Morphometric Characteristics of Cardiac Hypertrophy Induced by Long-Term Inhibition of NO Synthase

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Summary

Morphometry of cardiomyocytes and capillary domains in the left ventricle myocardium was performed in control rats and in rats treated with nitro-L-arginine methyl ester 50 mg/kg/day p.o. for a period of 8 weeks. The myocardial hypertrophy accompanying the NO-deficient hypertension induced by chronic inhibition of NO synthase is characterized by an increase in thickness of myocardial fibres and by relative rarefaction of the capillary bed, e.g. an alteration in myocardial structure which is typical for pressure overload hypertrophy.

Key words

NO deficiency – Hypertension – Morphometry – Cardiac hypertrophy – Myocyte – Capillary domain

Introduction

The early reluctance concerning the effect of NO* on cardiac performance (Klabunde et al. 1992, Weyrich et al. 1994, Kirstein et al. 1995) has been also reflected by ambiguous data dealing with cardiac hypertrophy in NO-deficient hypertension (Arnal et al. 1993, Morton et al. 1993, Bernátová et al. 1994). It seems, however, that the issue has slowly cleared, a number of reports proving the presence of cardiac hypertrophy has increased (Morton et al. 1993, Delacretaz et al. 1994, Kristek and Gerová 1995). The aim of the study was to characterize the type of cardiac hypertrophy accompanied the NO-deficient hypertension induced by chronic inhibition of NO synthase in rats. Namely two points were studied: (i) the cross-section area of myocytes and (ii) the capillary domains and/or density of capillaries, in particular in relation to myocyte cross-section area.

Methods

Fifteen male Wistar rats (10 weeks of age) were used for the study. Eight experimental rats were administered NO synthase inhibitor nitro-L-arginine methyl ester (L-NAME 50 mg/kg/day) in drinking water for eight weeks. Systolic blood pressure was measured each week in both groups by the tailplethysmographic method and recorded on a RV Recorder 8005 (W + W) Electronic INF.

After 8 weeks of L-NAME administration the animals were sacrificed by a high dose of pentobarbital (100 mg/kg b.w. intraperitoneally). The chest was opened, the heart excised, weighed and the weight was normalized by the body weight. The ventricular cavities were filled up with 6% solution of carboxymethyl cellulose (CMC), the heart was embedded in CMC and frozen by plunging into liquid propane chilled to about −170°C with liquid nitrogen. The ventricles were sectioned on a cryomicrotome from apex to base. Four 10 μm sections were mounted. The sections were stained for detection of alkaline phosphatase and dipeptidyl peptidase IV activities in the capillary endothelium according to the method of Lojda (1979). This double-staining procedure ensures that all capillaries were visualized.

Morphometry of capillaries

Morphometric measurements of capillaries were done with the aid of a Nikon Type 104 microscope using 20x objective. The image was digitized by means of an image analyzing system LUCIA S 3.10, and processed by image analyzing software DIPS 4.0 (SOFO, Brno, Czech Republic).
Five fields (200 x 250 \( \mu \text{m} \)) were selected from the subendomyocardium of each heart. The x and y coordinates of capillaries found inside the field were measured using the image analyzer DIPS. The capillary density was evaluated by the method of capillary domains (Rakušan et al. 1992). The capillary domain is the polygonal area enclosed by equidistant border lines between each of the neighbouring capillaries and its average size is indirectly proportional to the capillary density. The capillary domains were constructed by means of computer program CSS Statistica using Voronoi tessellation method (Turek et al. 1972) and their areas were determined automatically by the image analyzer DIPS. Only those domains which did not touch the edges of the field were evaluated.

**Morphometry of myocytes**

The myocyte morphometry was performed in the same fields used for morphometry by means of phase contrast. The measurements of dimensions and area of myocyte transversal sections were performed by means of semiautomatic image analysis (DIPS). Thirty randomly chosen myocytes within the field were evaluated.

**Myocyte capillary supply**

The number of capillaries which nourish each individual myocyte was determined. The capillary domains were superimposed over the image of the field of transversely cut heart muscle with traced myocyte contours. The number of domains falling into each chosen myocyte was counted by means of the image analyzing software DIPS.

**Results**

Blood pressure at the end of the experiment was 187.2 ± 7.2 mm Hg in experimental and 132.0 ± 1.2 mm Hg in control animals (\( P < 0.001 \)). Heart weight and the heart/body weight ratio significantly increased from 2.1 ± 0.04 in the controls to 3.0 ± 0.15 in experimental animals (\( P < 0.001 \)).

Figure 1 presents the area of myocyte transversal sections. Whereas in control animals the average value of myocyte transversal section represented 256.0 ± 41.55 \( \mu \text{m}^2 \), in experimental animals the value was significantly higher and reached 343.81 ± 58.86 \( \mu \text{m}^2 \) (\( P = 0.0054 \)).

Figure 2 presents the size of the capillary domain. In control animals, the average size of the capillary domain in the left ventricle myocardium was 326.75 ± 30.28 \( \mu \text{m}^2 \), in experimental animals the capillary domain increased significantly to 438.01 ± 73.20 \( \mu \text{m}^2 \) (\( P = 0.0046 \)).

Distribution of the number of capillaries which nourish each respective myocyte is presented as the histogram in Figure 3. The median value in experimental animals did not differ significantly from the value found in the controls. The finding indicates that no capillary proliferation had occurred.
Discussion

It is well known that cardiac myocytes are completely differentiated in the early postnatal period and no proliferation could be found in adult cardiac muscles. In the overloaded adult heart, the growth of myocytes is realized either by an increase in length, or by an increase in cross-section area of the individual myocytes. Polyploidy could be found accompanying the overload. Increase in afterload induces concentric hypertrophy associated with an increase in cross-section area of myocytes. Increase in afterload, induced by long-lasting hypertension as a consequence of NO• deficiency, is in agreement with the above view. Indeed, a significant increase in average cross-section area of myocytes was found in the left ventricle.

Different processes have been described in coronary capillaries in the hypertrophic myocardium. In the pressure-overloaded heart characterized by an increase in cross-section area of cardiomyocytes the relative rarefaction of the capillary bed with consequent diminishing of oxygen supply was described (Rakušan et al. 1992). However, in certain type of cardiac hypertrophy (exercise, hyperthyroidism, high altitude hypoxia) an actual growth of new capillaries was observed (Turek et al. 1972, Ziada et al. 1984, Chilian et al. 1985, Anversa et al. 1987, Hudlicka et al. 1989). Our measurements indicated a clear increase in size of the capillary domain, concomitantly with an increase in cross-section area of myocytes. The calculated ratio of myocyte cross-section area/capillary domain area in control and experimental animals did not differ significantly. This finding rather suggests a relative passive capillary rarefaction due to the increase in cross-section area of myocytes and no increase in the number of new capillaries.

It may thus be concluded that the myocardial hypertrophy accompanying the NO-deficient hypertension induced by chronic inhibition of NO synthase has some typical characteristics of hypertrophy induced by pressure overload, i.e. increased size of myocardial fibres and relative rarefaction of the capillary bed.

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