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MINIREVIEW

The Potential Role of Nitric Oxide in the Hypertrophic Growth of the Left Ventricle

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Summary

Left ventricular hypertrophy (LVH) is the result of interaction between a chronic hemodynamic overload and non-hemodynamic factors. There are several lines of evidence presented in this work suggesting that nitric oxide (NO) may participate in the hypertrophic growth of the myocardium. First, endothelial NO production was shown to be decreased in several types of hemodynamically overloaded circulation both in animals and humans. Second, compounds stimulating NO production were able to diminish the extent or modify the nature of LVH in some models of myocardial hypertrophic growth. Third, arterial hypertension can be induced by inhibition of nitric oxide synthase activity. This NO-deficient hypertension is associated with the development of concentric LVH, myocardial fibrosis and protein remodeling of the left ventricle. The mechanism of LVH development in NO-deficient hypertension is complex and involves decreased NO production and increased activation of the renin-angiotensin-aldosterone system. Cardiovascular protection *via* ACE inhibition in NO-deficient hypertension may be induced by mechanisms not involving an improvement of NO production. In conclusion, the hypertrophic growth of the LV appears to be the result of interaction of vasoconstrictive and growth stimulating effects of angiotensin II on the one hand and of vasodilating and antiproliferative effects of nitric oxide on the other.

Key words

Nitric oxide • Cardiac hypertrophy • Angiotensin II • L-NAME

Introduction

Despite the many decades of intensive research, left ventricular hypertrophy (LVH) has remained in the center of interest of clinical and experimental cardiology (Šimko 1994, 1996). Several aspects have helped to maintain this attractivity: altered gene expression in the hypertrophied myocardium, a number of non-hemodynamic factors participating in the growth process, the biological ambivalence of LVH and the biological value of LVH regression.

Among numerous non-hemodynamic factors potentially participating in the development and maintenance of LVH, the renin-angiotensin-aldosterone system seems to be of exceptional value (Weber and Brilla 1991, Pelouch *et al.* 1994, 1997, Schlaich and

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Schmieder 1998, Šimko and Šimko 1999a, Šimko *et al.* 2000). Recently, many studies have been focused on the relation of the nitric oxide (NO) molecule and different cardiovascular diseases, such as systemic hypertension (Lüscher 1990, Dominiczak and Bohr 1995, Raij 1998), atherosclerosis, myocardial ischemia and heart failure (Dusting and Macdonald 1995). There are some indications suggesting that impairment of the L-arginine-NO pathway may be involved in hypertrophic myocardial growth.

Nitric oxide is an ubiquitous radical generated by nitric oxide synthase (NOS). Three isoforms of NOS have been identified in different cells: in neuronal cells (NOS I, nNOS), in macrophages (NOS II, iNOS) and in endothelial cells (NOS III, eNOS). From the three described isoforms of this enzyme, isoform III, known as constitutive NOS (cNOS), is responsible for basal unstimulated NO synthesis in the vascular tree (Förstermann *et al.* 1991). NO acts as a second messenger converting GTP to cGMP, which decreases intracellular calcium concentration in a cascade of events, thus stimulating smooth muscle cell relaxation and vasodilatation (Sanders *et al.* 1995). Besides its vasodilating effect, the NO molecule has strong antigrowth and antiproliferative properties. NO-generating substances were shown, for example, to inhibit mitogenesis and proliferation (Garg and Hassid 1989) as well as total protein and collagen synthesis (Kolpakov *et al.* 1995) in cultured vascular smooth muscle cells and to abolish angiotensin II (Ang II)-induced hypertrophy of cultured rat cardiomyocytes (Ritchie *et al.* 1998). Nevertheless, further evidence obtained by different approaches has to be established *in vivo* to consider the involvement of the NO molecule in the myocardial hypertrophic process. It is especially important to show that a) NO production is attenuated in conditions associated with LVH, b) addition of substances related to NO production, can modify the extent and type of LVH, and c) inhibition of NO can induce hypertrophic myocardial growth.

