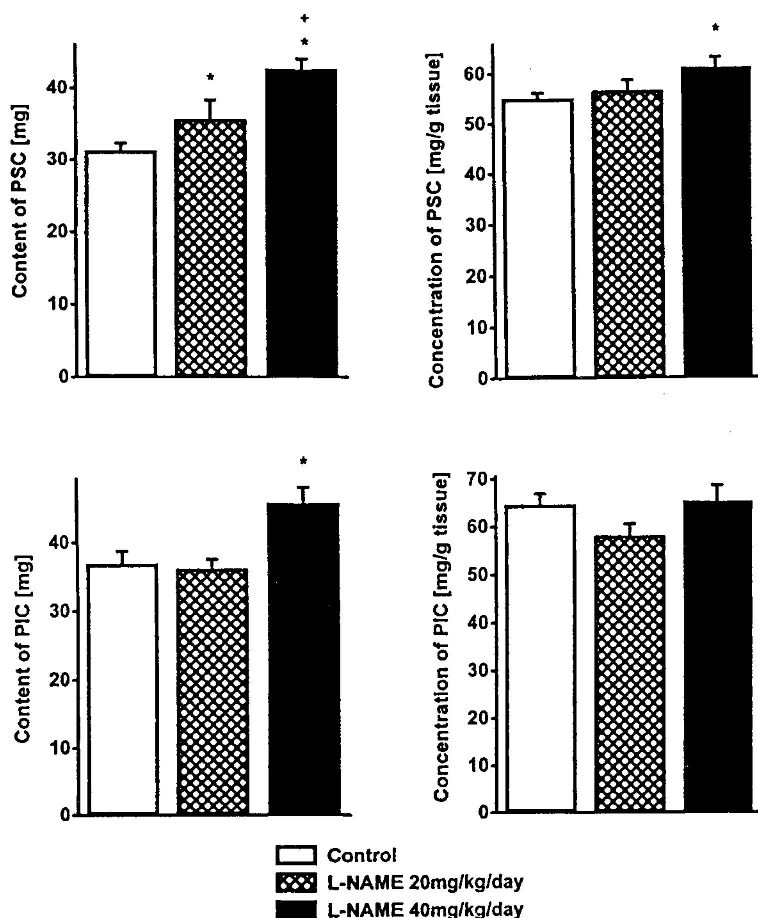

Fig. 2. *Effect of 4-week L-NAME treatment on the content and concentration of collagenous proteins in the left ventricle. Pepsin-soluble collagenous proteins (PSC) (top panel); Pepsin-insoluble collagenous proteins (PIC) (bottom panel). * $p<0.05$ as compared to control, + $p<0.05$ as compared to 20 mg/kg/day L-NAME group.*

The content of pepsin-soluble collagenous proteins (PSC) was 31.36±0.91 mg and the concentration of PSC was 54.04±1.15 mg/g tissue in the left ventricle of the control group. In the 20 mg/kg/day L-NAME group,

the content of PSC increased by 14 % ($p<0.05$) compared to controls and the concentration of PSC did not change. In the 40 mg/kg/day L-NAME group, the content of PSC increased by 37 % vs. controls ($p<0.05$) and by 17 % vs. the 20 mg/kg/day group ($p<0.05$), whereas the concentration of PSC increased by 11 % ($p<0.05$) vs. controls (Fig. 2, top panel).

The content of pepsin-insoluble collagenous proteins (PIC) was 37.01±1.83 mg and the concentration of PIC was 66.17±2.24 mg/g tissue in the left ventricle of the control group. In the L-NAME group receiving 20 mg/kg/day, the content of PIC did not differ from controls and the concentration of PIC was even decreased by 12 % ($p<0.05$). In the L-NAME group on the dose of 40 mg/kg/day, the content of PIC increased by 24 % ($p<0.05$) vs. controls and the concentration of PIC did not change. It was, however, increased significantly compared to the 20 mg/kg/day group (Fig. 2, bottom panel).

