# Effect of NO Synthase Inhibitor L-NAME on DNA Synthesis in the Myocardium of Albino Rats during Early Postnatal Ontogeny

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Effects of repeated treatment (5 times) with L-NAME, a NO synthase (eNOS) inhibitor on DNA synthesis in various zones of the heart in newborn albino rats was studied using $^3$H-thymidine autoradiography. This compound decreased (by 1.22-1.59 times) the count of labeled cardiomyocyte nuclei in various myocardial zones, particularly, in the myocardium of the left atrium, left ventricle, and interventricular septum. Changes in tissue proliferative activity were accompanied by activation of lipid peroxidation and inhibition of the antioxidant system in the myocardium.

**Key Words:** *nitric oxide synthase; DNA synthesis; myocardium; lipid peroxidation*

Left ventricular hypertrophy and concomitant metabolic disturbances in the heart are the most severe complication of arterial hypertension. Previous studies showed that the severity of tissue metabolic disorders considerably varies in patients with similar blood pressure. These differences can be explained by different activity of the nitric oxide/nitric oxide synthase (NO—NOS) system in the heart [7]. Increased cardiomyocyte ploidy is associated with lower risk of trophic and functional disturbances in the myocardium: the greater is the total genome, the higher are myocardial reserves under pathological conditions [1].

The cardiac NO—NOS system plays an important role in the embryogenesis of mammalian heart. Treatment with NOS inhibitors reversibly blocks differentiation of embryonic cardiomyocytes in culture [8]. These data suggest that the cardiac NO—eNOS system is involved in the formation of polyploid cardiomyocytes during early postnatal ontogeny.

Here we evaluated the role of endogenous cardiac NO in the regulation of proliferative processes in mammalian myocardium during early postnatal ontogeny.

## MATERIALS AND METHODS

Experiments were performed on 45 newborn random-bred albino rats. Control and experimental groups were composed by the method of litter separation to reduce genetically determined differences between litters. The eNOS inhibitor L-NAME (Nω-nitro-L-arginine methyl ester) was injected intraperitoneally in a daily dose of 25 mg/kg at 11.00 from the 2nd to 6th day of life. Control animals received an equivalent volume of 0.9% NaCl. DNA synthesis in the myocardium was determined auto-radiographically 24 h after the last treatment. $^3$H-Thymidine (1 μCi/g body weight, 1570 TBq/mol) was injected intraperitoneally 1 h before euthanasia. Histological preparations of the myocardium and autoradiographs were prepared by routine techniques.

Labeled cardiomyocytes were separately counted in the left and right atria and subendocardial and intramural layers of the left ventricle, interventricular septum, and right ventricle. The index of labeled nuclei reflecting the ratio of DNA-synthe-

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sizing nuclei (ILN) and labeling intensity showing the mean number of silver grains over the nucleus (LI) were calculated.

In special series we evaluated the effects of L-NAME on lipid peroxidation (LPO) in myocardial tissues. $H_2O_2$-induced luminol-dependent chemiluminescence (CL) was measured on an LS 50B chemiluminometer (Perkin Elmer) [5]. The intensity of spontaneous and $H_2O_2$-induced CL and maximum flash amplitude ($I_{max}$) were measured at room temperature, calculated per 1 mg lipids, and expressed in arbitrary units. The contents of total lipids, lipid hydroperoxides, malonic dialdehyde (MDA), and α-tocopherol were measured as described elsewhere [4].

The results were analyzed by Student's *t* test.

## RESULTS

L-NAME considerably inhibited DNA synthesis in various myocardial zones (Table 1). The count of DNA-synthesizing nuclei in the myocardium of the left atrium, subendocardial and intramural layers of the left ventricle, and subendocardial layer of the interventricular septum decreased by 1.5, 1.47, 1.52, and 1.56 times, respectively ($p<0.05$). ILN reflecting the number of proliferating cardiomyocytes tended to decrease in the myocardium of the right atrium, intramural layer of the interventricular septum, and subendocardial and intramural layers of the right ventricle (by 1.38, 1.47, 1.59, and 1.22 times, respectively). Thus, proliferative activity decreased in all myocardial zones. It should be emphasized that LI reflecting the rate of DNA synthesis remained unchanged in these cardiac regions. The inhibition of proliferative processes in the myocardium after blockade of the NO—eNOS system can be realized via various mechanisms. Previous studies showed that NO *in vitro* stimulates proliferation of myocardial cells, which is associated with the activation of ornithine decarboxylase and intensification of polyamine biosynthesis [13]. Thus, L-NAME abolishes stimulation of cardiomyocyte proliferation.

