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Long-term Inhibition of NO Synthase Induces Cardiac Hypertrophy with a Decrease in Adrenergic Innervation

M. GEROVÁ[1], B. HARTMANNOVÁ[3], S. DOLEŽEL[3], L. JEŽEK[2]

[1]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, [2]Slovakofarma Joint Stock Company, Hlohovec, Slovak Republic, and [3]Department of Pathological Physiology, Medical Faculty, Brno, Czech Republic

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Summary

Data concerning the effect of NO on the function and structure of the heart are controversial. We have studied two main questions: (i) Does the heart muscle reflect the hypertension induced by long-term inhibition of NO synthase? (ii) Since the arginine-NO pathway is also operative in the autonomic nervous system, the second goal was to ascertain the possible changes of the adrenergic nervous system in the heart after long-term NO synthase inhibition. Wistar rats were administered L-NAME in drinking water (50 mg/kg bw/day) for 8 weeks. Systolic blood pressure and heart rate were monitored weekly. The heart/body weight ratio were determined at the end of experiment. The adrenergic nerve terminals visualized by histochemistry were counted according to Haug's point counting method. Blood pressure increased significantly in L-NAME-treated rats. No changes were found in the heart rate. Heart/body weight ratio increased markedly. Surprisingly, the density of adrenergic nerve terminals did not alter accordingly. The density of adrenergic nerve terminals in the left ventricle and septum decreased but no significant changes were found in the left atrium and the right ventricle. Hypertension due to NO deficiency induced cardiac hypertrophy that was characterized by a decline in the density of adrenergic innervation of the overloaded left ventricle and septum.

Key words

Nitric oxide – Hypertension – Cardiac hypertrophy – Adrenergic innervation

Introduction

The discovery of the role of NO in the control of vascular smooth muscle tone led very soon to the development of a novel experimental model of hypertension induced by long-term inhibition of NO synthase, the enzyme triggering the arginine-NO pathway (Bayliss *et al.* 1992, Ribeiro *et al.* 1992). Dananberg *et al.* (1993) suggested the term "NO-deficient hypertension." Recently, trials were undertaken to define the structural and functional characteristics of NO-deficient hypertension. Surprisingly, Arnal *et al.* (1993) found cardiac hypertrophy only in a few rats of the experimental group administered an inhibitor of NO synthase. On the other hand, Morton *et al.* (1993) did find cardiac hypertrophy under similar conditions. We attempted to contribute to the question whether NO-deficient hypertension may represent such an unexpected model which would not be accompanied by cardiac hypertrophy.

The arginine-NO pathway has, however, been found to be operative also in the central and peripheral nervous system, including those parts that are relevant for cardiovascular control (Shapoval *et al.* 1991, Tagawa *et al.* 1994, Gerová *et al.* 1995). Since the sympathetic nervous system has been found to be involved in those types of hypertension accompanied by cardiac hypertrophy (Westfall and Meldrum 1985), we addressed the problem of adrenergic innervation of hypertrophic myocardium in NO-deficient hypertension. Using the quantitative point counting method, the volume density of adrenergic nerve terminals was determined in the myocardium.

Methods

Wistar male rats were used in this study. Twelve 10-week-old animals were divided into two groups. Six control animals were given tap drinking water. Six experimental animals received a daily dose of 50 mg L-nitro-arginine methyl ester (L-NAME) per kg b.w. in the drinking water for a period of eight weeks.

Systolic blood pressure was measured each week in both groups by the tail-plethysmographic method and recorded on a RV Recorder 8005 (W+W) Electronic INF.

The animals were sacrificed by an overdose of pentobarbital (100 mg/kg b.w) at the end of the experimental period. The chest was opened, the heart excised and weighed. Both ventricles were filled with a carboxy-methyl-cellulose solution (CMC) containing: 2 g CMC, 30 ml HEPES buffer, pH 7.15. An approximately 3 mm thick sample oriented transversely to the long axis of the heart was excised from the upper third of the ventricles. The sample which included the left ventricle, right ventricle and the interventricular septum was glued with the CMC solution onto a small cork plate, and frozen immediately in a propane-butane mixture at −170 °C. A sample from the left auricle was processed similarly. The procedure was completed within 15 min after sacrificing the animal. The samples were stored in liquid nitrogen. Slices 15 μm thick were sectioned in a cryostat at −28 °C and were processed according to Falck's method (Falck and Owman 1965). The volume density of sympathetic innervation of the myocardium was determined by the point counting technique according to Haug (1962) and Doležel *et al.* (1984). The mean number of hits per one field, obtained from two hundred counted fields, was taken as representative of the volume density of sympathetic nerve terminals of each myocardium.