The content of hydroxyproline in PIC was 0.15±0.01 mg and the concentration of hydroxyproline in PIC was 0.25±0.03 mg/g tissue in the left ventricle of the control group. In the L-NAME group receiving 20 mg/kg/day, the content as well as the concentration of hydroxyproline in PIC did not differ from controls. In the L-NAME group on the dose of 40 mg/kg/day, the content of hydroxyproline in PIC increased by 102 % ($p<0.05$) and the concentration of hydroxyproline in PIC increased by 64 % ($p<0.05$) compared to controls (Fig. 3).

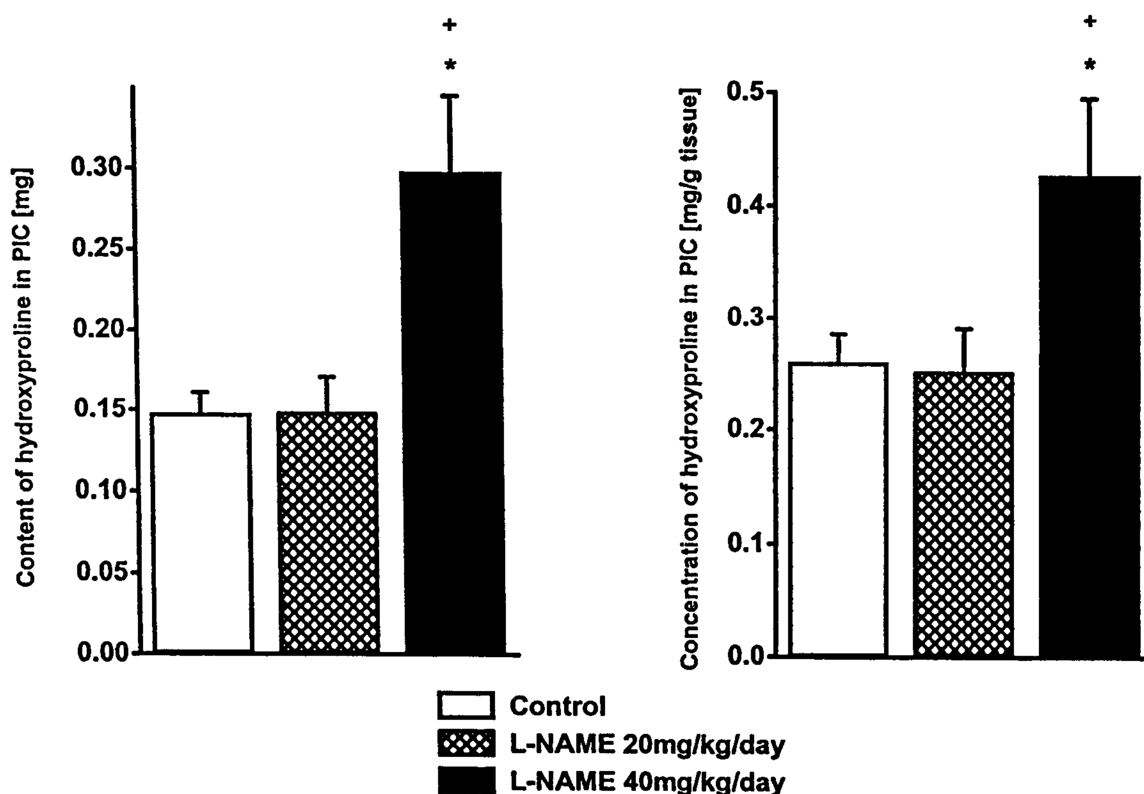

Fig. 3. *Effect of 4-week L-NAME treatment on the content and concentration of hydroxyproline in pepsin-insoluble collagenous proteins (PIC) in the left ventricle. * $p<0.05$ as compared to control, + $p<0.05$ as compared to 20 mg/kg/day L-NAME group.*

Histology and immunohistochemistry

Slides stained with hematoxylin and eosin revealed large areas of fibrosis in the myocardium of rats treated with L-NAME. These changes were emphasized with Van Gieson's staining of collagen, except for lesions with necrosis. Maximum changes were found in the subendocardial location of the left ventricle, the papillary muscles and in the interventricular septum (Fig. 4b). Similar changes were also seen in the right ventricle. Areas of acute necrosis with inflammatory cell accumulation in close vicinity immediately next to the foci of fibrosis documented the ischemic changes in the myocardium of L-NAME treated animals.