Endogenous tissue NO attenuates the effects of β-adrenergic stimulation in the heart by auto- and paracrine mechanisms [10]. Inhibitors of the NO—eNOS system potentiate catecholaminergic stimulation of the myocardium and, probably, decrease the count of proliferating cardiomyocytes in newborn animals [6].

L-NAME stimulates expression and release of atrial natriuretic peptide in animals with insufficiency of the cardiac NO system [9]. Our previous experiments demonstrated that atrial natriuretic peptide markedly inhibits proliferative processes in the myocardium of albino rats during early postnatal ontogeny [2].

Peroral administration of NOS inhibitors to adult albino rats for 3-6 weeks leads to the appearance of heterogeneous cardiomyocytes and to a decrease in their absolute count [11], which is probably related to the initiation of apoptosis [14]. The regulation of cardiomyocyte proliferation and death is realized via LPO activation [3]. Long-term inhibition of NOS with L-NAME is followed by oxidative stress in rats [12]. In rabbits, L-NAME decreases plasma level of zinc, which is involved in enzymatic protection of the myocardium from peroxidative processes [15].

We studied the effect of repeated treatment with 25 mg/kg L-NAME on free radical oxidation in the myocardium of albino rats during early post-

**TABLE 1.** Effect of Repeated Treatment with NOS Inhibitor L-NAME on DNA Synthesis in the Myocardium of Albino Rats during Early Postnatal Ontogeny (*M*±*m*)

| Myocardial zone | Control | | L-NAME | |
|---|---|---|---|---|
| | ILN | LI | ILN | LI |
| Left atrium | 10.86±1.44 | 19.53±2.21 | 7.29±0.91* | 17.92±1.39 |
| Right atrium | 11.26±1.46 | 19.05±2.74 | 8.16±1.44 | 16.60±1.38 |
| Left ventricle | | | | |
| subendocardial layer | 12.48±1.33 | 18.92±2.09 | 8.47±1.23* | 16.37±1.48 |
| intramural layer | 13.42±1.42 | 14.47±1.41 | 8.81±1.54* | 13.04±0.75 |
| Interventricular septum | | | | |
| subendocardial layer | 13.90±1.71 | 19.41±1.90 | 8.90±1.45* | 17.51±1.54 |
| intramural layer | 14.10±1.61 | 16.10±1.99 | 9.62±1.60 | 13.40±0.81 |
| Right ventricle | | | | |
| subendocardial layer | 12.53±1.87 | 19.33±2.70 | 7.86±1.47 | 17.51±1.89 |
| intramural layer | 10.36±1.88 | 13.13±1.76 | 8.50±2.06 | 12.10±1.17 |

**Note.** *$p<0.05$ compared to the control.

**TABLE 2.** Effect of Repeated Treatment (5 Times) with the NOS Inhibitor L-NAME on Free Radical Oxidation in the Myocardium of Albino Rats during Early Postnatal Ontogeny (*M*±*m*)

| Parameter | Control | L-NAME |
|---|---|---|
| Total lipids, mg/g tissue | 165.91±3.37 | 154.79±4.19 |
| α-Tocopherol, mg/g tissue | 11.46±1.14 | 6.05±0.50* |
| MDA, fluorescence units/mg lipids | 12.09±0.45 | 14.74±0.65** |
| Lipid hydroperoxides, optical density units/mg lipids | 0.05±0.01 | 0.085±0.010** |
| CL intensity | | |
| spontaneous | 0.56±0.05 | 0.55±0.06 |
| $H_2O_2$-induced | 16.89±1.39 | 18.83±1.74 |
| $I_{max}$ | 14.48±1.66 | 18.47±1.70 |

**Note**. *$p$<0.01 and **$p$<0.05 compared to the control.

natal ontogeny. Administration of the eNOS inhibitor was followed by considerable changes in the LPO—antioxidant defense system in the myocardium of newborn animals (Table 2). The contents of MDA and lipid hydroperoxides increased by 18 and 70%, respectively, while the concentration of vitamin E (α-tocopherol) decreased by 47%. Changes in parameters of CL were less pronounced. $H_2O_2$-induced CL and $I_{max}$ increased by 11.5 and 27.5%, respectively (insignificantly). It remains unclear, why LPO was considerably intensified, but CL remained practically unchanged. This phenomenon is probably associated with the nonlipid compensatory inhibition of free radical oxidation.

Our results suggest that functional insufficiency of the cardiac NO—NOS system in the early postnatal period determines structural homeostasis in the myocardium during ontogeny. The decrease in proliferative activity of cardiomyocytes is at least partially related to changes in the LPO—antioxidant defense system.

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