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Short communication

Inhibition of nitric oxide synthase causes cardiac phenotypic modulation in rat

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Abstract

Cardiac gene expressions of collagen and contractile proteins were examined in rats treated with a nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), for 3 weeks. The rats became hypertensive, which caused left ventricular hypertrophy. Among the mRNAs examined, β-myosin heavy chain was increased and α-myosin heavy chain was decreased in both left and right ventricles, whereas skeletal α-actin and atrial natriuretic polypeptide were increased in the left ventricle only. Furthermore, coadministration of losartan with L-NAME lowered blood pressure and caused regression of left ventricular hypertrophy, but did not affect β- and α-myosin heavy chain mRNA levels, indicating that L-NAME directly regulates β- and α-myosin heavy chain mRNA.

Keywords: Cardiac remodeling; Nitric oxide (NO); Collagen; Myosin heavy chain

1. Introduction

It is well known that inhibitors of nitric oxide (NO) synthase such as N^G-nitro-L-arginine methyl ester (L-NAME) cause vascular contraction and sustained hypertension, indicating that there is a continuous release of NO from the vascular endothelium (Moncada et al., 1991; Pollock et al., 1993). NO also inhibits proliferation of vascular smooth muscle cells (Kolpakov et al., 1995) and modulates myocardial contraction (Finkel et al., 1992). However, the role of cardiac NO in chronic hypertensive cardiac disease remains to be elucidated.

In general, sustained hypertension causes not only cardiac hypertrophy but also cardiac remodeling, such as cardiac fibrosis (Weber and Brilla, 1991) and phenotypic modulation of cardiac myocytes (Izumo et al., 1988). In vivo and in vitro studies have demonstrated that angiotensin II (Weber and Brilla, 1991) is involved in pathological cardiac changes. However, the detailed mechanism of these cardiac changes is poorly understood.

The present study was undertaken to evaluate the role of NO in the development of pathological cardiac hypertrophy. For this purpose, L-NAME was orally administered to Wistar rats for 3 weeks, and the expression of collagen and cardiac contractile protein genes in the left and right ventricles of these rats was examined. Furthermore, losartan, a well established long-acting angiotensin II type 1 receptor antagonist (Wong et al., 1990), was coadministered with L-NAME to examine whether the altered cardiac gene expression in this model is caused by ventricular overload or inhibition of NO synthesis.

2. Materials and methods

2.1. Drugs

L-NAME was purchased from Sigma (St. Louis, MO, USA). Losartan was a gift from Banyu (Tokyo, Japan).

2.2. Experimental protocol

Eighteen-week-old male Wistar rats (Wistar/Jcl Clea, Japan) were randomly divided into three groups and were fed on standard laboratory chow (CE-2, Clea, Japan). Group 1 ($n = 7$), which served as a control group, was given distilled water ad libitum for 3 weeks. Group 2 ($n = 7$) was given L-NAME (0.7 g/l; 70 mg/kg) in the drinking water for 3 weeks. Group 3 ($n = 7$) was given the

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mixture of L-NAME (0.7 g/l; 70 mg/kg) and losartan (0.2 g/l; 20 mg/kg) for 3 weeks. Systolic blood pressure was measured by the tail-cuff method (PS-8000, Riken Kaihatsu, Tokyo, Japan). After treatment, all rats were decapitated and the heart was rapidly excised and rinsed in cold saline. The left ventricle, intraventricular septum and right ventricle were separately weighed, frozen in liquid nitrogen and stored at −80°C until the extraction of total RNA. Left ventricular weight was defined as the sum of left ventricular and intraventricular septal mass. Cardiac hypertrophy was assessed by the left or right ventricular weight corrected for the body weight.

2.3. Extraction of total RNA

Total RNA was extracted from the left and right ventricles by the acid guanidium thiocyanate-phenol-chloroform method, as described earlier (Kim et al., 1994).

