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Long-Term NO Synthase Inhibition Affects Heart Weight and Geometry of Coronary and Carotid Arteries

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Summary

The heart weight and the structure of coronary and carotid arteries were studied in NO-deficient hypertension. Wistar rats were administered L-NAME (50 mg/kg/day) in drinking water for a period of 8 weeks. The blood pressure and heart rate were recorded weekly. In one group of control and experimental animals the heart weight was assessed and the heart/body weight ratio (relative heart weight) was calculated. In the other group of control and experimental animals, the cardiovascular system was perfused by a fixative under constant perfusion pressure. The inner circumference and the wall thickness (tunica intima and tunica media) of the coronary (septal branch) and carotid artery were measured using light microscopy and the wall/diameter ratio was calculated. Inhibition of NO synthase induced a significant increase in blood pressure (187.2±4.2 mm Hg compared to 131.4±1.9 mm Hg in the controls, $p<0.01$). The heart rate decreased (334.4±7.0 beats/min compared to 352.6±4.1 beats/min in the controls, $p<0.05$). The heart weight increased in NO-deficient rats (1.32±0.08 g versus 1.10±0.03 g, $p<0.05$); the heart/body weight index increased remarkably (3.09±0.15 compared to 2.10±0.04 in the controls, $p<0.01$). Morphometry of the septal branch of the left coronary artery indicated a decrease of the inner circumference (664±24 μm versus 832±30 μm, $p<0.01$), the increased wall thickness (21.15±0.84 μm compared to 12.47±0.62 μm in the controls, $p<0.01$) and the remarkably changed wall/diameter ratio (1:10 versus 1:21 in the controls). Similar alterations were found in the carotid arteries: the inner circumference was decreased (2456±39 μm versus 2732±66 μm, $p<0.01$), the wall thickness increased (45.14±0.41 μm compared to 26.08±1.23 μm, $p<0.01$) and the wall/diameter ratio was changed to 1:17 in comparison with 1:33 in the controls. In conclusion, cardiac hypertrophy and structural alterations of the coronary artery and carotid artery accompany NO-deficient hypertension.

Key words
Nitric oxide – Cardiac hypertrophy – Artery – Remodelling

Introduction

Soon after Rees *et al.* (1990) had demonstrated that the acute blockade of NO synthase increased blood pressure in anaesthetized animals, several authors described sustained hypertension after long-term inhibition of NO synthase in conscious animals (Baylis *et al.* 1992, Ribeiro *et al.* 1992, Jover *et al.* 1993, Pollock *et al.* 1993, Gerová *et al.* 1995). Dananberg *et al.* (1993) suggested the term "NO-deficient hypertension" for the above experimental model. Studies of the new model of hypertension revealed some unexpected features. Arnal *et al.* (1993) demonstrated that hypertension caused by long-lasting NO synthase inhibition was not accompanied by cardiac hypertrophy in the majority of experimental animals. Their results were confirmed by Bernátová and Pecháňová (1995). These findings contrasted with the unequivocal cardiac hypertrophy in renal hypertension.

As far as vascular smooth muscle is concerned, Garg and Hassid (1989) and Nakaki *et al.* (1990) demonstrated that NO inhibited mitogenesis and proliferation. Thus the blockade of the NO producing process, followed by an increase of blood pressure, could induce remodelling of the vessel wall. Surprisingly, in rats with NO-deficient hypertension, Dunn and Wilson (1993) did not find any changes in either conduit arteries or resistant mesenteric vessels, which are responsible for increased blood pressure. On the other hand, Morton *et al.* (1993) found structural changes in the mesenteric vascular bed after 4 weeks of NO synthase blockade. Recently, Delacretaz *et al.* (1994) described an increase in the intima-media thickness of the rat carotid artery without changes in the inner diameter. They hypothesized the development of very early vascular hypertrophy.