Attenuated production of NO in conditions associated with LVH

The production of NO has been found to be decreased in different models of hemodynamic overloading. In the coronary circulation of spontaneously hypertensive rats (SHR) with left ventricular hypertrophy, endothelium-mediated dilation was reduced. As endothelial NOS expression was also diminished, the reduced coronary flow might have been associated with decreased NO production (Crabos *et al.* 1997). The exhaustion of NO production seems to impair coronary circulation even in the developing stage of hypertension in SHR (Fujita *et al.* 1995). Attenuated endothelium-dependent relaxation was also observed in the aorta of Dahl rats (Lüscher *et al.* 1987), in mesenteric arterioles (Watt and Thurston 1989) and cerebral arteries (Mayhan 1990) of SHR and in deoxycorticosterone/salt-induced hypertension in mesenteric arteries (K-Laflamme *et al.* 1998). The vascular hypercontractile response to norepinephrine in Dahl salt-sensitive rats was also explained by the impairment in endothelial NO production (Nishida *et al.* 1998). Moreover, the concentration of plasmatic L-arginine, which is a substrate for nitric oxide synthase (NOS) producing NO (Palmer *et al.* 1988), was reduced after a stress stimulus in SHR compared to Wistar Kyoto rats (WKY), although the basal L-arginine concentration was normal (Hasegawa *et al.* 1992). Cardiac cGMP levels and cGMP protein kinase activity were lower in SHR than in normotensive rats (Kuo *et al.* 1976). NO production may be disturbed even in the volume-overloaded circulation. In rabbits, four-month aortic insufficiency impaired acetylcholine-induced relaxation in the aorta and renal artery (Holecyová *et al.* 1995).

The acetylcholine-induced relaxation of peripheral arteries was diminished in both primary and secondary forms of human hypertension (Chowienczyk *et al.* 1993, Taddei *et al.* 1993), suggesting impaired NO production. However, this indirect evidence of NO depletion in a hemodynamically overloaded circulation is rather non-specific. Direct evidence was presented in patients with untreated essential hypertension, where inorganic nitrate as a stable end-product of NO oxidation was shown to be diminished in the urine (Forte *et al.* 1997).

The crucial question has, however, remained open: Is impaired synthesis of NO in hemodynamically overloaded circulation the cause or consequence of increased blood pressure?

Modification of LVH by L-arginine

As L-arginine is the substrate for NO synthesis, it has been postulated that chronic L-arginine administration could modify the hypertrophic growth of hemodynamically overloaded myocardium.

Twelve-week treatment with L-arginine did not influence the increased blood pressure in SHR, but it reduced the extent of hypertrophy of the LV, attenuated the increased left ventricular alpha-actin mRNA expression and increased the cGMP content and nitrate/nitrite level, compared to vehicle-treated SHR. Thus, L-arginine administration attenuated LVH of SHR independently of its effect on blood pressure (Matsuoka *et al.* 1996). The inability of chronic L-arginine treatment to decrease arterial blood pressure in SHR is surprising, because in salt-sensitive rats, chronic L-arginine treatment decreased the blood pressure to normotensive level (Chen and Sanders 1991). Moreover, acute L-arginine administration induced hypotension in hypertensive humans (Nakaki *et al.* 1990). In SHR, some counteracting mechanism may have eliminated the hypotensive effect of L-arginine during chronic treatment. The reduction of LVH by chronic L-arginine treatment is probably time-dependent, because neither Kristek (1998) in SHR nor Stier *et al.* (1991) in stroke-prone SHR observed attenuation of LVH after L-arginine treatment lasting for 6 weeks and one month, respectively.

An attractive study with L-arginine administration was performed in the model of ascending aortic stenosis, where LVH is not the result of genetically modulated hemodynamic alteration but of pure mechanical intervention. Ascending aortic stenosis induced LVH without a change in cGMP level, cNOS activity or protein content in the left ventricle. Chronic L-arginine treatment increased left ventricular cGMP and cNOS protein levels, decreased left ventricular systolic pressure, but it had no effect on the extent of LVH (Bartunek *et al.* 1998).

