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Induction of Angiogenesis in NO-Deficient Rat Heart

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Summary

Angiogenesis is known to be triggered by various stimuli including hypertension. It was previously found that NO-deficient hypertension is accompanied by structural remodeling of the cardiac muscle and large coronary arteries. This study was aimed to examine the qualitative subcellular alterations of capillaries in the heart of the rats treated with L-NAME (40 mg/kg/day for 4 weeks). The results showed that long-lasting inhibition of NO production induced an apparent activation of fibroblast function. This was associated with enhancement of fibrotization as well as with the induction of angiogenesis. Accordingly, fibroblasts were frequently located in the vicinity of capillary pericytes, which was followed by their detachment and migration. Moreover, besides inactive or even injured capillaries, the other ones exhibited extensive proteosynthetic activity linked to capillary growth, proliferation and migration of endothelial cells. The results strongly indicate enhanced triggering of the angiogenesis in L-NAME-induced NO-deficient hypertension.

Key words

Nitric oxide deficiency • Angiogenesis • Capillary • Rat • Ultrastructure

Introduction

Angiogenesis is the process of new vessel formation from a pre-existing capillary network (Folkman 1982). Endothelial cell proliferation and migration are critical events for angiogenesis and they are tightly regulated by a large number of various angiogenic factors. Nitric oxide (NO), produced mainly by endothelial cells, is considered to be one of mediators of the angiogenesis of diverse types of cells. Controversial data have been found concerning the role of NO in the angiogenesis of endothelial cells. Ziche *et al.* (1994) and Morbidelli *et al.* (1996) demonstrated that NO mediates the angiogenesis of endothelial cells, however, their findings contradicted the results of Pipili-Synetos *et al.* (1993) and Murohara *et al.* (1999) who showed that NO exerted antiproliferative effect.

Angiogenesis as an important adaptive response has also been observed in the heart hypertrophy due to renal hypertension. Myocardial hypertrophy as a result of NO-deficient hypertension induced by L-arginine analogues has been characterized by an enhancement of vascular and myocardial proteosynthesis (Babál *et al.* 1997, Gerová *et al.* 1998) underlying vascular (Kristek *et al.* 1996, Babál *et al.* 1997) and myocardial remodeling (Babál *et al.* 1997, Okruhlicová *et al.* 1999, Pecháňová *et al.* 1997, 1999, Tribulová *et al.* 2000).

In the light of the above mentioned findings and some controversial results with respect to cardiac hypertrophy, the aim of this work was to examine,

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whether long-lasting inhibition of NO production is accompanied at the subcellular level with angiogenesis of capillaries in the myocardium of NO-deficient rats.

Material and Methods

Model of NO-deficient hypertension

Male Wistar rats (aged 12-13 weeks) were divided into 2 groups (each n=6). The first group served as a control, second one was given L-NAME in a daily dose of 40 mg/kg in a drinking water for 4 weeks. The systolic blood pressure and heart rate were measured by tail-cuff plethysmography every day. The left ventricle weight/body weight (LVW/BW) ratio was calculated for each animal. After 4 weeks, the animals were sacrificed by decapitation.

Transmission electron microscopy

At the end of the experiments, small blocks of the tissue from the left ventricle were fixed with 2.5 % glutaraldehyde in 100 mmol/l cacodylate buffer (pH 7.4) for 3 h. After washing in a cacodylate buffer, the tissue was postfixed in 1 % OsO_4 buffered with 100 mmol/l sodium cacodylate. The tissue was dehydrated *via* an ethanol series, infiltrated by propylene oxide and embedded in Epon 812. Toluidine blue-stained 1 μm thick sections were examined by light microscopy and appropriate areas were selected for cutting of thin sections. The sections were stained with uranyl acetate and lead citrate and examined using an electron microscope Tesla BS 500.