The aim of this study was to find whether a long-lasting load of the cardiovascular system, induced by hypertension due to inhibition of NO synthase, results in remodelling of the cardiovascular system. As a first approach, we studied the weight of the heart and the geometry of the coronary and carotid artery, i. e. vessels supposed to be most frequently affected by high blood pressure.

Methods

Wistar male rats were taken for the study. Two groups, each containing 14 animals, were used. At the beginning of the experiment the animals were 10 weeks old. Control animals were given tap drinking water. The experimental animals were given L-nitro-arginine methyl ester (L-NAME, 50 mg/kg b.w./day) into the drinking water for 8 weeks. Systolic blood pressure was measured in both groups by the tail plethysmographic method and registered on a BP Recorder 8005 W+W Electronic each week.

At the end of the experiment, the animals were sacrificed by an overdose of Pentobarbital (100 mg/kg b.w., i.p.). In seven control rats and in the same number of animals of the experimental group the chest was opened, the heart excised and weighed. In other seven controls and seven experimental animals the chest was opened and the cardiovascular system was perfused under constant perfusion pressure of 120 mm Hg for 10 min *via* a cannula placed in the left ventricle. As a fixative, 3 % glutaraldehyde in 0.1 M phosphate buffer was used. After 10 min of perfusion, the middle part of the common carotid artery and the upper half of the septal branch of the left coronary artery were excised. The septal branch was used because its upper part runs on the surface of the myocardium, is well defined and easily accessible. Both arteries were divided into three segments (1 mm long) and postfixed with 2 % osmium tetroxide. After double fixation, the specimens were stained en block with 2 % uranyl acetate, dehydrated through ascending concentrations of alcohol and embedded in Durcupan ACM. Two randomly selected blocks of each artery were cut perpendicularly to the long axis. Two representative areas of each block approximately 0.5 μm thick were sectioned and stained by methylene blue solution. Both the inner circumference and thickness of the arterial wall (tunica intima and tunica media) of the respective vessel were measured in the light microscope. Arterial wall thickness was measured at about 45° intervals around the vessel circumference. Thus, the average of 4 measurements of the inner circumference and 32 measurements of arterial wall thickness from each animal were taken as representative of each parameter. From the data measured, the inner diameter and the arterial wall area (tunica intima and tunica media) were calculated. The parameters studied were expressed as means ± S.E.M. ANOVA, Bonferroni test and Student's t-test for unpaired variables were used for statistical evaluation. Values were considered to be significant at $p<0.05$.

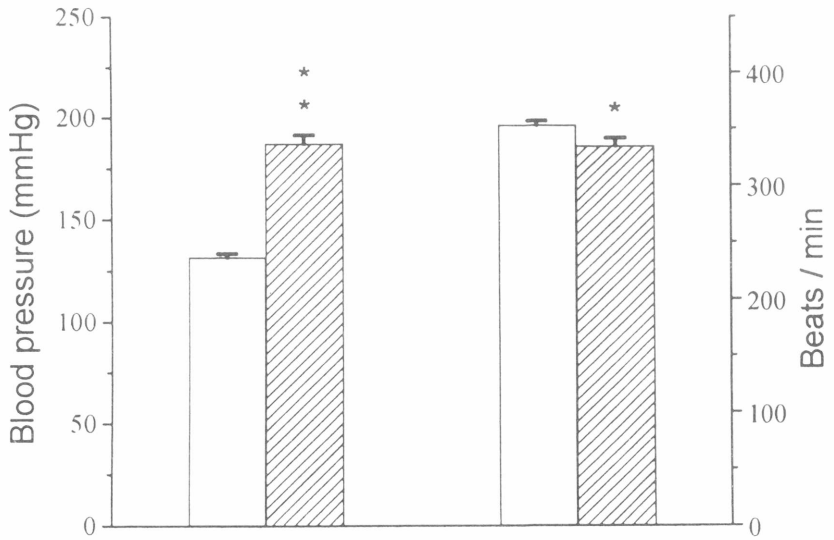

Fig. 1
*Blood pressure and heart rate in control rats (open columns) and L-NAME treated rats (hatched columns). * $P<0.05$; ** $P<0.01$.*