The evaluations were performed without the observer's knowledge of the origin of the sample.

The parameters studied were expressed as means ± S.E.M. The significance of differences between the means of corresponding samples of the control and experimental group was determined by Student's t-test. $P<0.05$ values were considered to be significant.

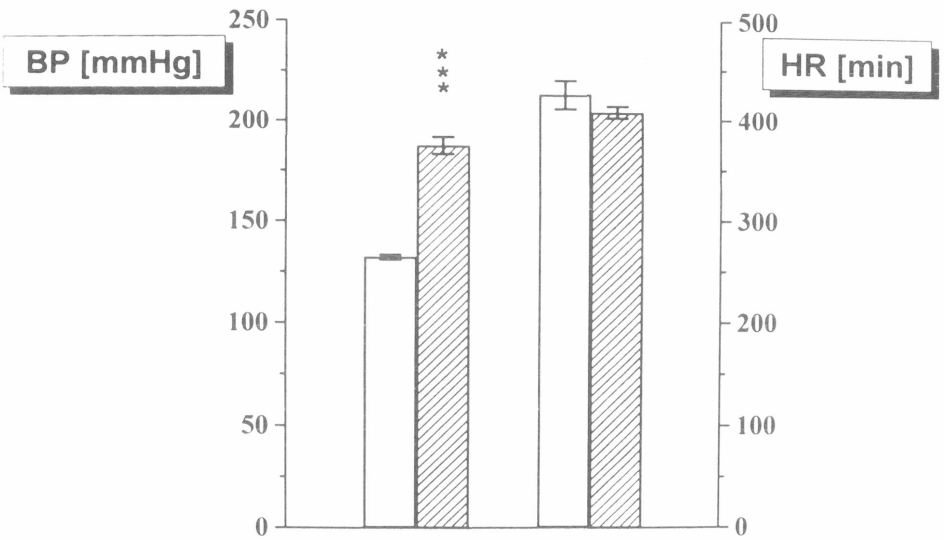

Fig. 1
Systolic blood pressure and heart rate of control rats (open columns) and rats administered L-NAME for a period of 8 weeks, 50 mg/kg bw/day (hatched columns).

Results

The systolic blood pressure in control age-matched animals was 132.0±1.2 mm Hg. The systolic blood pressure in animals treated with L-NAME for eight weeks was significantly higher than in control rats (187.2±4.2 mm Hg, $P<0.001$) (Fig. 1).

The heart rate in hypertensive animals was 408.0±5.8 beats/min which was somewhat lower than in the controls (426.0±14.3 beats/min). This difference was not significant (Fig. 1).

The heart weight of control rats aged 18 weeks was 1.10±0.03 g whereas in hypertensive rats it amounted to 1.32±0.09 g ($P<0.05$). The calculated heart/body weight ratio increased from 2.10±0.04 in the control animals to a value of 3.00±0.15 in hypertensive rats ($P<0.001$) (Fig. 2).