There was a remarkable thickening of the perivascular fibrous tissue and of the arterial media resulting in narrowing of blood vessel lumina (Fig. 5b).

In the L-NAME treated animals, PCNA immunohistochemistry disclosed numerous positively stained nuclei in the areas of reparative changes in the left ventricle and in the media of arteries (Figs 4c and 5c). No thrombosis was detected.

None of the described histological findings were observed in rats of the control group (Figs 4a and 5a).

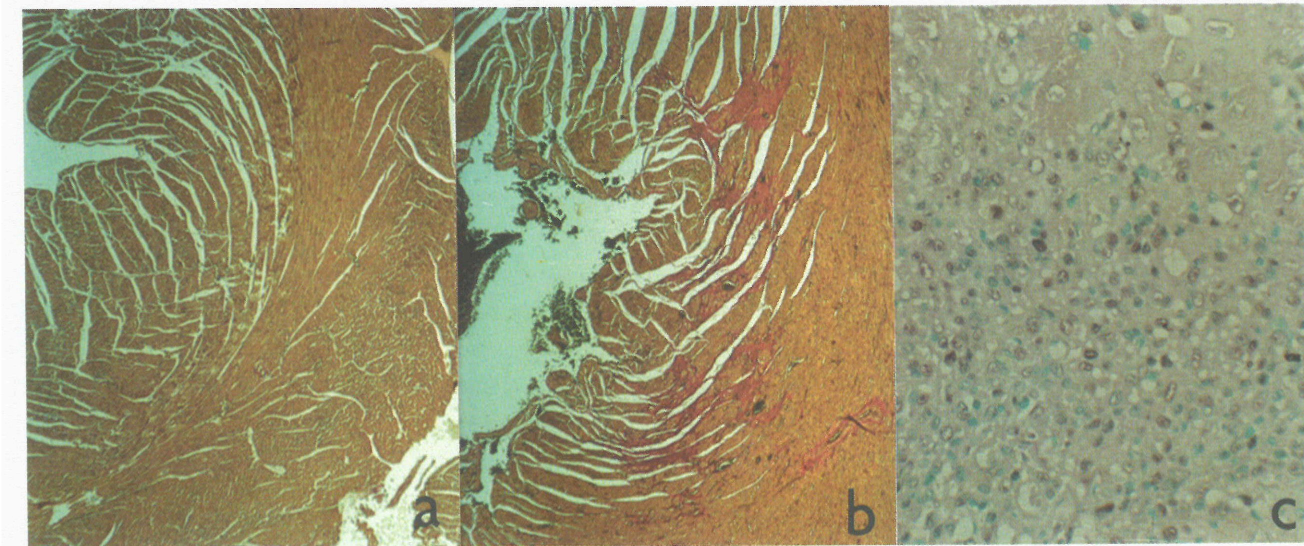

Fig. 4. *Left ventricle of a control rat (a), focal myocardial fibrosis in the subendocardial area of the left ventricle in a L-NAME treated rat receiving 40 mg/kg/day (b), and PCNA immunohistochemistry uncovering numerous positive nuclei (dark) of cells in an area of reparative changes in the left ventricle of a L-NAME rat treated with the dose of 40 mg/kg/day (c). a,b: Van Gieson, 40x; c: PCNA, immunoperoxidase, diaminobenzidine, counterstained with methyl green, 200x.*