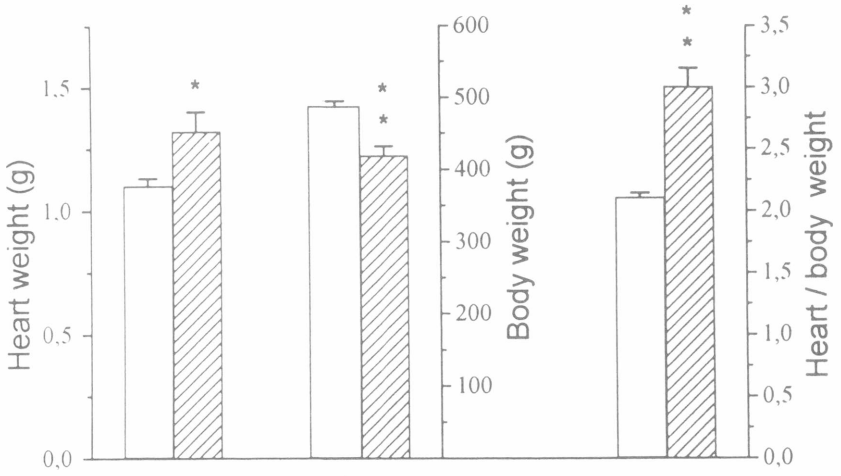

Fig. 2
*Heart weight, body weight and heart/body weight ratio in control rats (open columns) and L-NAME treated rats (hatched columns). * $P<0.05$; ** $P<0.01$.*

Results

In rats treated with L-NAME for a period of eight weeks the average systolic blood pressure was 187.3±4.2 mm Hg and in age-matched control rats 132.5±1.2 mm Hg ($p<0.01$). The heart rate was 334.4±7.0 beats/min in experimental animals and 352.6±4.1 beats/min in the controls ($p<0.05$) (Fig. 1). The body weight of experimental animals was significantly lower than in the controls (418.6±13.3 g versus 487.1±7.8 g, $p<0.01$) (Fig. 2).

Contrary to the body weight, the heart weight in experimental animals increased to 1.32±0.08 g, while in control animals it was 1.10±0.03 g ($p<0.05$). The relative heart weight (heart/body weight index) increased significantly from 2.10±0.04 to 3.09±0.15, $p<0.01$ (Fig. 2).

