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Chronic Disturbances in NO Production Results in Histochemical and Subcellular Alterations of the Rat Heart

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Summary

The mechanisms and myocardial alterations associated with NO-deficient hypertension are still far from clear. The aim of the present study was to focus on the enzyme histochemical and subcellular changes in the heart of L-NAME treated rats, as well as to examine the influence of captopril treatment. Wistar rats were administered either L-NAME (40 mg/kg/day) alone or together with captopril (100 mg/kg/day) for a period of 4 weeks. A significant increase of blood pressure confirmed the reliability of the model. The results showed that long-lasting L-NAME administration was accompanied by a decrease of endothelial NO-synthase activity and by a significant local decrease of the following enzyme activities: capillary-related alkaline phosphatase, 5'-nucleotidase and ATPase (but not dipeptidyl peptidase IV) and cardiomyocyte-related glycogen phosphorylase, succinic dehydrogenase, β-hydroxybutyrate dehydrogenase and ATPases. No activity of these enzymes was found in the scar, whereas a marked increase of alkaline phosphatase and dipeptidyl peptidase IV activities was found in the foci of fibrotization. Histochemical changes correlated with subcellular changes, which were characterized by 1) apparent fibroblast activation associated with interstitial/perivascular fibrosis, 2) heterogeneous population of the normal, hypertrophic and injured cardiomyocytes, 3) enhancement of the atrial granules and their translocation into the sarcolemma, and 4) impairment of capillaries as well as by induction of angiogenesis. Similar alterations were also found in the heart of captopril co-treated rats, despite of the significant suppression of blood pressure. The results indicate that NO-deficient hypertension is accompanied by metabolic disturbances and ultrastructural alterations of the heart and these changes are probably not induced by the renin-angiotensin system only.

Key words

Nitric oxide • Hypertension • Histochemistry • Ultrastructure • Captopril

Introduction

In contrast to essential hypertension characterized by functional, metabolic and structural changes of the heart (Bing *et al.* 1995, Hojo *et al.* 1992) and by the involvement of renin-angiotensin-aldosteron systems in the development of myocardial dysfunction (Weber and Brilla 1991, Griendling *et al.* 1993, Šimko and Šimko 1999), the mechanisms as well as alterations related to NO-deficient hypertension (Ribeiro *et al.* 1992) are still incomplete. It was reported that NO deficiency is associated with increased contractility (Török and Gerová

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1996) and decreased vascular relaxation (Holecyová *et al.* 1996) accompanied by increase of blood pressure (Pecháňová and Bernátová 1996) as well as by enhancement of vascular and myocardial proteosynthesis (Babál *et al.* 1997, Gerová *et al.* 1998) underlying vascular (Kristek and Gerová 1996, Kristek *et al.* 1996, Bernátová *et al.* 1999) and myocardial remodeling (Pecháňová *et al.* 1997). The latter was characterized particularly by left ventricular hypertrophy and profound interstitial fibrosis. All these changes might account for the decreased cardiac output (Amrani *et al.* 1992). While searching for the mechanisms involved in the development of this type of hypertension, it has been found that besides restriction of NO production, activation of the sympathetic nervous and renin-angiotensin system can play a certain role (Rees *et al.* 1989, Jover *et al.* 1993, Sander *et al.* 1995, Pecháňová *et al.* 1997, 1999). However, the kallikrein-kinin and renin-aldosteron systems cannot also be excluded (Weber and Brilla 1991, Sigush *et al.* 1996).

When the above mentioned data are taken into consideration together with some controversial findings concerning cardiac hypertrophy (Arnal *et al.* 1993) and possible factors involved in myocardial remodeling (Weber and Brilla 1991, Moreno *et al.* 1995), it is evident that additional studies are still needed. We focused, therefore, on the *in situ* detection of the qualitative alterations in the heart of NO-deficient rat, i.e. the histochemical demonstration of selected enzymes activities as well as the subcellular myocardial changes. We also assessed whether these qualitative alterations could be affected by the ACE inhibitor captopril.

Material and Methods

Male adult Wistar rats were divided into three groups. The first group consisted of animals treated by L-NAME in a the daily dose of 40 mg/kg in drinking water for 4 weeks. The second group simultaneously received L-NAME (40 mg/kg) plus 100 mg/kg captopril, both in drinking water for the same period. The third group comprised age-matched control animals. Systolic blood pressure and heart rate were measured by tail-cuff plethysmography. After 4 weeks, the animals were killed by cervical translocation, the heart was quickly excised and the left ventricle was weighed for calculating the left ventricle-to- body weight ratio (LVW/BW).

For histochemical examination, the left ventricle was immediately frozen in liquid nitrogen followed by cutting into 10 μm thick cryostat sections. NO synthase activity was demonstrated by the NADPH diaphorase reaction (Klimaschewski *et al.* 1992). Oxidative and glycolytic metabolism-related alterations were monitored by estimating the activity of succinic and β-hydroxybutyrate dehydrogenases. The capillary network changes were monitored by assessing the activities of alkaline phosphatase (arterial part), dipeptidyl peptidase IV (venular part) and by 5'-nucleotidase (both parts). Furthermore, myocardial activities of membrane and myofibrillar ATPases were detected. Histochemical reactions were performed on cryostat sections according Lojda *et al.* (1976).

For electron microscopic examination, small blocks of ventricular tissue were fixed by immersion in 2.5 % glutaraldehyde and buffered with 0.1 M sodium cacodylate for 3 h. After postfixation in buffered 1 % osmium tetroxide for 1 h, the tissue was washed in a cacodylate buffer followed by dehydration in series of ethanol, infiltration with propylenoxide and embedding in Epon 812. Semithick sections stained with methylene blue were examined in the light microscope and appropriate areas were chosen for preparing of the ultrathin sections. These were stained with lead phosphate and uranyl acetate and examined in the electron microscope Tesla BS 500.

Results

General changes

At the end of the first week, a significant increase of systolic blood pressure by 30 % and a decrease of heart rate by 23 % were detected in the rats administered 40 mg/kg/day L-NAME compared to age-matched controls. These changes persisted during the following three weeks. In the L-NAME plus captopril group, significant suppression of hypertension was detected. The LVW/BW ratio was significantly increased by 29 % in the L-NAME group compared to the controls, whereas it did not change significantly in the L-NAME plus captopril group vs. control. The difference between L-NAME and L-NAME plus captoril was significant (for further details see Pecháňová *et al.* 1997).