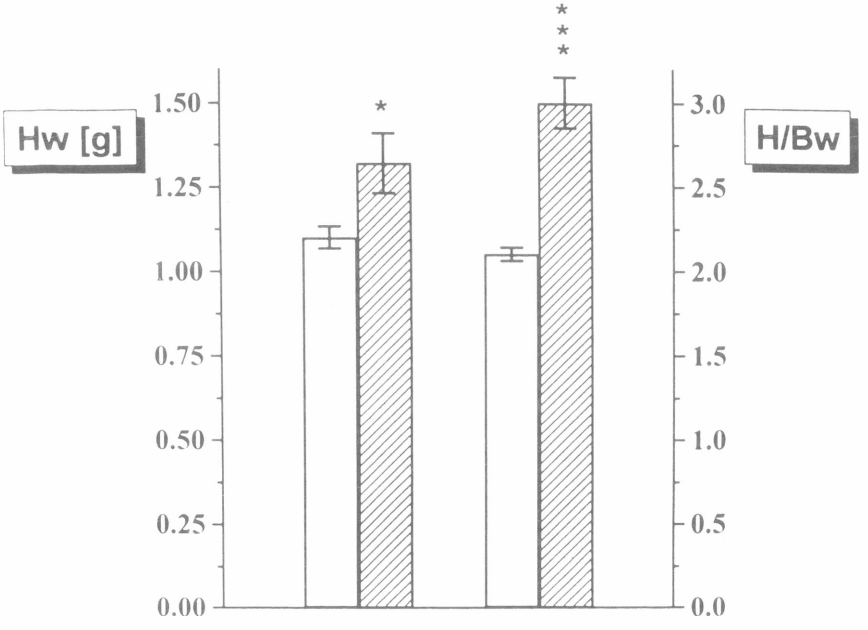

Fig. 2
Heart weight and heart/body weight index in control rats (open columns) and rats administered L-NAME for a period of 8 weeks, 50 mg/kg bw/day (hatched columns).

Table 1
Volume density of adrenergic nerve fibres in the myocardium of control rats and of rats administered L-NAME (50 mg/kg bw/day) for 8 weeks

Group	Left ventricle	Right ventricle	Septum	Left auricle
Controls	1.14	1.28	0.98	1.37
	0.75	0.83	0.67	0.99
	0.86	1.28	1.17	1.16
	1.19	1.51	1.05	1.57
	0.84	0.98	0.89	1.37
	0.94	1.10	0.99	–
Mean	0.95	1.16	0.95	1.29
S.E.M	±0.07	±0.09	±0.06	±0.09
L-NAME	0.26	–	0.28	0.73
	0.67	0.89	0.38	0.79
	0.62	1.34	1.19	1.27
	0.35	1.12	0.29	1.00
	0.47	0.74	0.31	1.87
	0.73	1.52	0.65	1.30
Mean	0.51**	1.12	0.52**	0.99
S.E.M.	±0.07	±0.14	±0.14	±0.09

*The data represent number of hits of adrenergic nerve fibres per field. Each value is a mean from two hundred counted fields. Asterisks indicate statistical significance of the value compared to the corresponding value in control animals. ** $P<0.01$.*

Table 1 represents the volume density of the adrenergic nerve terminals related to the myocardium in individual control and L-NAME-treated animals. This table shows the number of hits of adrenergic nerve fibres per one field in the left ventricle, right ventricle, interventricular septum and in the left auricle. The above data document a significant decrease in volume density of adrenergic nerve terminals to 54.16 % in the left ventricle ($P<0.01$) and to 54.73 % in the septum ($P<0.01$). A decreasing non-significant trend was found in the left auricle (76.74 %) and in the right ventricle (94.91 %) (Fig. 3).

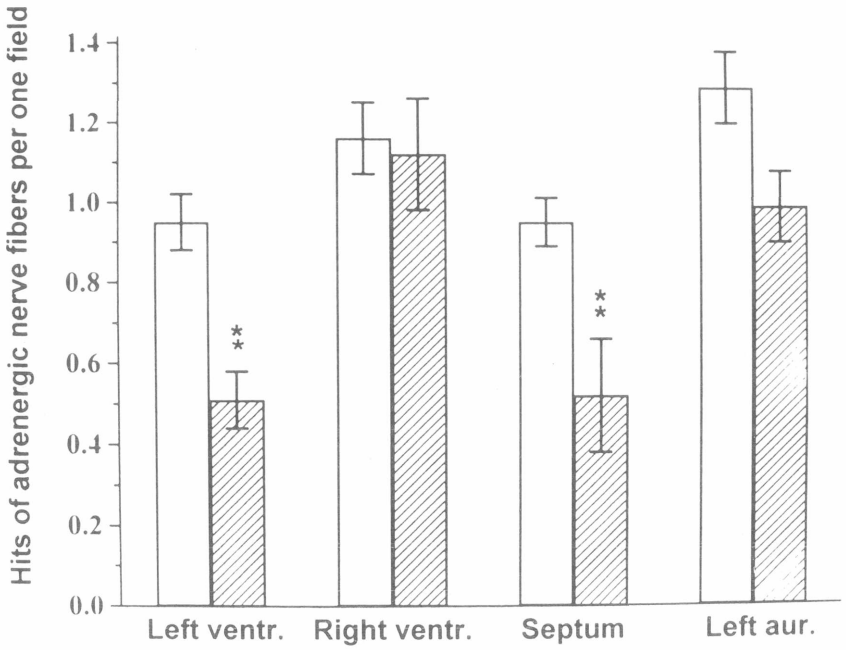

Fig. 3
Volume density of adrenergic nerve fibres in individual parts of the myocardium of control rats (open columns) and rats administered L-NAME for a period of 8 weeks, 50 mg/kg bw/day (hatched columns).