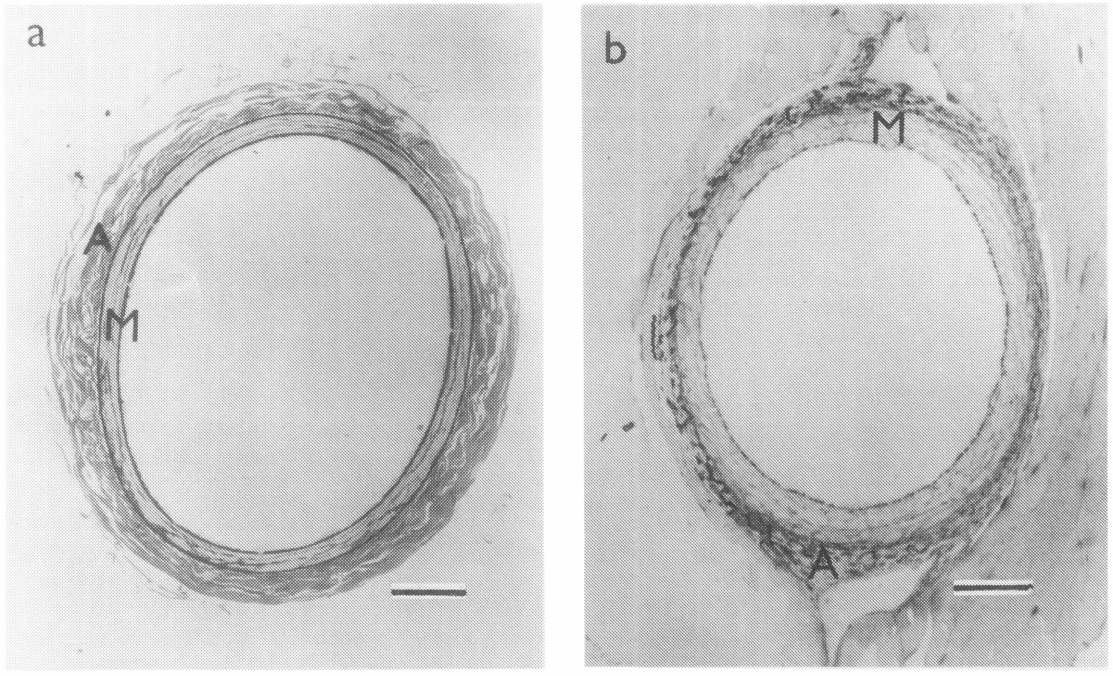

Fig. 3
Cross-section of the septal branch of the left coronary artery. a – artery from control rat, b – artery from L-NAME treated rat. Tunica media (M), tunica adventitia (A). Bar 50 μm.

Semithin cross-sections of the arterial wall from rats administered L-NAME clearly indicated an increase in the wall thickness of both the septal branch of the left coronary artery (Figs 3a, 3b) and of the carotid artery (Figs 4a, 4b). The morphometric measurements of the septal branch of the coronary and the carotid artery in controls and in animals after administration of L-NAME yielded the following data.

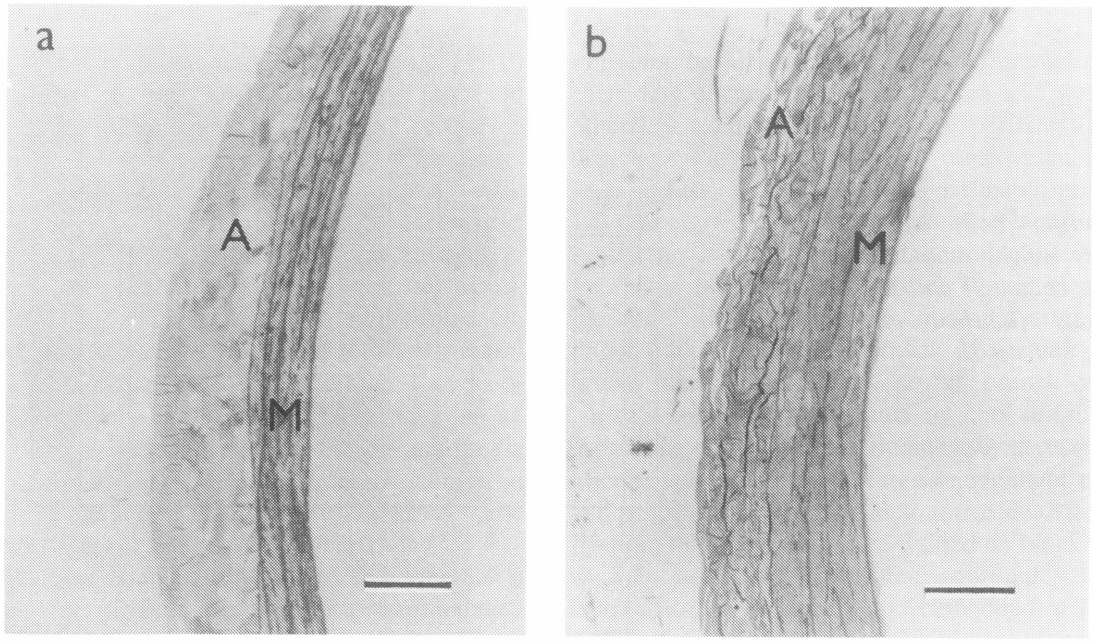

Fig. 4
Cross section of the carotid artery. a – artery from control rat, b – artery from L-NAME treated rat. Tunica media (M), tunica adventitia (A). Bar 50 μm.