Histochemical changes

Endothelial NADPH diaphorase/nitric oxide synthase activity was apparently decreased in the coronary arteries, capillary network and cardiomyocytes of L-NAME treated animals (Figs 1A and 1B). The

activities of succinic and β-hydroxybutyrate dehydrogenases as well as glycogen phosphorylase were locally markedly decreased and absent in the fibrotic areas (Figs 2A and 2B). The activity of dipeptidyl peptidase IV, a marker of the venous part of capillaries, was almost unchanged, and differed from that of alkaline phosphatase, a marker of the arterial part of the capillary bed, which was locally diminished. The activities of both enzymes were strongly enhanced in the foci of fibrotization (Figs 3A and 3B). The reaction for

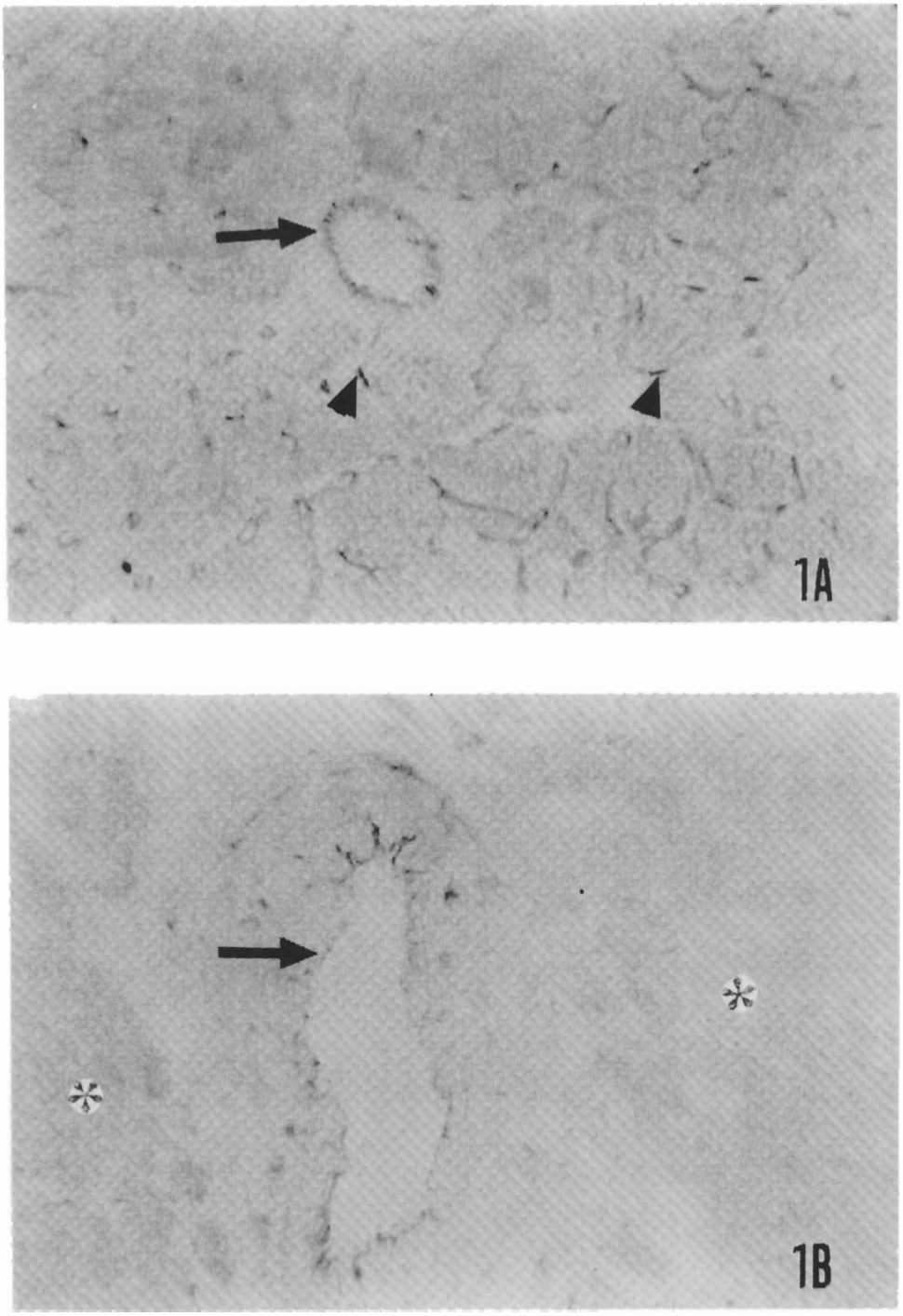


Fig. 1. *Histochemical demonstration of NO synthase activity (coupled to NADPH diaphorase) in the myocardium. In the control* ***[A]****, the activity was shown in endothelial cells of the artery and capillaries (arrow) and in the cardiomyocytes (arrow head). After chronic L-NAME administration* ***[B]****, the activity was apparently decreased in the endothelial cells as well as in the cardiomyocytes (asterisk). Magnification 80x*

5'-nucleotidase was locally decreased. Almost no enzyme activity examined was found in the scar. Myofibrilar and membrane Ca^{2+}-dependent and Mg^{2+}-dependent ATPases were focally slightly decreased and absent in the areas of replacement fibrosis. The same feature of histochemical changes was observed in the heart of the L-NAME plus captopril group.