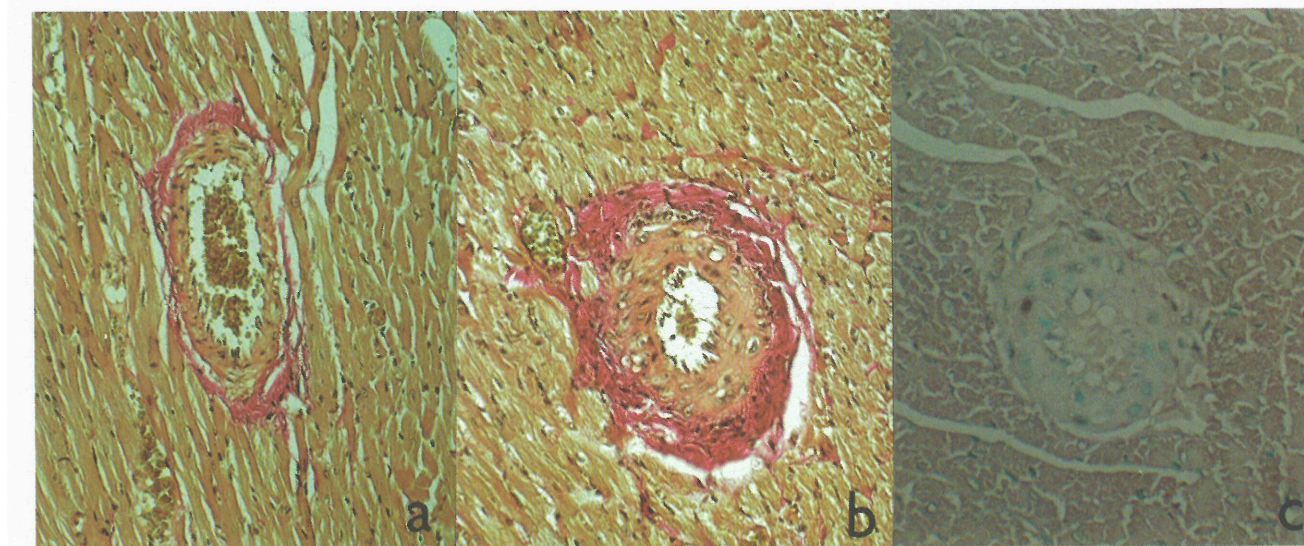

Fig. 5. *Artery in the anterior segment of the interventricular septum in a control rat (a), 40 mg/kg/day L-NAME treated rat showing thickening of the media and pronounced periarterial fibrosis (dark) (b), with numerous PCNA positive nuclei (arrow) of smooth muscle cells in the hyperplastic media (c). a,b: Van Gieson; c: PCNA, immunoperoxidase, diaminobenzidine, counterstained with hematoxylin; 360x.*

Morphometry

Fibrous tissue represented 1.30±0.07 % of the myocardium area in slices from control group hearts, while in the 20 mg/kg/day L-NAME group it was 4.70±0.31 % ($p<0.05$) and in the 40 mg/kg/day L-NAME group 10.54±0.46 % ($p<0.05$). The amount of fibrosis in the hearts produced by the two doses of L-NAME differed significantly (Table 1).

Discussion

The present study has shown that besides an increase in blood pressure and a decrease in heart rate, L-NAME administration (20 and 40 mg/kg/day) resulted in the development of left ventricular hypertrophy, in changes of protein profile of the left ventricle and in myocardial fibrosis. A significantly greater decrease of NO synthase activity as well as of cGMP concentration in the left ventricle was recorded in the group with the higher dose of L-NAME. The changes in collagenous proteins and the extent of myocardial fibrosis corresponded well with the dose-dependent decrease of NO synthase activity and cGMP concentration. On the other hand, there were no significant changes in SBP, HR and the LVW/BW ratio between the lower and higher doses of L-NAME groups.