Hypertension and LVH induced by inhibition of NO production

Cardiovascular remodeling in NO-deficient hypertension: left ventricular hypertrophy

At the beginning of the 90's several laboratories reported that chronic inhibition of NO synthase by L-arginine analogues induced sustained systemic hypertension in normotensive rats (Arnal *et al.* 1992, Baylis *et al.* 1992, Ribeiro *et al.* 1992, Pollock *et al.* 1993). This soon became one of the most attractive models of hemodynamic overload in recent years (for review see Pecháňová and Bernátová 1998). In this model, NO synthase activity was shown to be inhibited in the left ventricle, aorta, brain and kidney (Bernátová *et al.* 1996, 1999a), and the cGMP level was depressed in all four organs investigated (Pecháňová and Bernátová 1996, 2000). The inhibition of NO synthase activity and of cGMP levels were dependent on the dose of L-NAME used (Bernátová *et al.* 1996).

Many authors (Hropot *et al.* 1994, Rhaleb *et al.*1994, Pecháňová *et al.* 1997, Mandarim-de-Lacerda and Pereira 1997, Takaori *et al.* 1997, Devlin *et al.* 1998, Nakamura *et al.* 1998, Anderson *et al.* 1999, Uhlenius *et al.* 1999) reported that chronic inhibition of NO synthesis resulted in hypertrophy of the LV, and Bernátová *et al.* (1999b) described the hypertrophy of the aorta. However, several research groups did not observe LVH during chronic NO deficiency (Banting *et al.* 1997, Wickman *et al.* 1997, Ledingham and Laverty 1997, Matsubara *et al.* 1998). LVH development seems to depend on the time and dosage of L-NAME administration (Michel *et al.* 1996, Pereira *et al.* 1998, Pessanha *et al.* 1999) and perhaps on other (hitherto unknown) modulating factors (age and strain of animals, mode of their handling).

The pressure overload, as a consequence of NO deficiency, induced enlargement of the cross-sectional area of myocytes (Sládek *et al.* 1996, Pereira *et al.* 1998), a typical alteration of concentric LVH.

Cardiovascular remodeling in NO-deficient hypertension: left ventricular fibrosis

Hypertrophic growth of the left ventricle is characterized by increased RNA and DNA concentration, increased concentration of metabolic and soluble collagenous proteins and a higher content of contractile and insoluble collagenous proteins (Pecháňová *et al.* 1997, 1999). Nevertheless, the proportion of collagen I and III was not altered (Mandarim-de-Lacerda and Pereira 1997). Increased left ventricular RNA concentration and leucine incorporation into proteins of the LV reflects enhanced protein synthesis, typical for the period of developing hypertrophy (Fízeľová and Fízeľ 1971, Gerová *et al.* 1998, Šimko *et al.* 1998). Enhancement of DNA concentration, reflecting an increase in nucleic mass, can probably be accounted for fibrocytes, as cardiomyocytes are not supposed to divide in the myocardium of adult individuals. This assumption corresponds with the stereologically demonstrated expansion of the cardiac interstitium (Pereira and Mandarim-de-Lacerda 1998) and with histologically proved perivascular and interstitial fibrosis in the model of NO-deficient hypertension (Moreno *et al.* 1995, Babál *et al.* 1997, Akuzawa *et al.* 1998).

These fibrotic myocardial alterations seem to be at least partly the result of ischemia (Moreno *et al.* 1995, 1996), because anerobic metabolism with increased lactate production was observed in the LV of L-NAME treated rats (Hropot *et al.* 1994). The acute intracoronary L-NAME administration, which was associated with a reduction in acetylcholine-induced coronary vasodilatation, reduced both baseline coronary artery blood flow and its response to isoprenaline in dogs (Kaneko *et al.* 1996). The potential functional alterations appear to be associated with morphological remodeling of coronary arteries (Pereira and Mandarim-de-Lacerda 1999). The increased length-density of coronary arteries (Mandarim-de-Lacerda and Pereira 1997) as well as increased proteosynthetic activity and proliferation of the endothelial and smooth muscle cells of coronary arterioles (Okruhlicová *et al.* 2000) indicates neovascularization in the overloaded left ventricle. On the other hand, the increased wall to lumen ratio (Numaguchi *et al.* 1995) as a result of thickening of the tunica media and tunica intima of intramyocardial arteries (Pereira *et al.* 1998), along with an increase in the extracellular matrix of the tunica media of the coronary artery (Kristek *et al.* 1996), may result in attenuation of coronary artery reserve in NO deficiency, potentially inducing ischemic changes in the LV. One should also consider that the well-known antiaggregatory effect of NO (Forte *et al.* 1997) may be attenuated in NO deficiency, thus enhancing intracoronary blood clotting with irreversible ischemic damage of the myocardial tissue.