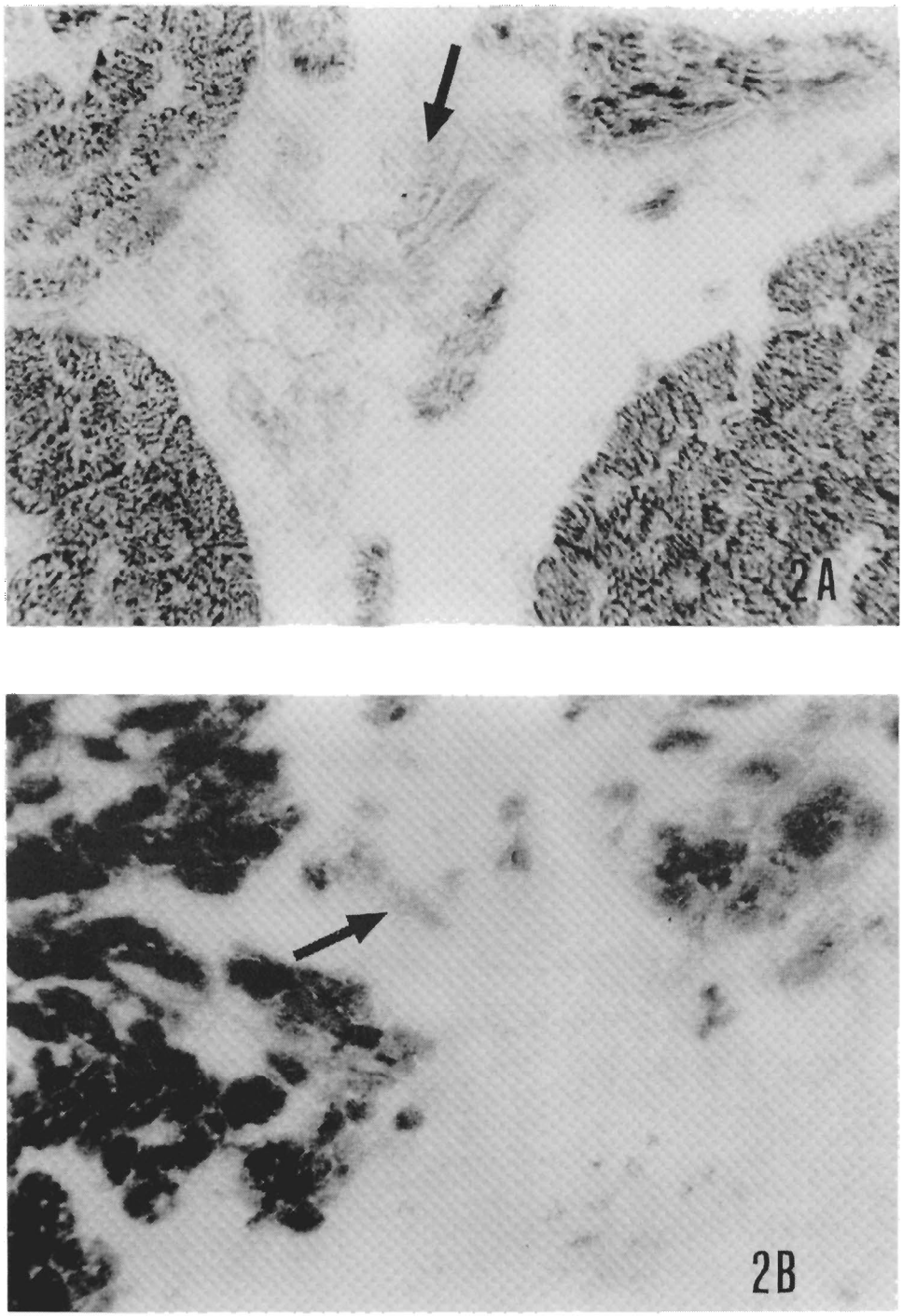


Fig. 2. *The activities of succinic dehydrogenase* ***[A]*** *and glycogen phosphorylase* ***[B]*** *in the heart of L-NAME treated rats are markedly decreased or absent in group cardiomyocytes (arrows) in comparison with basal activities of surrounding tissue. Magnification 80x*

Ultrastructural alterations

In the heart of L-NAME treated rats, the activation of fibroblasts associated with profound interstitial and perivascular fibrosis was found (Fig. 4A). Moreover, apparent thickening of the external lamina of the sarcolemma as well as the basal membrane of capillaries were observed. Injured capillaries and extravasation were found (Fig. 4B) but, on the other hand, proliferation and migration of endothelial cells and neoformation of capillaries were frequent (Fig. 4C).