Septal branch of the left coronary artery. (Fig. 5). The inner circumference was 664.4±24.7 μm in the experimental rats and 832±30.3 μm in age-matched controls ($p<0.01$). The calculated diameter was 211.5±7.8 μm in experimental rats and 264.9±9.65 μm in the controls ($p<0.01$). The wall thickness (tunica intima and tunica media) was found to be 21.15±0.84 μm in experimental animals, whereas it was 12.47±0.62 μm in the controls ($p<0.01$). Since wall thickness depends on intravascular pressure, we also calculated the wall area in both groups of animals. The calculated wall area was 15.5±0.8 x 10^3 μm^2 in experimental rats and 11.2±0.9 x 10^3 μm^2 in the controls ($p<0.01$). The wall/diameter ratio 1:10 (106.5±3.7 x 10^{-3}) in experimental rats changed from 1:21 (48.0±1.9 x 10^{-3}) in control animals ($p<0.01$).

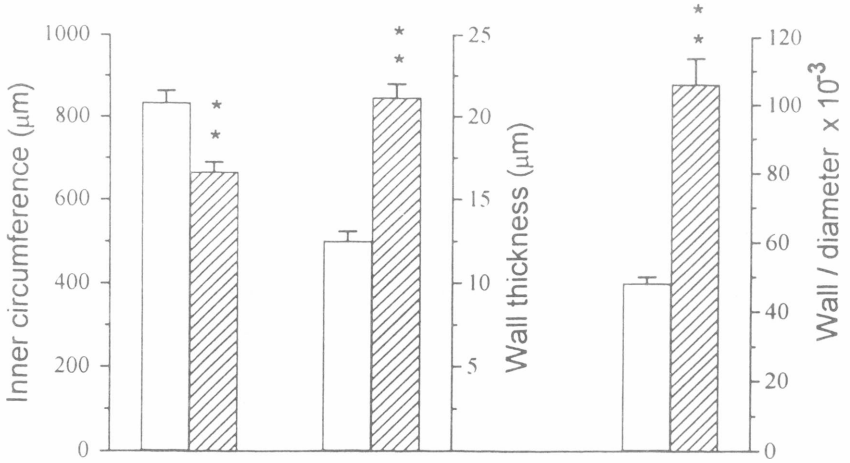

Fig. 5
*Inner circumference, wall thickness (tunica intima and tunica media) and wall/diameter ratio of septal branch of the left coronary artery in control rats (open columns) and L-NAME treated rats (hatched columns). ** P<0.01.*

Carotid artery. (Fig. 6). The inner circumference of the experimental arteries was 2456±39 μm, in the controls 2732±67 μm ($p<0.01$). The calculated inner diameter was 781.8±1.4 μm in experimental rats and 869.8±21.2 μm in the controls ($p<0.01$). The thickness of the arterial wall in experimental rats was 45.14±1.41 μm and 26.08±1.23 μm in the control animals ($p<0.01$). The calculated wall area was 117.6±4.6 x 10^3 μm^2 and 73.3±3.6 x 10^3 μm^2 ($p<0.01$). The wall/diameter ratio changed markedly to about 1:17 (57.9±1.7 x 10^{-3}) in hypertensive rats from the control value of about 1:33 (30.2± 1.7 x 10^{-3}) ($p<0.01$).