2.4. Northern blot hybridization

Northern blot hybridization was performed as described previously (Kim et al., 1995). Briefly, samples (20 μg) of total RNA were denatured and electrophoresed, and the RNAs in the gel were transferred to a GeneScreen Plus nylon membrane (DuPont, Boston, MA, USA). Then, hybridization with cDNA probes or oligonucleotide probes was carried out, and the filters were washed and exposed to Kodak XAR-5 film at −70°C. For all RNA samples, the density of an individual mRNA band was divided by that of the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA band, to correct for the difference in RNA loading and/or transfer.

2.5. cDNA and oligonucleotide probes and their labeling

cDNA probes used were as follows. Rat α1(I) collagen cDNA was kindly donated by Dr. D. Rowe (Genovese et al., 1984), mouse cDNAs for α1(III) collagen (Liau et al., 1985) and α1(IV) collagen (Oberbaumer et al., 1985) were from Dr. Y. Yamada, rat GAPDH was from Dr. P. Fort (Fort et al., 1985). A 0.825-kb fragment of rat atrial natriuretic polypeptide (ANP) was synthesized by using the polymerase chain reaction, as described by Ohta et al. (1995). The cDNA probes were labeled with [^{32}P]deoxycytidine 5′-triphosphate tetra(triethylammonium) salt (New England Nuclear, Boston, MA, USA) and the oligonucleotide probes for α-myosin heavy chain, β-myosin heavy chain and skeletal α-actin were synthesized and labeled with [^{32}P]γ-ATP (New England Nuclear) as described by Kim et al. (1995).

2.6. Statistics

All values were expressed as means ± S.E.M. Differences between groups were tested by analysis of variance (ANOVA) followed by Duncan's multiple range test. A statistically significant difference was defined as a value of $P < 0.05$.

3. Results

3.1. Effects of L-NAME and losartan on blood pressure and cardiac left and right ventricular weights

Systolic blood pressure of group 2 (L-NAME-treated group) was significantly higher than that of group 1 (control group) (189 ± 5 vs. 143 ± 2 mmHg, $P < 0.01$). Left ventricular weight of group 2 was greater than that of group 1 (193 ± 7 vs. 179 ± 2 mg/100 g body weight, $P < 0.01$), whereas there was no difference in right ventricular weight between group 2 and group 1 (37 ± 1 and 41 ± 1 mg/100 g body weight, respectively). Coadministration of losartan with L-NAME for 3 weeks significantly

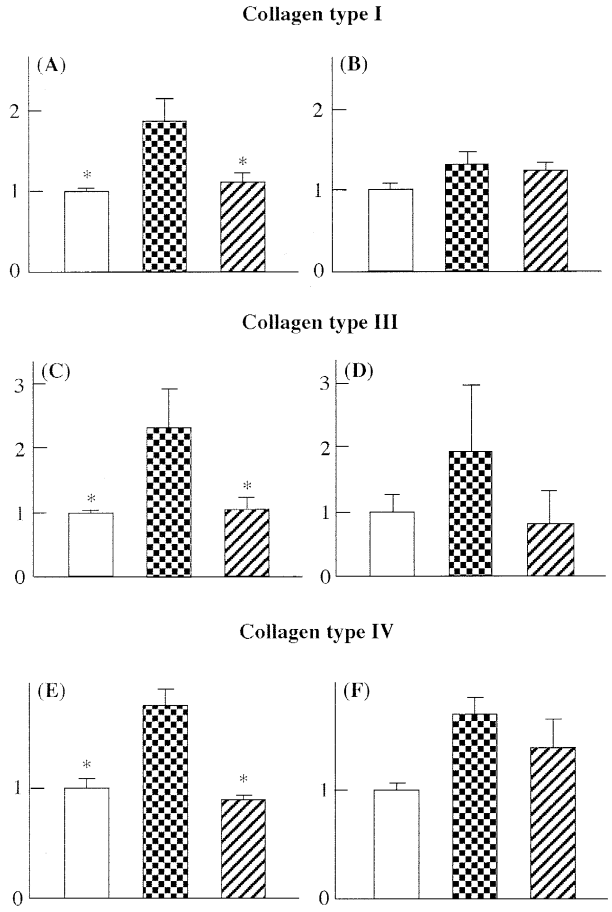

Fig. 1. Bar graphs show left (A, C, E) and right (B, D, F) ventricular mRNA levels for types I, III and IV collagen, corrected for GAPDH mRNA levels, in control Wistar rats ($n = 7$, left bar), Wistar rats treated with L-NAME for 3 weeks ($n = 7$, middle bar), and Wistar rats treated with L-NAME plus losartan for 3 weeks ($n = 7$, right bar). The mean value of mRNA in the control group is represented as 1. Each bar represents mean ± S.E.M. * $P < 0.05$, $^{+}$ $P < 0.01$ vs. L-NAME-treated rats.