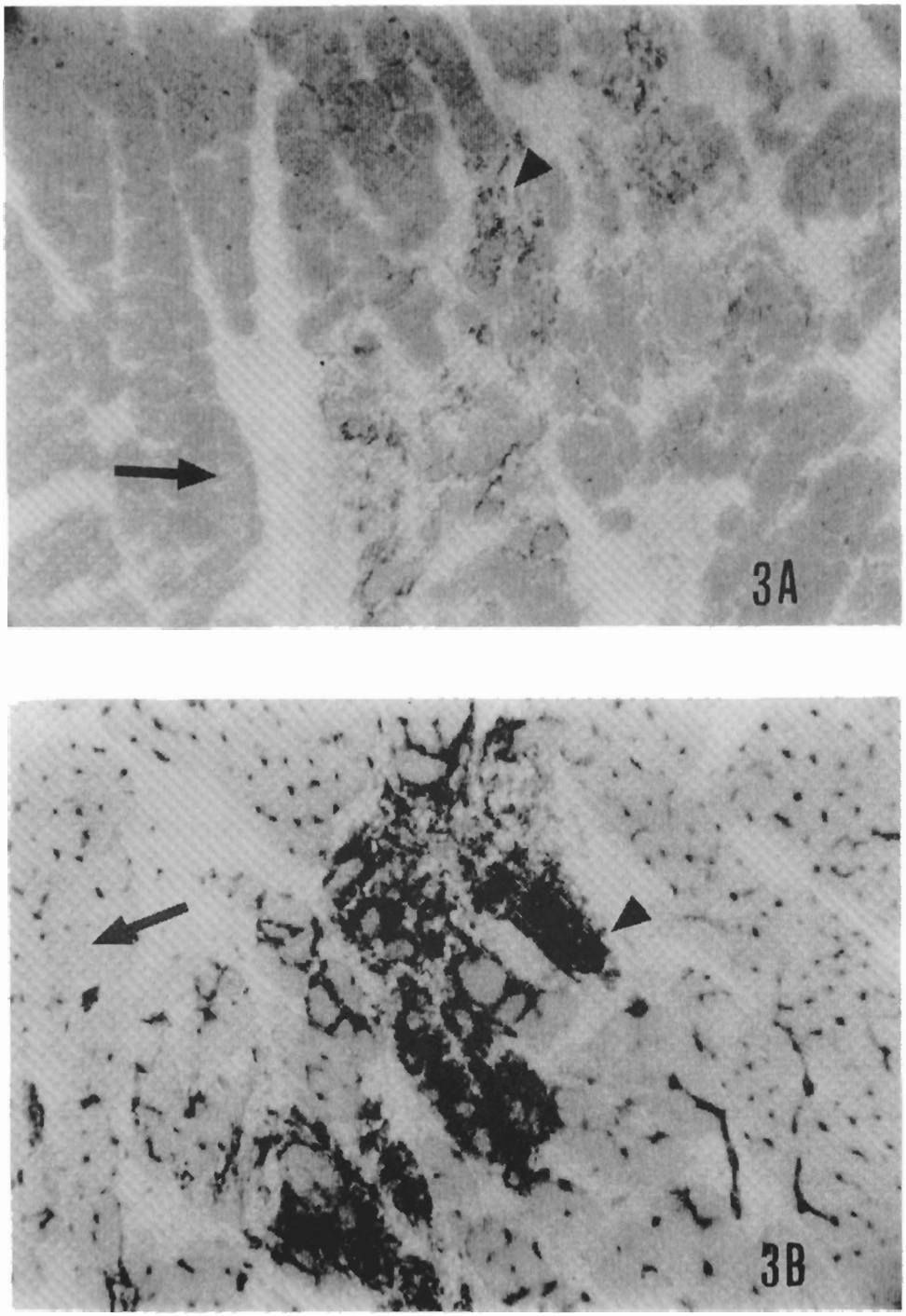
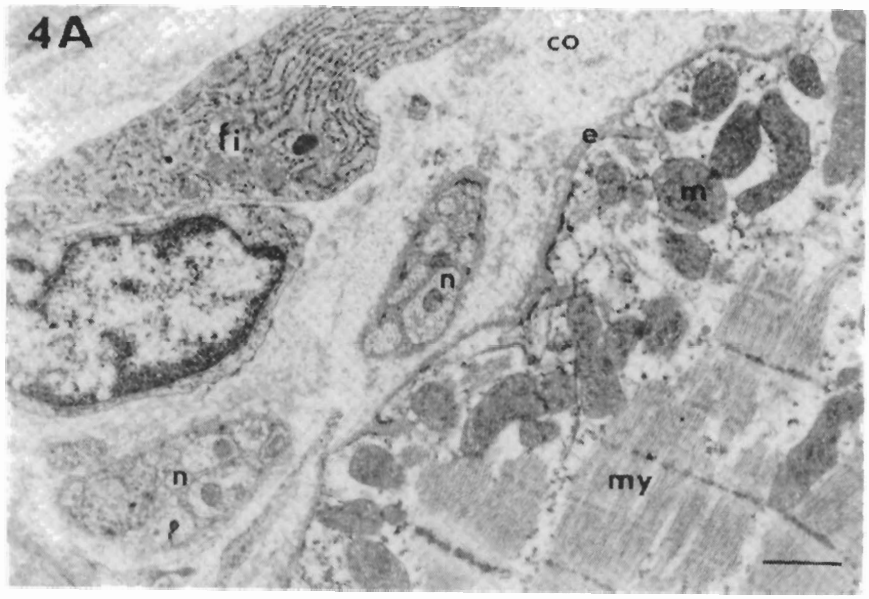
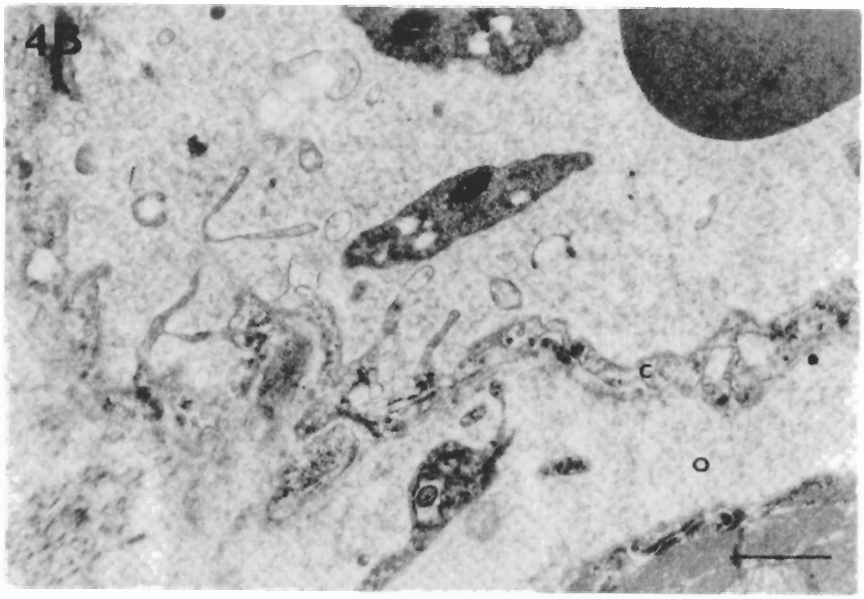


Fig. 3. *The reaction for dipeptidyl peptidase IV activity* ***[A]*** *and alkaline phosphatase activity* ***[B]*** *is slightly decreased in some capillaries (arrows), but markedly increased in the area of replacement fibrosis (arrow heads). Majority of capillaries revealed basal activity similar to that in the control tissue. Magnification 80x*





