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Review

L-NAME in the cardiovascular system – nitric oxide synthase activator?

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Abstract:
L-arginine analogues are widely used inhibitors of nitric oxide synthase (NOS) activity both *in vitro* and *in vivo*, with N^{ω}-nitro-L-arginine methyl ester (L-NAME) being at the head. On the one hand, acute and chronic L-NAME treatment leads to changes in blood pressure and vascular reactivity due to decreased nitric oxide (NO) bioavailability. However, lower doses of L-NAME may also activate NO production *via* feedback regulatory mechanisms if administered for longer time. Such L-NAME-induced activation has been observed in both NOS expression and activity and revealed considerable differences in regulatory mechanisms of NO production between particular tissues depending on the amount of L-NAME. Moreover, feedback activation of NO production by L-NAME seems to be regulated diversely under conditions of hypertension. This review summarizes the mechanisms of NOS regulation in order to better understand the apparent discrepancies found in the current literature.

Key words:
L-arginine analogues, N^{ω}-nitro-L-arginine methyl ester, nitric oxide synthase, feedback regulation, nuclear factor κB

Abbreviations: BHR – borderline hypertensive rats, EDRF – endothelium-derived relaxing factor, eNOS – endothelial nitric oxide synthase, iNOS – inducible nitric oxide synthase, L-NA – N^{ω}-nitro-L-arginine, L-NAME – N^{ω}-nitro-L-arginine methyl ester, L-NMMA – N^{ω}-monomethyl-L-arginine, NF-κB – nuclear factor κB, NO – nitric oxide, NOS – nitric oxide synthase, ROS – reactive oxygen species, SHR – spontaneously hypertensive rats, WKY – Wistar-Kyoto rats

Introduction

The great and well-known nitric oxide (NO) story began in 1987, when two experimental groups [31, 50] finally identified chemical basis of endothelium-derived relaxing factor (EDRF) discovered formerly by Furchgott and Zawadzki in 1980 [21]. After 6-year long hard work of identifying EDRF, one year sufficed to reveal the intracellular source of NO, L-arginine [52] and at the same time, L-arginine analogue, N^{ω}-monomethyl-L-arginine (L-NMMA), which had been shown to inhibit NO-synthesizing enzyme [51].

Discovery of three types of NO synthase (NOS) in early 90's has brought interest to developing various inhibitors of NO synthesis. Soon, L-arginine analogues became widely used NOS inhibitors, providing useful tool for achieving NO-deficient conditions.

L-arginine analogues

Analogues of L-arginine serve as NOS inhibitors by virtue of their substitution at one or both of the terminal guanidino (^{G} or $^{\omega}$) nitrogen [68]. Generally, L-isomers solely are considered to be active inhibitors, although there is also evidence that N^{ω}-nitro-D-arginine might inhibit endothelial relaxation of the aortal rings and N^{ω}-nitro-D-arginine methyl ester inhibited NOS activity both in the rat heart and aorta [5, 83].

The mechanism of NOS inhibition by substrate analogues consists in competitive bonding to enzyme, but the molecular background of inhibitory action varies for particular analogues. In most cases, analogue-mediated inhibition of NO synthesis is *in vivo* reversible after L-arginine replenishment. However, there are some substances, such as L-NMMA, N^{ω}-iminoethyl-L-ornithine or N^{ω}-allyl-L-arginine that can be utilized by NOS forming intermediates tightly bound to enzyme molecule and thereby blocking the synthesis irreversibly – that is why they deserved the name "suicide inhibitors" [49, 68].

Reversible inhibitor and one of the most frequently used L-arginine substituents is N^{ω}-nitro-L-arginine (L-NA), or its esterified form N^{ω}-nitro-L-arginine methyl ester (L-NAME), which are considered to be non-selective inhibitors [43, 74]. Esterification of the carboxyl group of L-NA increases water solubility, which simplifies the experimental use of this analogue. On the other hand, specific esterase is needed to fully exhibit inhibitory action in the tissue and that may constrain the effect of L-NAME for particular tissues [27]. Despite 30–100-times lower inhibitory efficiency compared to L-NA, L-NAME is widely used in both acute and long-term *in vitro* and *in vivo* experiments, when the effects of NO production restriction are investigated.

L-NAME administration

In endothelium, NO production is continual, keeping balance between vasoconstriction and vasodilatation. NO acts as an antagonist of various constrictoric factors with sympathetic nervous system at the head [2]. Therefore, changes in arterial blood pressure or total peripheral resistance after acute L-NAME administration into vascular bed can be very quickly noticed and the intensity of reaction is determined by both the amount of L-NAME in the dose and the rate of organism dependence on NO [15, 29]. Similarly, application of both L-NAME and L-NMMA led to dose-dependent constriction of the isolated rings of the rat aorta and femoral artery *in vitro* [64].

The theory of "endothelial dysfunction", and consequential insufficient NO production in human essential hypertension, led to creation of an animal model of human hypertension due to NO deficiency, achieved by long-term L-NAME treatment of experimental animals. Long-term administration of NOS inhibitor in relatively high doses (10, 20, 25, 40, 50, 65, 80, 100 mg per kg of body mass per day) induced so-called "NO-deficient hypertension" in normotensive rats and this model became widely used tool for investigation of the NO participation in cardiovascular disorders [3, 11, 13, 18, 19, 35, 40, 48, 66, 86]. Nevertheless, the way of drug administration, the dose as well as the treatment period varies from case to case and sometimes ends in arguable findings, as the effects of L-NAME treatment on blood pressure may be inconsistent even in one laboratory using the same rat strain (compare [40, 48] and [77]).

Another controversial matter of long-term L-NAME treatment is L-NAME-induced left ventricular hypertrophy. In some cases left ventricular hypertrophy was present after 3-week-long treatment with the L-NAME in the dose of 10 mg/kg/day, but, on the other hand, hypertrophy was absent even after 8 weeks of 100 mg/kg/day L-NAME administration (Tab. 1).

Regarding vascular function, higher doses of L-NAME administered for 3–6 weeks reduced relaxant response of the aorta, femoral artery and small mesenteric arteries to carbachol or acetylcholine *in vitro* [10, 19, 39, 57]. In addition, the doses of 10 to 100 mg of L-NAME per kg/day led to dramatic decrease of cGMP content in the aorta of normotensive rats [4, 13, 26].

One of very specific cases is an administration of L-NAME to rat strain with spontaneous or borderline hypertension. These spontaneously hypertensive rats (SHR) develop blood pressure up to 180–200 mmHg in the 4^{th} – 6^{th} week of life on genetic basis without any pharmacological or physiological intervention, regardless of sodium diet [69]. Borderline hypertensive rats (BHR) are F1 offspring of one normotensive and one spontaneously hypertensive parent. Such genetic combination becomes manifested by blood pressure elevation to levels about 130–150 mmHg [8, 67]. Previously, SHR were thought to have impaired