suppressed the blood pressure (152 ± 3 mmHg, $P < 0.05$) and left ventricular weight (177 ± 4 mg/100 g body weight, $P < 0.05$) of L-NAME-treated rats.

3.2. Effect of L-NAME and losartan on cardiac gene expression

Fig. 1 shows that mRNAs for collagen types I, III and IV in the left ventricle were significantly higher in L-NAME-treated rats than in control rats. Coadministration of losartan with L-NAME suppressed the enhanced expression of types I, III and IV collagen in the left ventricles of L-NAME-treated rats to almost control levels. Neither L-NAME nor losartan affected the levels of collagen mRNA in the right ventricle.

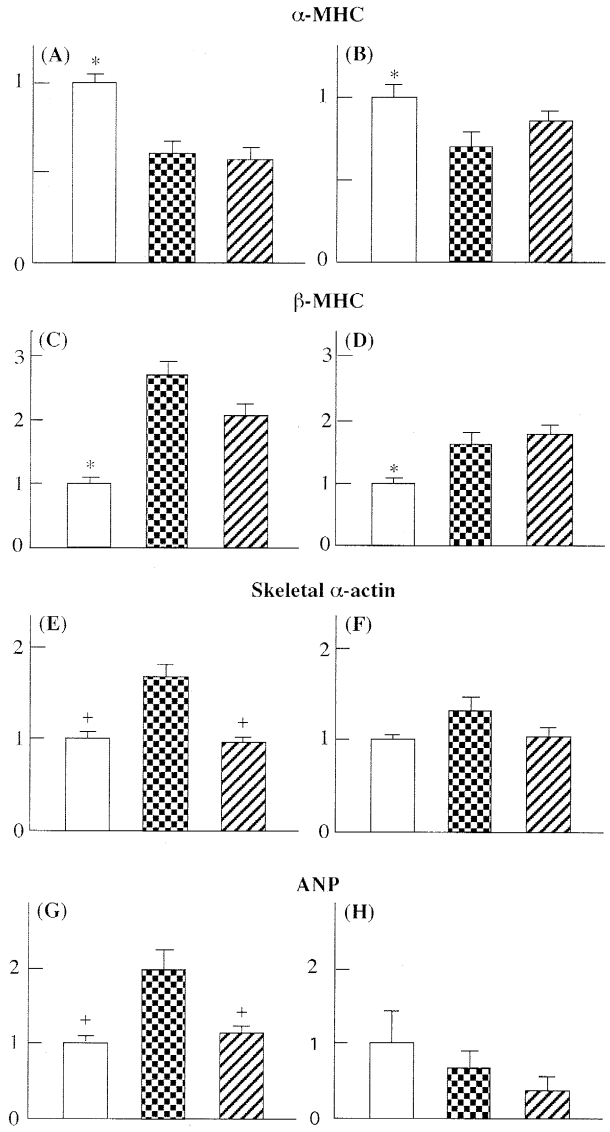

Fig. 2. Bar graphs show left (A, C, E, G) and right (B, D, F, H) ventricular mRNA levels for α-myosin heavy chain (α-MHC), β-myosin heavy chain (β-MHC), skeletal α-actin and ANP, corrected for GAPDH mRNA levels, in control Wistar rats ($n = 7$, left bar), Wistar rats treated with L-NAME for 3 weeks ($n = 7$, middle bar), and Wistar rats treated with L-NAME plus losartan for 3 weeks ($n = 7$, right bar). The mean value of mRNA in the control group is represented as 1. Each bar represents mean ± S.E.M. * $P < 0.05$, $^{+}$ $P < 0.01$ vs. L-NAME-treated rats.