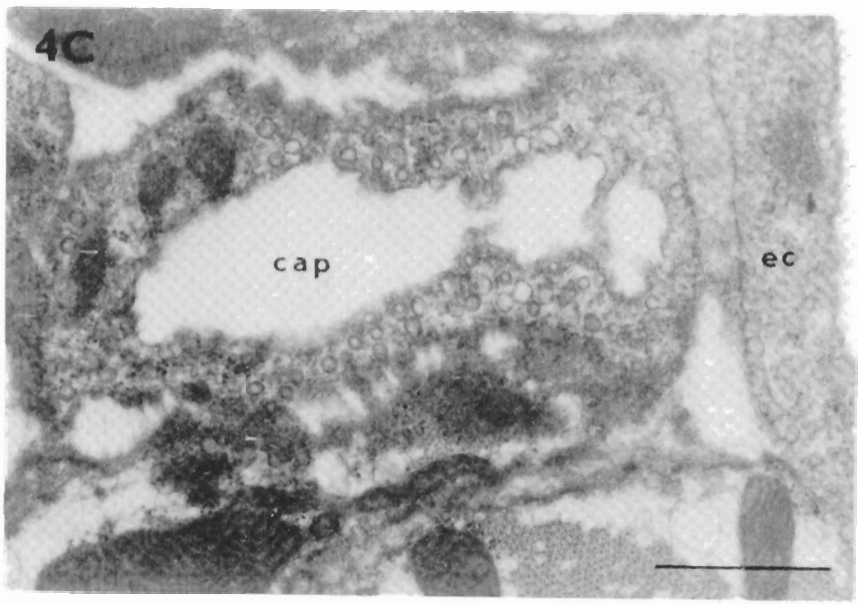


Fig. 4. ***[A]*** *Ultrastructural picture of the myocardial tissue of L-NAME treated rats shows the activation of fibroblasts (fi) associated with overproduction of collagen (co) and thickening of external lamina of sarcolemma (e). n – nerve profiles, my – myofibrils, mitochondria.* ***[B]*** *Impairment of the capillary (c) resulting in extravasation and extracellular edema (o). r – red blood cell.* ***[C]*** *Proliferation of the endothelial cells (ec) associated with neoformation of capillaries (cap), bar 1 μm.*

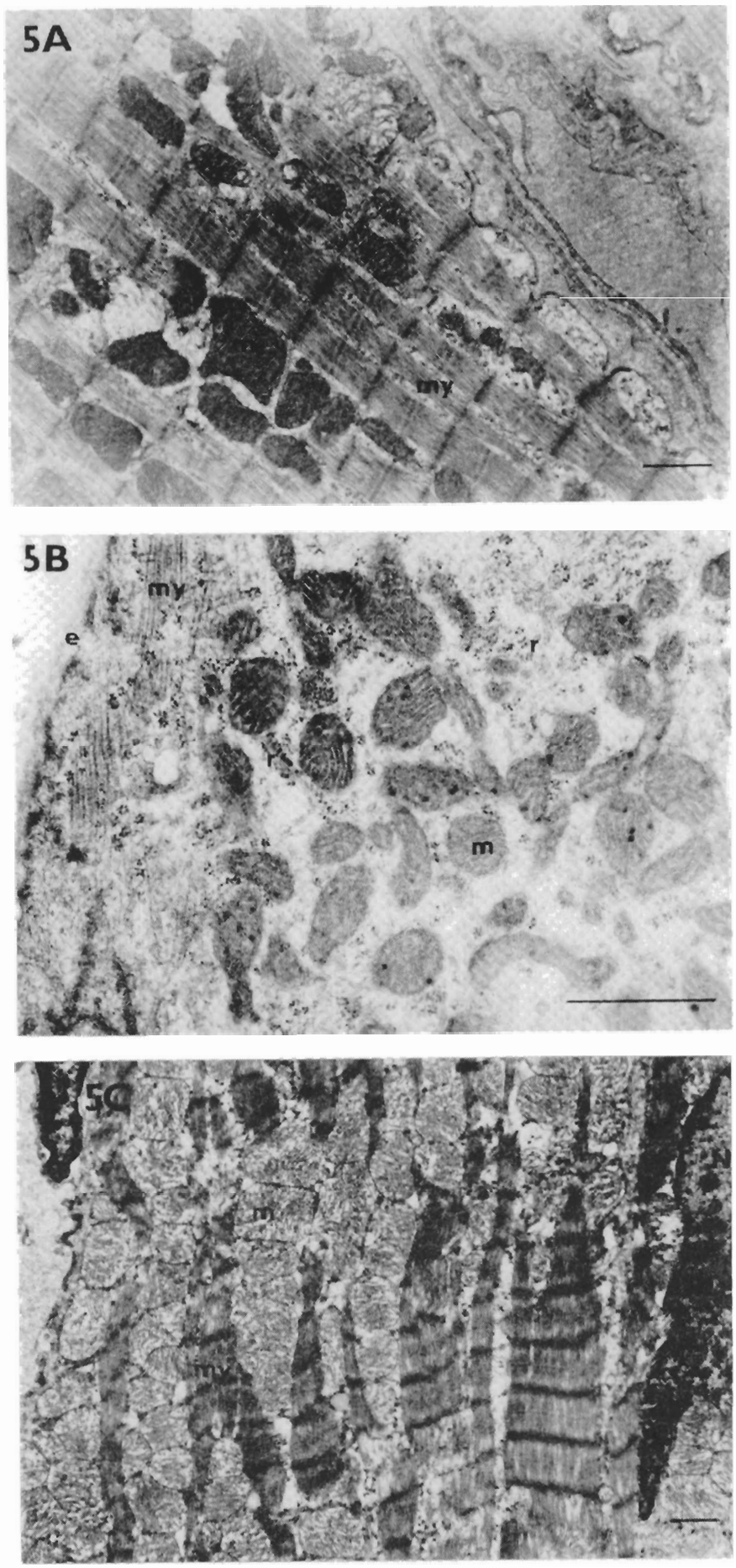


Fig. 5. ***[A]*** *Almost normal cardiomyocyte with electrondense mitochondria of regular size (m) and conventional pattern of myofibrils (my).* ***[B]*** *cardiomyocyte showing markers of hypertrophy: numerous mitochondria of various size (m), numerous polyribosomes (r), neoformation of myofilaments (my). Thickening of external lamina of the sarcolemma is apparent (e).* ***[C]*** *injured cardiomyocyte with edematous mitochondria (m), pycnotic nucleus (N) and degradation of myofibrils (my), bar 1 μm.*