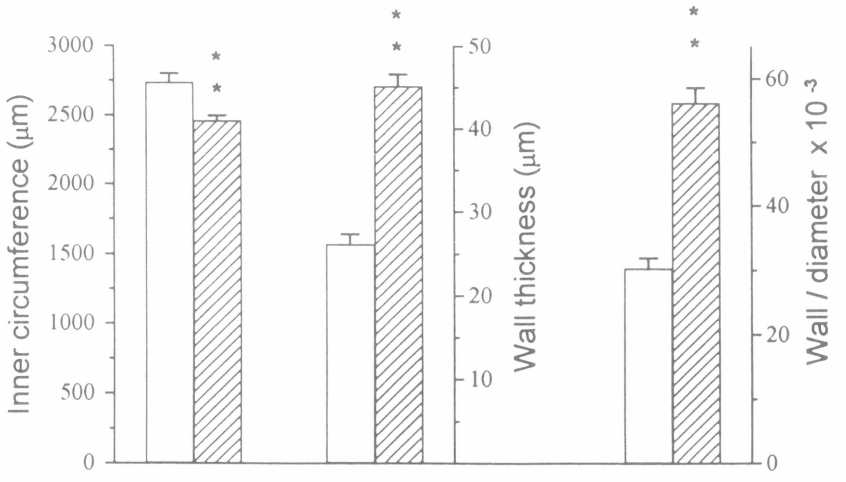

Fig. 6
*Inner circumference, wall thickness (tunica intima and tunica media) and wall/diameter ratio of carotid artery in control rats (open columns) and L-NAME treated rats (hatched columns). ** $P<0.01$.*

Discussion

Eight weeks' lasting inhibition of NO synthase induced a sustained increase in blood pressure and a slight decrease in heart rate. The hypertension was characterized by an increase in absolute and relative heart weight, both indicating cardiac hypertrophy. Our findings are in agreement with the data of Morton *et al.* (1993) and Delacretaz *et al.* (1994), but they contradict the data of Arnal *et al.* (1993) and the first data of Bernátová and Pecháňová (1995) who used the same inhibitor and similar duration of NO synthase inhibition. Recently, however, Bernátová *et al.* (1996) focussing in particular on the left ventricle also found an increase in left ventricle weight in L-NAME treated rats. It seems that the duration of NO deficiency plays a role in cardiac hypertrophy.

The effect of NO level on cardiac performance has been studied *in vitro* and in acute experiments but the results are controversial. Weyrich *et al.* (1994) reported no negative inotropic effect in cat and rat papillary muscles, in a fairly large range of NO levels. In their experiments, NO had an inhibitory effect on contractility only in those papillary muscles whose tone had been increased by noradrenaline. Kennedy *et al.* (1994) did not find any chronotropic effect of NO on the isolated rat heart. On the other hand, Finkel *et al.* (1995) reported a clear change in the force-frequency relation of hamster papillary muscles after inhibition of NO synthase. Brady *et al.* (1993) found that NO attenuated the contractility of cardiac myocytes. In experiments on conscious dogs, Lechevalier *et al.* (1994) reported a decrease in cardiac output together with increased blood pressure after intravenous L-NAME administration.

Chronic inhibition of NO synthase in our experiments, as well as in those of Morton *et al.* (1993) and Delacretaz *et al.* (1994), demonstrated reliably that NO-deficient hypertension was accompanied by cardiac hypertrophy. Nevertheless, the fundamental question still remains open. Is the triggering factor for cardiac hypertrophy a decreased level of NO and/or cGMP in cardiac myocytes or is it an increase in afterload? NO deficiency should, however, trigger a completely different pathway, for example the renin-angiotensin system, which in turn might trigger cardiac hypertrophy secondarily.

A further substantial question to be addressed concerns the state of myocytes, fibroblasts and the extracellular matrix of hypertrophic myocardium in NO-deficient hypertension.

As far as the coronary artery is concerned, no data are available about NO-deficient hypertension for comparison. Our results showed that the inner diameter of the coronary artery decreased and wall thickness increased. In fact, we used the same pressure of 120 mm Hg for perfusion in both control and experimental animals and we found a significant decrease of diameter in NO-deficient hypertensive animals. The finding that the diameter in experimental and control animals was different at the same pressure, as well as the increase in wall thickness justified us to suggest a coronary remodelling in this type of experimental hypertension. The significantly larger calculated wall area in experimental rats strongly supported the above consideration.