Mechanisms of LVH in the model of NO deficient hypertension

The mechanisms involved in this type of hypertension and LVH are rather complex and hitherto not completely understood. The experiments explaining mechanisms of NO-deficient hypertension and LVH aim to address two principal questions: Which of the humoral and/or autocrine-paracrine substances are responsible for cardiovascular alterations in NO-deficient hypertension? To what extent are hemodynamic and non-hemodynamic factors involved?

Role of NO and RAS in NO-deficient hypertension

As has already been mentioned, NO synthase activity is decreased in several organs after prolonged L-NAME treatment (Bernátová *et al.* 1996), thus resulting in decreased NO production (Akuzawa *et al.* 1998). As under normal circumstances NO is continuously released into the circulation, modulating thus arterial blood pressure (Lüscher 1990), the reduced NO production is considered to be one of the principal factors in L-arginine analogue-induced hypertension. However, the renin-angiotensin-aldosterone system, and to a lesser extent also the sympathetic system and endothelin, all possessing vasoconstrictor and growth promoting properties, can also participate in this process (Schiffrin 1995, Sventek *et al.* 1996).

Chronic NO deficiency is linked with the attenuation of endothelium-dependent vasorelaxation, along with a hypercontractility of various parts of the vascular bed (Holécyová *et al.* 1996). Of particular interest is the reduced acetylcholine-induced relaxation in the renal artery (Holécyová *et al.* 1996) associated with renal arterial fibrinoid necrotic lesions (Xu *et al.* 1995) and impaired renal structure and function (Hropot *et al.* 1994, Akuzawa *et al.* 1998, Uhlenius *et al.* 1999), which may result in increased renin release and renin-angiotensin system activation. Indeed, increased plasma renin activity was reported by several laboratories (Arnal *et al.* 1992, Baylis *et al.* 1992, Takemoto *et al.* 1997ab, Devlin *et al.* 1998, Nakamura *et al.* 1998). Although serum ACE activity was not increased in L-NAME hypertensive rats, local-tissue ACE activity was markedly stimulated in the LV and aorta (Takemoto *et al.* 1997a). Furthermore, an increase of plasma aldosterone concentration was observed in this model (Usui *et al.* 1998).

Protective effects of RAS inhibition in NO-deficient hypertension and LVH

The presented data suggest that, along with L-NAME-induced NO deficiency, activated ACE may also participate in NO-deficient myocardial hypertrophy. The growth effect of activated ACE may be achieved either by Ang II or aldosterone enhancement and/or by increased bradykinin breakdown with a further depression of NO synthesis and attenuation of prostacyclin formation (Linz *et al.* 1995). To differentiate which humoral or tissue systems related to ACE participate in NO-deficient hypertension and concomitant LVH, the effect of blockade at different levels of the RAS/kallikrein-kinin cascade was investigated in NO-deficient hypertension.

Administration of the ACE inhibitor captopril prevented LVH development, changes in nucleic acid content and collagen remodeling (Pecháňová *et al.* 1997),

although the decreased NOS activity in the heart, aorta, brain and kidney was not restored by captopril (Bernátová *et al.* 1996, 1999a). Similarly, other authors observed that the ACE inhibitory protection in L-NAME treated animals was independent of NO production. Trandolapril was reported to improve acetylcholine-induced relaxation of the aorta, yet without improving aortic NOS activity (Takase *et al.* 1996). Similarly, imidapril prevented LVH and nephrosclerosis development without improving the depressed nitrate/nitrite production (Akuzawa *et al.* 1998). Temocapril administered to L-NAME treated rats caused regression of spinal and cardiac ischemic damage, ameliorated renal impairment and improved the survival. Nevertheless, the depressed cGMP level in the aorta was not restored (Michel *et al.* 1996). It has been suggested that cardiovascular protection *via* ACE inhibition in NO-deficient hypertension may be caused by mechanisms not involving the improvement of NO production (Pecháňová *et al.* 1997).