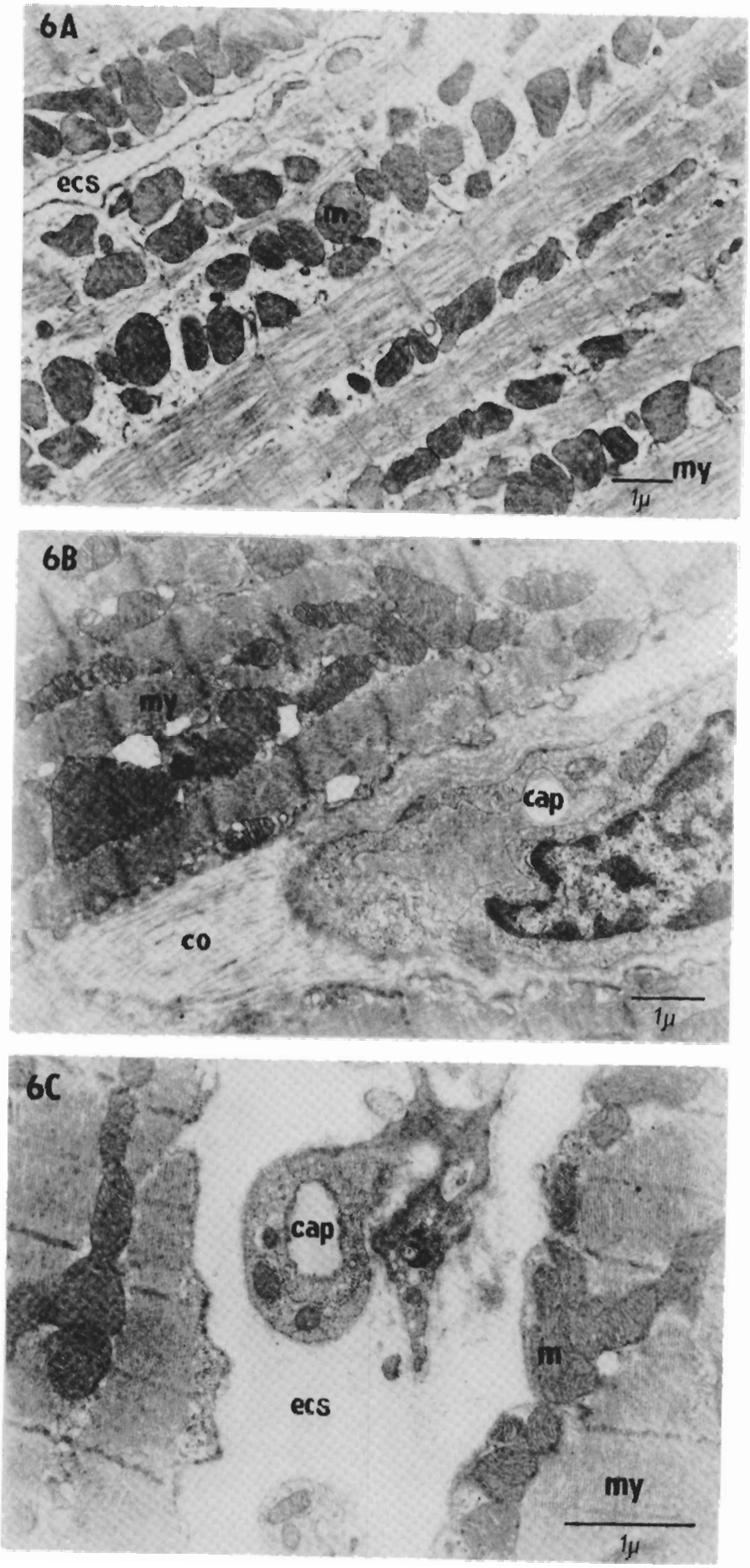


Fig. 6. ***[A]*** *Ultrastructure of an age-matched control heart with conventional architecture of the cardiomyocytes. m – mitochondria, my – myofibrils, ecs – extracellular space, bar 1 μm,* ***[B]*** *In the myocardium from the L-NAME plus captopril-treated rat perivascular fibrosis (co) still persists together with mild alterations of mitochondria (m). my – myofibrils, bar 1 μm,* ***[C]*** *Captopril treatment did not prevent the process of L-NAME-induced angiogenesis accompanied by endothelial proliferation and neoformation of the capillaries (cap). Widening of the extracellular space (ecs) can still be observed. m – mitochondria, my – myofibrils, bar 1 μm.*

Furthermore, besides almost normal cardiomyocytes with a conventional architecture (Fig. 5A), the majority of cardiomyocytes exhibited either subcellular markers of hypertrophy (Fig. 5B) and/or the ischemia-like injury (Fig. 5C). The former was characterized by a high number of electron-dense mitochondria of various sizes located not only between myofibrils, but frequently subsarcolemmally, and by enhanced amounts of ribosomes and rough sarcoplasmic reticulum as well as by neoformation of myofilaments. The latter were characterized by numerous edematous mitochondria, by pycnotic nuclei and by the disappearance of myofibrils. The atrial cardiomyocytes exhibited increased numbers of specific atrial granules and their translocation into the sarcolemma.

In comparison to the control hearts (Fig. 6A), the L-NAME induced ultrastructural alterations persisted even during captopril treatment. Both interstitial fibrosis (Fig. 6B) as well as a widening of extracellular space and angiogenesis were observed (Fig. 6C).

Discussion

The results of this study showed that NO-deficient hypertension is accompanied by apparent enzyme histochemical changes, which were heterogenously distributed within the myocardium and correlated with subcellular alterations, which were also nonuniform. Similar heterogeneity was observed in other experimental models of myocardial and endothelial dysfunction, such as diabetic cardiomyopathy or isoproterenol-induced injury (Tribulová *et al.* 1996, Slezák and Tribulová 1975). This points out that physiological heterogeneity of the heart is much more pronounced during pathophysiological conditions and that myocardial cells differ in their response to various stress factors or stimuli. The pathophysiological heterogeneity of the metabolic and structural alterations is linked with the electrical heterogeneity of the myocardium, thus creating an arrythmogenic substrate (Peters *et al.* 1997, Tribulová *et al.* 1998) as well as disturbances in the excitation-contraction process resulting in cardiac dysfunction and failure. In agreement with this, our later results have shown a higher vulnerability of the NO-deficient heart to ventricular fibrillations (Tribulová *et al.* 1999).