The inner diameter also decreased in the carotid artery. In NO-deficient hypertension, Delacretaz *et al.* (1994) reported a thickening of the wall of the carotid artery and Morton *et al.* (1993) of the mesenteric artery. Delacretaz *et al.* (1994) did not find a decrease in the inner diameter of the carotid artery. The difference in the inner diameter compared to our results, may be explained by differences in the methodical approaches employed. We measured the inner circumference of the coronary and carotid artery under constant perfusion pressure, whereas Delacretaz *et al.* (1994) worked on non-pressurized vessels. The

alterations in architecture of both coronary and carotid artery were found to be in agreement with various vessels in several models of hypertension, e.g. spontaneously hypertensive rats (Lew and Angus 1992), two-kidney, one-clip Goldblatt model (Owens and Schwartz 1983) or stroke-prone spontaneously hypertensive rats (Baumbach and Heistad 1989).

Contrary to cardiac myocytes, there is unequivocal evidence that NO inhibits the proliferation of vascular smooth muscle cells (Garg and Hassid 1989, Cornwell *et al.* 1994, Arnal *et al.* 1994). In addition, the basic haemodynamic stimulus – increase in blood pressure – already triggered an increase in proteosynthesis in the coronary wall after 4 hours (Gerová *et al.* 1994) without changes in DNA. Thus, in NO-deficient hypertension both stimuli, i.e. a decrease of NO levels and increased blood pressure, seem to operate synergically and are supposed to enhance the contribution of vascular smooth muscle to the increase of wall thickness.

As in the myocardium, future studies should focus on the inner structure of the coronary and carotid arterial wall. Namely, the important question is which component brings about the increase in wall thickness – cellular or non-cellular components. When considering the increased smooth muscle contractility induced by inhibition of endothelial NO production, the jeopardy of coronary supply may become evident. The same consideration holds true for the carotid artery and the areas which it supplies. The nature of alterations of vascular smooth muscle cells and the subsequent changes in the ultrastructure of the coronary and carotid wall remain open for study.

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References

ARNAL J.F., EL AMRANI A.I., CHATELLIER G., MENARD J., MICHEL J.B.: Cardiac weight in hypertension induced by nitric oxide synthase blockade. *Hypertension* **22:** 380–387, 1993.

ARNAL J.F., YAMIN J., DOCKERY S., HARRISON D.G.: Regulation of endothelial nitric oxide synthase mRNA, protein, and activity during cell growth. *Am. J. Physiol.* **267:** C1381–C1388, 1994.

BAUMBACH G.L., HEISTAD D.D.: Remodeling of cerebral arterioles in chronic hypertension. *Hypertension* **13:** 968–972, 1989.

BAYLIS C., MITRUKA B., DENG A.: Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J. Clin. Invest.* **90:** 278–281, 1992.

BERNÁTOVÁ I., PECHÁŇOVÁ O.: Experimental NO-deficient hypertension: metabolic changes in rats. *Pflügers Arch.* **430:** R120, 1995.

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.

BRADY A.J., WARREN J.B., POOLE-WILSON P.A., WILLIAMS T.J., HARDING S.E.: Nitric oxide attenuates cardiac myocyte contraction. *Am. J. Physiol.* **265:** H176–H182, 1993.

CORNWELL T.L., ARNOLD E., BOERTH N.J., LINCOLN T.M.: Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. *Am. J. Physiol.* **267:** C1405–C1413, 1994.

DANANBERG J., SIDER R.S., GREKIN R.J.: Sustained hypertension induced by orally administered nitro-L-arginine. *Hypertension* **21:** 39–363, 1993.