Although the ACE inhibitor temocapril decreased systolic blood pressure similarly as did the direct arteriolar dilator hydralazine, yet only temocapril decreased serum, cardiac and aortic ACE activity and prevented LVH, perivascular fibrosis and coronary artery remodeling in NO-deficient rats. Interestingly, these protective effects of ACE inhibition were also manifest when a low, non-antihypertensive dose of temocapril was used. Thus the protective effect of ACE inhibition seems to be independent of concomitant hemodynamic alterations (Takemoto *et al.* 1997a). The addition of bradykinin receptor antagonist HOE 140 to temocapril did not cause any reduction in the protective effect of ACE inhibition. Moreover, the Ang II type 1 (AT_1) receptor antagonist CS 866 was equally protective as temocapril in the L-NAME hypertension model. Inhibition of tissue Ang II activity mediated *via* AT_1 receptors was considered responsible for the beneficial effect of ACE inhibition against the remodeling during the long-term inhibition of NO synthesis (Takemoto *et al.* 1997b). Furthermore, although the α_1-adrenergic receptor blocker bunazosin restored blood pressure to the control value, aldosterone levels, AT_1 receptor number and myocardial fibrosis remained enhanced. This suggested that myocardial fibrosis induced by NOS blockade may have in part been determined by elevation of serum aldosterone *via* increased AT_1 receptor number in the adrenal gland (Usui *et al.* 1998).

Conclusions

The positive adaptive effects of LVH may be negatively counterbalanced by increased incidence of heart failure, myocardial infarction or sudden death. However, myocardial hypertrophy is not a uniform process. Its nature depends on the severity and mode of hemodynamic overload and on many non-hemodynamic factors. Therapeutically-induced regression of hypertrophy may also exhibit different aspects with different prognostic implications (Frohlich 1989, Šimko 1995, 1996). This potential variability of the biological value of LVH regression depends, beside the nature of LVH itself, also on disparate ability of antihypertensive agents to modify the neurohumoral acitivation (Šimko 1996). The achievement of "physiological regression of hypertrophy", with the myocardium being similar to that of the normal heart, is dependent on sufficient knowledge on the non-hemodynamic factors participating in a particular type of LVH.

Nitric oxide seems to be an important factor in modulating hypertrophic growth. It exerts a hypotensive effect *via* peripheral artery dilation, thus decreasing the afterload and also reducing the preload by dilation of the venous system. The direct antiproliferative effect of NO can also come into play. The hypertrophic process appears to be the result of continuous interaction of vasoconstricting and growth supporting factors on the one hand, and of vasodilating and antiproliferative factors on the other. Angiotensin II and nitric oxide, acting against each other with respect to blood pressure control and a direct tissue-growth effect, seem to be predominantly operative in maintaining the balance of the steady-state. Stimulated production of Ang II or inhibition of NO may result in hypertrophic growth.

As LVH is an undesirable process, NO could be considered to be a protective factor. This view is supported by the experimental data showing that in SHR, compared to normotensive controls, aortic NOS was increased by 106 % and cardiac NOS by 73 %, with no aortic hypertrophy and only slight hypertrophy of the LV (15 %). On the other hand, in Dahl salt-sensitive rats, where aortic cNOS was reduced by 73 % and cardiac cNOS was not changed, prominent hypertrophy of the LV and aorta (36 % and 88 %, respectively) were observed (Hayakawa and Raij 1997). The authors have suggested that increased tissue NOS activity may serve as a protective homeostatic mechanism (Haikawa and Raij 1997). Long-term stimulation of NO/cGMP signaling in rat aortic stenosis by chronic L-arginine treatment

depressed left ventricular systolic function *in vivo* (Bartunek *et al.* 1998), which could be seen as an inappropriate intervention on the hypertrophied heart. It should, however, be taken into consideration that beta-blockade, despite its negative inotropy, provides a remarkable benefit in the long-term treatment of patients with heart failure (Packer *et al.* 1997). Similarly, the negative inotropy of NO might be desirable in the chronic course of myocardial hypertrophy. Thus it does not seem unreasonable to speculate that the addition of NO donors to well established treatment of LVH (ACE inhibitors, calcium antagonists) might bring about a beneficial effect both with respect to the remodeling process and prognosis.

Much further experimental work will be required to elucidate the potential clinical benefit of nitric oxide with respect to left ventricular hypertrophy.

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