The lower activity of NO synthase, coupled with the NADPH diaphorase reaction (Klimaschewski *et al.* 1992), correlated with the biochemical findings (Pecháňová and Bernátová 1996, Bernátová *et al.* 1996). This was associated with capillary dysfunction demonstrated by both decreased alkaline phosphatase activity and by subcellular endothelial injury with consequent increased permeability and protein extravasation. The latter was even observed during intravenous injection of L-NAME (Filep *et al.* 1993). This defect can contribute to extracellular edema. Furthermore, the inhibition of NO synthase (resulting in a deficient NO production) induced marked proliferation of endothelial cells and neoformation of capillaries. These findings are in contradiction with the studies of Ziche *at al.* (1994) and Morbidelli *et al.* (1996), who suggested NO as a trigger for angiogenesis, but in agreement with Kaur *et al.* (1998) and Pipili-Synetos *et al.* (1993), who suggested NO as an antiangiogenic mediator. The decrease of NO synthase activity and angiogenesis were present even in the L-NAME plus captopril group, although the impairment of capillaries and extracellular edema were less pronounced. It seems that suppression of blood pressure by captopril is associated with an attenuation of the changes in permeability of the vessels and capillaries leading to a decrease of extracellular edema. This can contribute to the decrease of LVW/BW ratio observed in this group.

Non-specific alkaline phosphatase, which is known to be located in cell membranes, is characteristic for active transport processes (Lojda *et al.* 1976) and its activity was high in non-injured capillaries with proliferative activity and also in areas of replacement fibrosis with the intensive production of extracellular matrix compounds. In addition, these areas possessed a high activity of dipeptidyl peptidase IV, a protease known to be involved in the degradation of some vasoactive peptides as well as of collagen metabolism.

Accordingly, in L-NAME treated rats the apparent enhancement of perivascular/pericapillary and interstitial production of extracellular collagenous material was induced. Co-administration of captopril did not prevent the process of fibrotization, but diminished collagen production cannot be excluded, as it was found by quantitative evaluation of hydroxyproline (Pecháňová *et al.* 1997).

Decreased activities of mitochondrial succinic dehydrogenase, β-hydroxybutyrate dehydrogenase and cytoplasmic glycogen phosphorylase clearly indicate a breakdown of energetic metabolism in markedly affected cardiomyocytes, exhibiting severe subcellular ischemia-like injury. Locally decreased myofibrilar and

sarcolemmal ATPases activities might be associated with subcellular alterations of these structures in injured cardiomyocytes. Moreover, it can reflect a decreased function of Na,K-ATPase in this model (Vrbjar *et al.* 1998). These changes fit in very well with metabolic and structural alterations associated with the arterial hypertension and the compensated hypertrophy of different etiology (Torii *et al.* 1990, Ito *et al.* 1992)

Cardiomyocytes with high proteosynthetic activity associated with the hypertrophy, i.e. myofilament growth and intercalated disc formation, were characterized by high amounts of mitochondria and corresponding high activities of energy producing enzymes. Atrial cardiomyocytes exhibited an increased number of ANF-related granules and their transclocation to the sarcolemma indicating enhanced ANF release. It was reported that raised blood pressure stimulated ANF production and secretion in spontaneously hypertensive rats (Yokota *et al.* 1993).

In conclusion, the results of this study indicate that long-term inhibition of NO production is associated with cardiovascular metabolic alterations and the induction of extracellular matrix synthesis, cardiomyocyte hypertrophy and angiogenesis. These changes were only partially prevented by ACE inhibition. This suggests that most likely additional factors, e.g. bradykinin, which also regulate fibrous tissue formation, might be involved in NO deficiency-induced myocardial alterations.

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References

AMRANI M, ONSHEA J, ALLEN NJ, HARDING SE, JAYAKUMAR J, PEPPER JR, MONCADA S, YACOUB MH: Role of basal release of nitric oxide on coronary flow and mechanical performance of the isolated rat heart. *J Physiol Lond* **456:** 681-687, 1992.

ARNAL JF, EL AMRANI AI, CHATELLIER G, MENARD F, MICHEL JB: Cardiac weight in hypertension induced by nitric oxide synthase blockade. *Hypertension* **22:** 380-387, 1993.

BÁBAL P, PECHÁŇOVÁ O, BERNÁTOVÁ I, ŠTVRTINA S: Chronic inhibition of NO synthesis produces myocardial fibrosis and arterial media hyperplasia. *Histol Histopathol* **12:** 623-629, 1997.

BERNÁTOVÁ I, PECHÁŇOVÁ O, ŠIMKO F: Captopril prevents NO-deficient hypertension and left ventricular hypertrophy without affecting nitric oxide synthase activity in rats. *Physiol Res* **45:** 311-316, 1996.

BERNÁTOVÁ I, PECHÁŇOVÁ O, KRISTEK F: Mechanism of structural remodeling of the rat aorta during long-term L-NAME treatment. *Jpn J Pharmacol* **81:** 99-106, 1999.

BING OL, BROOKS WW, ROBINSON KG, SAWSKY MT, HAYES JA, LITWIN SE, SEN S, CONRAD CHH: The spontaneously hypertensive rat as a model of the transition from compensated left ventricular hypertrophy to failure. *J Mol Cell Cardiol* **27:** 383-396, 1995.

FILEP JG, FOLDES-FILEP E, SIROIS P: Nitric oxide modulates vascular permeability in the rat coronary circulation. *Br J Pharmacol* **108:** 323-326, 1993.

GEROVÁ M, PECHÁŇOVÁ O, BERNÁTOVÁ I, KITTOVÁ M, JURÁNI M: Early changes of protein synthesis in myocardium and coronary arteries by NO synthase inhibition. *Physiol Res* **47:** 405-412, 1998.

GRIENDLING KK. MURPHY TJ, ALEXANDER RW: Molecular biology of the renin-angiotensin system. *Circulation* **87:** 1824-1828, 1993.

HOJO Y, EBATA H, IKEDA U, TSURUYA Y, NATSUME T, SHIMADA K: Enhanced spontaneous calcium efflux and decrease of calcium-dependent calcium release from the isolated perfused heart of spontaneously hypertensive rats. *J Hypertens* **10:** 513-520, 1992.

HOLÉCYOVÁ A, TOROK J, BERNÁTOVÁ I, PECHÁŇOVÁ O: Restriction of nitric oxide rather than elevated blood pressure is responsible for alterations of vascular responses in nitric oxide-deficient hypertension. *Physiol Res* **45:** 317-321, 1996.

ITO H, TORII M, SUZUKI T: Myocardial vulnerability in cardiac hypertrophy: comparison of hypertensive and normotensive rat drug-induced myocardial changes. *Genetic Hypertension* **218:** 257-259, 1992.