3.3. Effect of L-NAME and losartan on cardiac contractile protein gene expression

Fig. 2 shows that mRNAs for β-myosin heavy chain, skeletal α-actin and ANP in the left ventricle were significantly higher in L-NAME-treated rats than in control rats, whereas α-myosin heavy chain mRNA level was significantly lower in L-NAME-treated rats than in control rats. In the right ventricle, α-myosin heavy chain was lower and β-myosin heavy chain was higher in L-NAME-treated rats than in control rats. Thus, inhibition of NO synthesis caused dedifferentiation of myocytes in both ventricles from an adult to a fetal phenotype. Coadministration of losartan with L-NAME suppressed the enhanced expression of skeletal α-actin and ANP gene in the left ventricles of L-NAME-treated rats to almost control levels. However, α- and β-myosin heavy chain mRNA levels in the L-NAME-treated rats were not normalized in either ventricle by losartan.

4. Discussion

Deposition of types I and III collagen is known to enhance cardiac stiffness (Weber and Brilla, 1991). The reinduction of β-myosin heavy chain and skeletal α-actin in adult heart also modulates cardiac performance by affecting cardiac systolic and diastolic function (Hewett et al., 1994). Thus, these pathological changes of the heart may be responsible for the onset of heart failure.

In the present study, Wistar rats were orally treated with L-NAME for 3 weeks and the expression of various genes related to cardiac remodeling was examined. The systolic blood pressure of the rats, measured indirectly by the tail cuff method, increased by 45 mmHg and the rats developed cardiac hypertrophy to a smaller extent than other rat models of experimental hypertension (Kim et al., 1994; Ohta et al., 1995), which is in good agreement with the reports by others (Rhaleb et al., 1994; Delacretaz et al., 1994). In the left ventricles of L-NAME-treated rats, mRNA levels for types I, III and IV collagen were increased compared with those in control rats. A recent morphological study (Numaguchi et al., 1995) showed that cardiac hypertrophy and fibrosis are observed in Wistar-Kyoto rats treated with L-NAME for 8 weeks, which is consistent with our findings.

Furthermore, re-expression of fetal isoforms of contractile proteins was observed in L-NAME-treated rats. mRNA for skeletal α-actin and β-myosin heavy chain was increased, and mRNA for α-myosin heavy chain, which is

the predominant isoform of adult rat heart, was decreased in the left ventricles. L-NAME also stimulated the gene expression of left ventricular ANP, which is another marker of phenotypic modulation of ventricular myocytes. Thus, inhibition of NO synthesis caused phenotypic modulation of myocytes in the left ventricles, as seen in hypertrophied hearts in other models of hypertension (Izumo et al., 1988; Kim et al., 1995). Interestingly, α-myosin heavy chain mRNA was decreased and β-myosin heavy chain mRNA was increased in the right ventricles of L-NAME-treated rats, indicating that these changes are not caused by ventricular overload but may be caused by a direct action of the NO synthase inhibitor.

To further examine whether the changes in mRNAs in L-NAME-treated rat hearts are due to ventricular overload or to inhibition of NO synthesis, losartan, a long-acting angiotensin II type 1 antagonist (effective for at least 24 h at 3 mg/kg p.o.; Wong et al., 1990) was coadministered with L-NAME for 3 weeks. Losartan significantly suppressed the L-NAME-induced hypertensive effect by 37 mmHg and the cardiac hypertrophy, in agreement with other reports (Pollock et al., 1993; Jover et al., 1993). mRNAs for skeletal α-actin and types I, III and IV collagen in the left ventricles were suppressed by this treatment. In contrast, α- and β-myosin heavy chain mRNA levels were unaffected by losartan in both ventricles. These data indicate that the altered expression of α- and β-myosin heavy chains is caused by the inhibition of NO synthesis and not by the increased ventricular overload.

In summary, we demonstrated that mRNAs related to cardiac remodeling are increased in the hearts of rats with hypertension induced by L-NAME. In particular, the alterations of α- and β-myosin heavy chain mRNAs are caused by inhibition of NO synthase.

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