Since guanylate cyclase has been considered to be an NO receptor, the concentration of cGMP can serve as an indirect marker for NO concentration in tissues (Lancaster 1992). Using the same experimental model, NO synthase activity and cGMP concentration in the L-NAME treated groups from our previous study were also found to be decreased in the aorta, brain and kidney (Bernátová *et al.* 1996). This decrease was associated with an increase in SBP, decrease in HR and elevation in the LVW/BW ratio, as confirmed in the present study. The hemodynamic changes and left ventricle weight alteration are in agreement with the findings of several other authors (Ribeiro *et al.* 1992, Delacretaz *et al.* 1994, Xie *et al.* 1996). Our previous findings of increased RNA concentration and [^{14}C]leucine incorporation into proteins of the left ventricle in the L-NAME treated group (Pecháňová *et al.* 1997) provided direct biochemical evidence of enhanced proteosynthesis, typical for the period of developing hypertrophy (Motz *et al.* 1983). Fibrocytes represent a substantial population of myocardial cells, so that an increase of DNA concentration after L-NAME treatment (Pecháňová *et al.* 1997) may logically be ascribed to fibrotic tissue enlargement. Indeed, our morphometric investigation revealed a significant proliferation of fibrotic tissue in the left ventricle. Moreover, we found that the amount of cardiac fibrosis differed significantly in the two L-NAME experimental groups. These morphological findings were supported by protein profile analysis, which showed a significant increase of hydroxyproline of the insoluble collagen fraction in the higher L-NAME dose group. On the other hand, the concentration of metabolic proteins (mitochondrial and cytoplasmic enzyme proteins) was comparably enhanced in both L-NAME groups. The adaptation response of the left ventricle to hemodynamic overload, which was comparable in both L-NAME groups, may be considered to reflect similar demands on energy production and calcium transport processes. The unchanged concentration together with a proportionate increase of contractile proteins content in the left ventricle of L-NAME treated animals was consistent with the findings of myocardial hypertrophy.

Several authors have addressed the question whether the structural alterations of the cardiovascular system during chronic NO synthase inhibition were caused by the lack of NO *per se* or by the hypertension which it induces. In the experimental model of Numaguchi *et al.* (1995), coadministration of hydralazine with L-NAME prevented arterial hypertension and significantly attenuated myocardial fibrosis but failed to affect cardiac hypertrophy and microvascular remodeling induced by chronic inhibition of NO synthesis. On the other hand, the calcium channel blocker diltiazem was not totally effective either in preventing arterial hypertension or in attenuating myocardial fibrosis associated with chronic NO synthase inhibition (Zappellini *et al.* 1997). During cotreatment of enalapril (ACE inhibitor) with L-NAME, prevention of arterial hypertension also prevented LV hypertrophy, but enalapril was not able to prevent LV fibrosis during chronic inhibition of NO synthase (Moreno *et al.* 1995). Another ACE inhibitor, ramipril, prevented the development of both cardiac hypertrophy and structural alterations of the left ventricle in the model of NO-deficient hypertension (Hropot *et al.* 1994, Linz *et al.* 1995). As inhibition of ACE is associated with increased bradykinin concentration accompanied by potential enhancement of subsequent NO and prostaglandin synthesis (Sigusch *et al.* 1996), all these substances should be taken into account when investigating blood pressure regulation and structural remodeling. Prevention of both the blood pressure increase and structural remodeling of the heart due to

chronic NO synthesis inhibition appears to be mainly dependent on the ability of the particular drug to affect hemodynamic processes and protein metabolism. Our experimental model, based on the fact that two different doses of L-NAME (20 and 40 mg/kg/day) caused practically to the same increase in SBP, allowed us to distinguish between the dose-induced effect of NO insufficiency and the hemodynamic effect without using other substances and thus to eliminate their potential influence on LV metabolism and structure.

As both L-NAME doses led to hypertrophy only in the left ventricle, myocardial fibrosis in the hypertrophied part of the heart (left ventricle) could be compared with that in the non-hypertrophied one (right ventricle). Our results showed maximal changes associated with myocardial fibrosis in the subendocardial location of the left ventricle and similar changes in the right ventricle. Thus in this particular model of hypertension, the hypertrophic growth of the left ventricle was probably regulated by a different mechanism than the fibrotic tissue enlargement. These results are in agreement with the observations of Moreno *et al.* (1996) who showed that chronic inhibition of NO synthesis causing cardiac ischemia was not related to cardiac hypertrophy. In agreement with our finding of unchanged RVW/BW ratio after long-term L-NAME treatment, Hampl *et al.* (1993) showed that chronic L-NAME treatment did not increase pulmonary arterial pressure. Taken together, it seems that hypertrophy is associated with hypertension and fibrotic changes with NO insufficiency.

On the basis of these observations we may conclude that in this model of L-NAME-induced hypertension the processes associated with NO insufficiency *per se*, rather than the hypertension, were responsible for the alterations in collagenous proteins of the left ventricle as well as for the fibrotic changes in the myocardium.

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