DELACRETAZ E., HAYOZ D., OSTERHELD M.C., GENTON C.Y., BRUNNER H.R., WAEBER B.: Long-term nitric oxide synthase inhibition and distensibility of carotid artery in intact rats. *Hypertension* **23:** 967–970, 1994.

DUNN W.R., WILSON V.G.: No evidence for vascular remodeling in an L-NAME-induced model of hypertension. *Abstracts of the XXXII Congress IUPS,* Glasgow, 1993, pp. 109–110.

FINKEL M.S., ODDIS C.V., MAYER O.H., HATTLER B.G., SIMMONS R.L.: Nitric oxide synthase inhibitor alters papillary muscle force-frequency relationship. *J. Pharmacol. Exp. Ther.* **272:** 945–952, 1995.

GARG U.C., HASSID A.: Nitric oxide generating vasodilators and 8-bromo cyclic GMP inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J. Clin. Invest.* **83:** 1774–1777, 1989.

GEROVÁ M., PECHÁŇOVÁ O., STOEV V., KITTOVÁ M., BERNÁTOVÁ I.: Protein metabolism in coronary wall affected by cardiac load. *Cell. Mol. Biol. Res.* **40:** 123–128, 1994.

GEROVÁ M., MAŠANOVÁ C., PAVLÁSEK J.: Inhibition of NO synthase in the posterior hypothalamus increases blood pressure in the rat. *Physiol. Res.* **44:** 131–134, 1995.

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

KENNEDY R.H., HICKS K.K., BRIAN J.E., SEIFEN E.: Nitric oxide has no chronotropic effect in right atria isolated from rat heart. *Eur. J. Pharmacol.:* 255: 149–156, 1994.

LECHEVALIER T., DOURSOUT M.F., CHELLY J.E., KILBOURN R.G.: Cardiovascular effects of N^G-methyl-L-arginine in chronically instrumented conscious dogs. *J. Appl. Physiol.* **77:** 471–475, 1994.

LEW M.J., ANGUS J.A.: Wall thickness to lumen diameter ratios of arteries from SHR and WKY: comparison of pressurized and wire-mounted preparations. *J. Vasc. Res.* **29:** 435–442, 1992.

MORTON J.J., BEATTIE E.C., SPEIRS A., GULLIVER F.: Persistent hypertension following inhibition of nitric oxide formation in the young Wistar rat: role of renin and vascular hypertrophy. *J. Hypertens.* **11:** 1083–1088, 1993.

NAKAKI T., NAKAYAMA M., KATO R.: Inhibition by nitric oxide and nitric oxide-producing vasodilators of DNA synthesis in vascular smooth muscle cells. *Eur. J. Pharmacol.* **189:** 347–353, 1990.

OWENS G., SCHWARTZ S.: Vascular smooth muscle cell hypertrophy and hyperploidy in the Goldblatt hypertensive rat. *Circ. Res.* **53:** 491–501, 1993.

POLLOCK D.M., POLAKOWSKI J.S., DIVISH B.J., OPGENORTH T.J.: Angiotensin blockade reverses hypertension during long-term nitric oxide synthase inhibition. *Hypertension* **21:** 660–666, 1993.

REES D.D., PALMER R.M.J., SCHULZ R., HODSON H.F., MONCADA S.: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.* **101:** 746–752, 1990.

RIBEIRO M.O., ANTUNES E., DE NUCCI G., LOVISOLO S.M., ZATZ R.: Chronic inhibition of nitric oxide synthesis: a new model of arterial hypertension. *Hypertension* **20:** 298–303, 1992.

WEYRICH A.S., MA X.-L., BUERKE M., MUROHARA T., ARMSTEAD V.E., LEFER A.M., NICOLAS J.M., THOMAS A.P., LEFER D.J., VINTEN-JOHANSEN J.: Physiological concentrations of nitric oxide do not elicit an acute negative inotropic effect in unstimulated cardiac muscle. *Circ. Res.* **75:** 692–700, 1994.

Reprint Requests

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