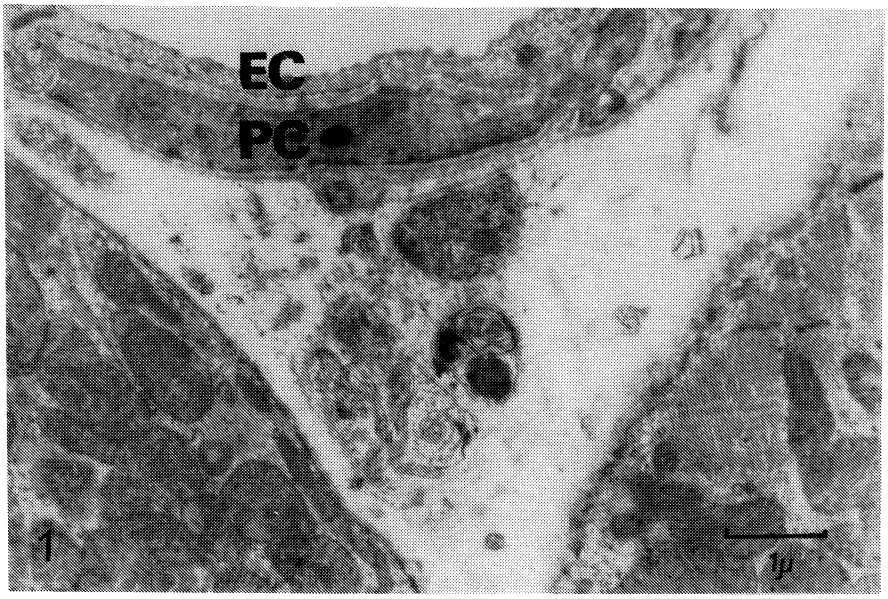

Fig. 1. *Conventional architecture of a capillary consisting of endothelial cells (EC) which are covered over with a pericyte (PC) in the myocardium of a hypertensive rat.*

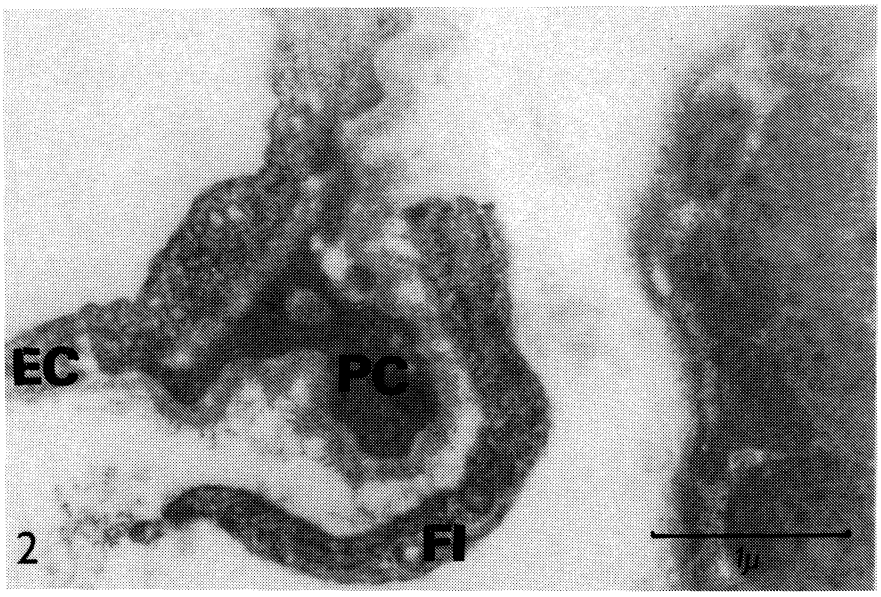

Fig. 2. *The communication of a pericyte (PC) with a fibroblast (Fl) iniciates the process of angiogenesis, i.e. the peeling of a pericyte (PC) from an endothelial cell (EC).*

Results

General profile

Oral administration of L-NAME in the dose 40 mg/kg/day increased systolic blood pressure by 30 % and decreased the heart rate by 26 % after the first week as compared with the controls ($p<0.05$). The changes of blood pressure and the heart rate persisted during the following three weeks. After 4 weeks, the LVW/BW ratio was significantly elevated (+27 %) in the L-NAME treated group.