JOVER BA, HERITZI A, VENTRE F, DUPONT M, MIMRAN A: Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. *Hypertension* **21:** 944-948, 1993.

KAUR K, YAO J, PAN X, MATTHEWS C, HASSID A: NO decreases phosphorylation of focal adhesion proteins via reduction of Ca in rat aortic smooth muscle cells. *Am J Physiol* **274:** H1613-H1619, 1998.

KLIMASCHEWSKI L, KUMMER W, MAYER B, COURAUD JY PREISSLER U, PHILIPPIN B, HEYM CH: Nitric oxide synthase in cardiac nerve fibers and neurons of rat and guinea pig heart. *Circ Res* **71:** 1533-1537, 1992.

KRISTEK F, GEROVÁ M: Long-term NO synthase inhibition affects heart weight and geometry of coronary and carotid arteries. *Physiol Res* **45:** 361-367, 1996.

KRISTEK F, GEROVÁ M, DEVÁT L, VARGA I: Remodeling of septal branch of coronary artery and carotid artery in L-NAME treated rats. *Physiol Res* **45**: 329-333, 1996

LOJDA Z, GOSSRAU R, SCHIEBLER TH: *Enzym-histochemische Methoden*. Springer, Berlin, 1976.

MORBIDELLI L, CHANG CH, DOUGLAS JG, GRANGER HJ, LEDDA F, ZICHE M: Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. *Am J Physiol* **270:** H411-H415, 1996.

MORENO H JR, PIOVERSAN NATHAN L, PEREIRA COSTA SK, METZE K, ANTUNES E, ZATZ R, DE NUCCI G: Enalapril does not prevent the myocardial ischemia caused by the chronic inhibition of nitric oxide synthesis. *Eur J Pharmacol* **287:** 93-96, 1995.

PECHÁŇOVÁ O, BERNÁTOVÁ I: Effect of long-term NO synthase inhibition on cyclic nucleotide content in rat tissue. *Physiol Res* **45**: 305-309, 1996.

PECHÁŇOVÁ O, BERNÁTOVÁ I, PELOUCH V, ŠIMKO F: Protein remodelling of the heart in NO-deficient hypertension: the effect of captopril. *J Mol Cell Cardiol* **29:** 3365-3374, 1997.

PECHÁŇOVÁ O, BERNÁTOVÁ I, PELOUCH V, BABÁL P: L-NAME-induced protein remodeling and fibrosis in the rat heart. *Physiol Res* **48:** 353-362, 1999.

PETERS NS, COROMILAS J, SEVERS NS, WIT AL: Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. *Circulation* **95:** 988-996, 1997.

PIPILI-SYNETOS E, SAKKOULA G, HARALABOPOULOS P, ANDIOPUOLOU P, PERISTERIS P, MARAGOUDAKIS ME: Evidence that nitric oxide is an endogenous antiangionenic mediator. *Br J Pharmacol* **111:** 894-902, 1993.

REES DD, PALMER RMJ, MONCADA S: Effect of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* **86:** 3375-3378, 1989.

RIBEIRO MO, ANTUNES E, DE NUCCI G, LOVISOLO SM, ZATZ R: Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* **20:** 298-303, 1992.

SANDER M, HANSEN PG, VICTOR RG: Sympathetically mediated hypertension caused by chronic inhibition of nitric oxide. *Hypertension* **20:** 691-695, 1995.

SIGUSCH HH, CAMPBELL SE, WEBER KT: Angiotensin II-induced myocardial fibrosis in rats: role of nitric oxide, prostaglandins and bradykinin. *Cardiovasc Res* **32:** 546-554, 1996.

ŠIMKO F, ŠIMKO J: Heart failure and angiotensin converting enzyme inhibition: problems and perspectives. *Physiol Res* **48**: 1-8, 1999.

SLEZÁK J, TRIBULOVÁ N: Morphological changes after simulatenous administration of isoproterenol and K,Mg-aspartate as a physiological Ca^{2+} antagonist. *Arzneimittelforsch* **25:** 918-923, 1975.

TORII M, ITO H: Some enzyme characteristics of spontaneously hypertensive rats myocardium. *Jpn Circ J* **54:** 688-694, 1990.

TÖRÖK J, GEROVÁ M: Vascular responses after long-term inhibition of nitric oxide synthesis in newborn dogs. *Physiol Res* **45:** 323-328, 1996.

TRIBULOVÁ N, RAVINGEROVÁ T, VOLKOVOVÁ K, ZIEGELHOFFER A, OKRUHLICOVÁ L, ZIEGELHOFFER B, STYK J, SLEZÁK J: Resistance of diabetic rat hearts to Ca overload-related injury. Histochemical and ultrastructural study. *Diabetes Res Clin Pract* **31:** 113-122, 1996.

TRIBULOVÁ N, STYK J, SOSNER I, VARON D, OKRUHLICOVÁ L, SLEZÁK J, MANOACH M: Attenuation of Ca overload by class III antiarrhytmic drugs. *Exp Clin Cardiol* **4:** 64-72, 1998.

TRIBULOVÁ N, MANOACH M, OKRUHLICOVÁ L, ŠTETKA R, PANCZA D, BERNÁTOVÁ I, PECHÁŇOVÁ O: Increased sensitivity of NO-deficient rat heart to low K^+ induced ventricular fibrillation. *Physiol Res* **48:** 37P, 1999.

VRBJAR N, DZURBA A, BERNATOVÁ I, PECHÁŇOVÁ O: NO-deficient hypertension causes inhibition of the function of Na,K-ATPase in myocardium by decreasing of its sodium binding site. *Cardiol*ogy **7:** 37-40, 1998.

WEBER KT, BRILLA CG: Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation* **83:** 1849-1865, 1991.

YOKOTA N, YAMAMOTO Y, KITAMURA K, KANGAWA K, MINAMINO N, MATSUO H, ETO T: Alterations in circulating and cardiac tissue concentrations of brain natriuretic peptide in spontaneously hypertensive rats. *Cardiovasc Res* **27:** 1312-1315, 1993.

ZICHE M, MORBIDELLI L, MASINI E, AMERINI S, GRANGER HJ, MAGGI CA, GEPPETTI, LEDDA F: Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. *J Clin Invest* **94:** 2036-2044, 1994.

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