Discussion

The results of the experiments provided evidence that the inhibition of NO synthase for eight weeks induced sustained hypertension, with a tendency to decrease the heart rate which, however, was not significant. The hypertension was accompanied by cardiac hypertrophy, the heart weight being increased by 20 % and the heart/body weight ratio by 42 %. The unequivocal cardiac hypertrophy observed in the present study is controversial with respect to the variability of results of *in vitro* experiments or of acute experiments on anaesthetized animals. Various authors reported all three possible responses: NO was reported to have an inhibitory effect on the heart muscle (Klabunde *et al.* 1991, Brady *et al.* 1993), a positive inotropic effect (Kirstein *et al.* 1995), or no effect (Weyrich *et al.* 1994, Kennedy *et al.* 1994).

Our results are in agreement with the *in vivo* experiments of Morton *et al.* (1993) who described cardiac hypertrophy after chronic inhibition of NO synthase in rats. They are, however, controversial to the findings of Arnal *et al.* (1993) mentioned in the Introduction.

In the *in vivo* experiments, it is necessary to consider at least two substantial stimuli: in addition to the shift in NO levels, an increase in afterload may affect the myocardium.

The answer to the second question of our study, namely on the changes of the density of adrenergic innervation in the myocardium, was unexpected. The volume density of adrenergic nerve terminals in the increased mass of the myocardium did not alter correspondingly. On the contrary, a remarkable decrease in volume density of adrenergic nerve terminals was found in the left ventricular myocardium and in the septal myocardium, while no significant changes occurred in the volume density of adrenergic nerve terminals in the right ventricle and left auricle.

After long-term inhibition of NO synthase, the shift in NO• levels must conceivably influence all the cell elements in the heart, including the autonomic nerve supply of the heart. Indeed, the autonomic ganglia as well as the autonomic nerve terminals were shown to contain NO synthase (Klimaschewski *et al.* 1992, Ursell and Mayes 1993), i.e. NO• might be directly involved in the autonomic control of the heart and also of the coronary vascular tree.

Indirect evidence indicates that NO• contributes positively to the activity and also to the differentiation of the autonomic nervous system. Thus e.g. Tagawa *et al.* (1994) described an increase in neuronal activity of the nucleus tractus solitarius brought about by nitric oxide. On the basis of their experiments, Peunova and Enikolopov (1995) have

suggested that NO•, by initiating the growth arrest of pheochromocytes, may be a prerequisite for final differentiation, i.e. for the growth of neurites in pheochromocytes. Having in mind these data, one could speculate that a decline in NO• levels might compromise the growth and/or normal function of the autonomic nerves supplying the heart.

After 8 weeks of treatment with L-NAME a non-significant declining trend of adrenergic innervation density was found in the right ventricle and left auricle. Yet, in the left ventricle and septal myocardium a remarkable and significant decrease of adrenergic innervation was revealed. It thus seems plausible that the compromising process might be manifested only in the myocardium exposed to the overload.

To explain the decreased density of adrenergic nerve fibres in the hypertrophic myocardium due to inhibition of NO synthase, two possibilities may be considered: (i) the decline might be a matter of simple depletion of the transmitter in the nerve terminals, or (ii) it might be an actual "rarefaction" of adrenergic nerve fibres in the growing hypertrophic myocardium. The method used in the present experiments (Falck's method, light microscopy) did not allow to solve this problem. Nevertheless, the total number of nerve terminals containing monoaminergic transmitters obviously lagged behind the increase of the cardiac mass.

The decrease in cardiac output reported by Lechevalier *et al.* (1994) after inhibition of NO synthase is consistent with the decrease in the density of adrenergic nerve terminals.

In conclusion, eight weeks' lasting inhibition of NO synthase induced hypertension in rats. Cardiac hypertrophy accompanied this type of hypertension. The volume density of adrenergic nerve terminals declined in the hypertrophic myocardium. These findings indicate that a low NO• level may have a compromising effect on adrenergic innervation of the heart.

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Reprint Requests

M. Gerová, M.D., D.Sc., Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1, 833 01 Bratislava, Slovak Republic.