Electron microscopic evaluation of L-NAME group

Some cardiomyocytes from the left ventricle were without evident ultrastructural alterations. Some cardiomyocytes revealed ultrastructural signs of hypertrophy, e.g. the presence of polyribosomes, neoformation of myofilaments and increased amounts of mitochondria of different shape and size. Some myocytes displayed markers of ischemia-like injury with edematous mitochondria. In the extracellular space, we observed an activation and proliferation of fibroblasts with a high number of ribosomes, associated with increased extracellular matrix production resulting in focal interstitial and perivascular fibrosis. Endothelial cells of some capillaries were injured with decreased electrondensity of the nucleus and increased permeability due to impaired intercellular connections. The architecture of noninjured capillaries was typical, however, we always found a thickening of their basal lamina. The capillaries were covered with pericytes (Fig. 1). NO synthase inhibition with L-NAME was accompanied by morphological changes of the pericytes. Their elongated shape was shortened and they appeared to peel off the endothelial cells. Thus, the close contact between endothelial cells and pericytes became gradually lost. During this process, a close interaction of fibroblasts with pericytes was observed (Fig. 2). In this fibroblast-pericyte contact, a flocculent thickening of the capillary basal membrane was detected. We frequently observed the migration of endothelial cells (Fig. 3) as the next step of angiogenesis and the forming of new capillaries with a small lumen (Fig 4). At the same time, endothelial cells were enriched with ribosomes and pinocytic vesicules.

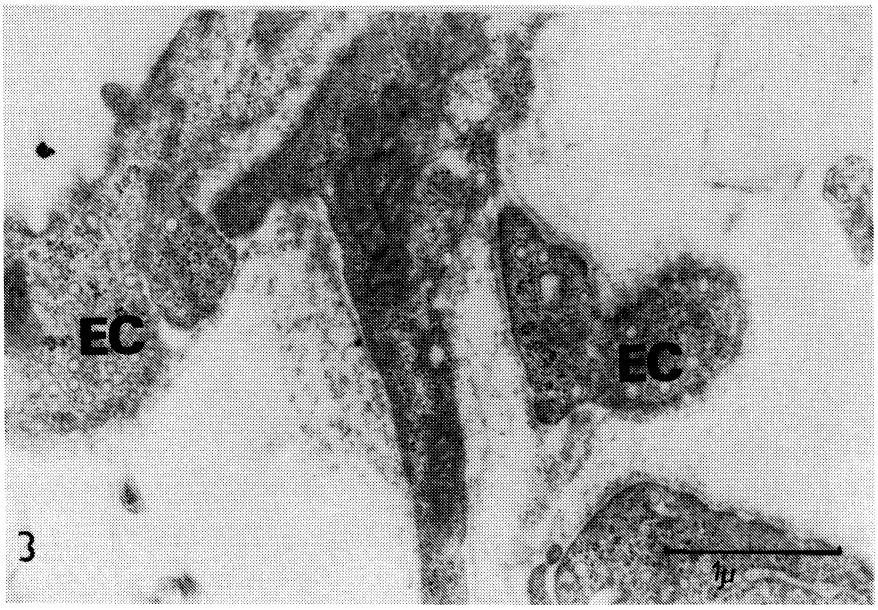

Fig. 3. *The migration of endothelial cell (EC) into the extracellular space.*

Discussion

The model of NO-deficient hypertension has been described by a number of authors (Arnal *et al.* 1993, Ribeiro *et al.* 1992). In spite of this, data concerning the development of myocardial hypertrophy are still controversial. In the myocardium of L-NAME treated rats, a significant increase of total RNA and DNA content has been observed (Pecháňová *et al.* 1995). This was a result of stimulated myocytes and enhanced proliferation of smooth muscle and endothelial cells as well as of fibrous tissue as was observed at the light microscopy level (Babál *et al.* 1997).

It is known that the capillaries are composed of endothelial cells surrounded by a basal lamina and a single layer of pericytes (Sims 1991, Herman 1993). It has been suggested that pericytes subserve several specific roles, e.g. they may regulate capillary blood flow

and capillary growth, and serve as specific precursors to vascular smooth muscle cells and endothelial cells (for review see Hirschi and D'Amore 1996). The location of pericytes on the abluminal surface of capillary endothelial cells allows them to communicate with each other, and thereby, to influence their behavior *via* bidirectional extracellular exchange of soluble mediators, such as the vascular endothelial growth factor, the platelet-derived growth factor and the fibroblast growth factor, which appear to promote pericyte proliferation and/or migration (Montesano *et al.* 1986). Furthermore, the transforming growth factor-β1 appears to be involved as a potent inhibitor of endothelial cell proliferation *in vitro* (Heimark *et al.* 1986, Sato and Rifkin 1989).

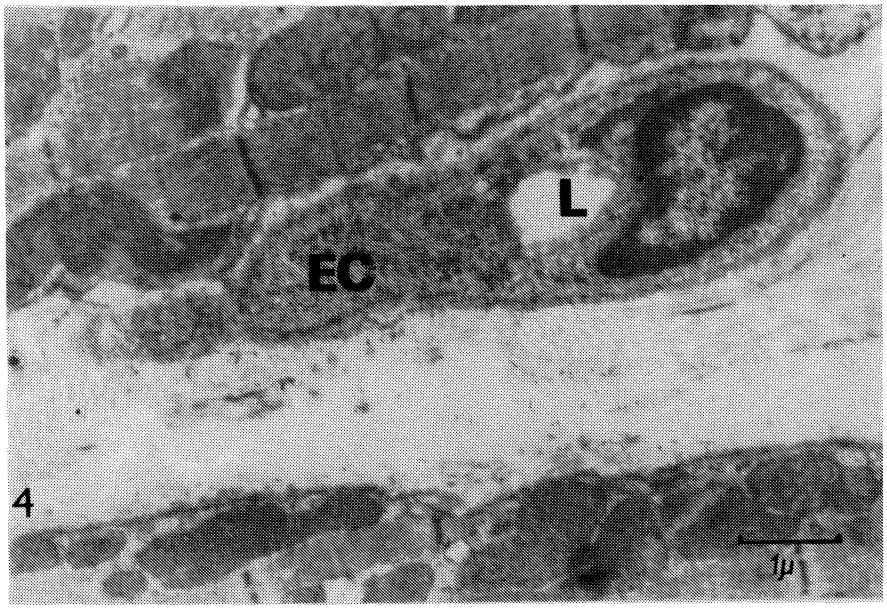

Fig. 4. *The newly-formed capillary with a narrow lumen (L); endothelial cell (EC) containing a high amount of ribosomes and small pinocytic vesicles.*

We observed capillaries in the myocardial tissue of NO-deficient rats which were in a very close contact with the pericytes as has been observed in the normal heart. Pericytes of some capillaries, however, changed their shape so that the connection between endothelial cells and pericytes was gradually lost. This allowed the endothelial cells to proliferate, migrate and to form new capillaries. The inhibitory effect of pericytes on the proliferation of endothelial cells is interrupted by fibroblasts (Montesano *et al.* 1986). The interaction of fibroblasts with pericytes, that we demonstrated at the subcellular level, substantiates these data. This was accompanied by increased amounts of ribosomes and pinocytic vesicles in endothelial cells that are typical for enhancement of proteosynthetic processes and transport activity.

In the light of the data mentioned above, our ultrastructural findings clearly indicate that the angiogenesis of capillary endothelial cells in the myocardium of NO-deficient rats is increased and implicate the role of pericytes and fibroblasts in this process. Angiogenesis was most likely induced by the lack of NO resulting from L-NAME inhibition of NO synthase. Our results thus confirm the data of Pipili-Synetos *et al.* (1993) who showed that NO suppresses angiogesis. On the other hand, it has been suggested that NO might initiate angiogenesis *in vitro* (Ziche *et al.* 1994, Morbidelli *et al.* 1996).

The application of L-NAME impaired the function of large vessels and adversely affected vascular relaxation (Holécyová *et al.* 1996). This can lead to local myocardial ischemia (Avontuur and Ince 1995) which can explain ischemia-like mitochondrial changes detected in the NO-deficient myocardium. Metabolic disturbances were detected on the basis of decreased activity of various enzymes involved in energy metabolism (Tribulová *et al.* 2000). Moreover, significant inhibition of NO synthase in this model of hypertension was detected by both biochemical (Pecháňová and Bernátová 1996) and histochemical methods (Tribulová *et al.* 2000).

In conclusion, our results strongly indicate that chronic application of L-NAME in rats induces hypertension, which is accompanied by structural remodeling of the myocardium as well as by triggering the process of angiogenesis which are associated with myocardial hypertrophy.

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Reprint requests

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