Mechanism of Hypertension Induced by Chronic Inhibition of Nitric Oxide in Rats

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In order to clarify the mechanism of hypertension induced by a nitric oxide (NO) synthase inhibitor, l-1-N\textsuperscript{6}-nitro-l-arginine (LNNA), metabolites of NO, catecholamines, and hemodynamic parameters were measured during 7 days of oral administration of LNNA in rats. Control rats received either l-arginine (L-Arg) or the vehicle. Systolic blood pressure, measured by the tail-cuff method was elevated throughout the period of LNNA administration, but that in the two control groups was not influenced by treatment. Heart rate decreased on the second day only in LNNA-treated rats. Although L-Arg treatment had no influence, LNNA markedly decreased the plasma level and the urinary excretion of nitrate ions (NO\textsubscript{3}⁻). Urinary excretion of noradrenaline was significantly decreased on the second day of LNNA administration and returned to the control level thereafter. When hemodynamic changes were measured by using radioactive microspheres, LNNA was found to increase blood pressure by markedly increasing total peripheral resistance. Cardiac output was decreased by LNNA. L-Arg, again, did not influence the hemodynamic variables as compared with the vehicle control group. The regional vascular resistance was increased by LNNA in many tissues and organs, except the brain and the heart. Regional blood flow, on the other hand, was significantly decreased only in the liver and skin by LNNA. The marked reduction in NO\textsubscript{3}⁻ in urine by LNNA-treatments may indicate that the measured NO\textsubscript{3}⁻ is exclusively of endogenous origin, and that inhibition of NO production causes elevation of blood pressure by constricting peripheral arteries. Sympatholytic responses by the baroreceptor reflex were thereby evident only on the second and the third days, which was indicated by bradycardia and suppression of noradrenaline excretion into urine. These results indicate that the inhibition of NO synthase actually decreases production of endogenous NO, and that the hypertension caused by decreases in NO production is due to elevation of total peripheral vascular resistance. (Hypertens Res 1995; 18: 319-324)

Key Words: nitric oxide, nitric oxide synthase inhibitor, hypertension, nitrate ion, catecholamine, hemodynamics

A potent vasorelaxing factor of endothelium origin (EDRF) is thought to be endogenous nitric oxide (NO), produced enzymatically from l-arginine by a NO synthase (1-4). L-1-N\textsuperscript{6}-monomethyl l-arginine (LMMA) and L-1-N\textsuperscript{6}-nitro-l-arginine (LNNA) inhibit the NO synthase, and are believed to reduce the production of endogenous NO (1). NO is unstable and supposed to be degraded into nitrite (NO\textsubscript{2}⁻) and nitrate (NO\textsubscript{3}⁻) ions (5, 6). In fact, relatively large amounts of NO\textsubscript{3}⁻ exist in plasma and urine. Therefore, it may be reasonable to measure NO\textsubscript{2}⁻ and NO\textsubscript{3}⁻ as indices of endogenous NO production. However, there are many probable sources of NO\textsubscript{3}⁻ besides endothelial NO, such as that in diet, inspired air (7), drinking water, leukocytes (8) and autonomic nerve endings (9). The source of NO\textsubscript{2}⁻ and NO\textsubscript{3}⁻ in biological fluids has not been clarified.

To determine whether and to what extent endogenous NO is involved in the pathophysiology of hypertension induced by chronic inhibition of a NO synthase in rats, we measured urinary excretions of NO\textsubscript{2}⁻ and NO\textsubscript{3}⁻ ions with high-performance liquid chromatography before and during administration of a NO synthase inhibitor, LNNA, in rats. Effects of inhibition of NO production by LNNA on the hemodynamic variables were also measured by using tracer microspheres with a reference sample method to determine the direct cause of hypertension.

Materials and Methods

Eight-week-old male Wistar rats (n=40) were used. They were divided into three groups, and each
Assay for Catecholamines

Catecholamines in urine samples were extracted with activated aluminum oxide (Alumina, activated, Wako Pure Chemical Industries Ltd., Osaka, Japan). Adrenaline and noradrenaline were assayed by a HPLC (HLC 10A, Shimadzu, Kyoto) system with electrochemical detection (Coulochem II, ESA Inc., Bedford, MA). The recovery ratio of plasma samples was 68.0 ± 2.03% for noradrenaline and 61.7 ± 3.58% for adrenaline. Coefficients of variation for noradrenaline and adrenaline were 3.99% and 6.92%, respectively.

Measurement of Hemodynamics

All experiments were performed under anesthesia with urethane (1.1 g/kg, i.p.). Catheters were inserted into the femoral artery and vein, and into the left cardiac ventricle through the right carotid artery. Blood pressure was recorded continuously during the experiments by connecting the femoral arterial catheter to a small volume displacement transducer (TP-200T, Nihon Kohden, Tokyo), and pulse rate triggered by the phasic arterial pulse was calculated automatically with a tachometer (SEN-6104, Nihon Kohden, Tokyo). After recording blood pressure and heart rate for more than 10 min until the stabilization of these variables, radioactive microspheres (15 μm in diameter, labeled with 57Co and suspended in normal saline plus 0.01% Tween 80, NEM-022A, New England Nuclear) were injected into the left ventricle, and a reference sample, 0.45 ml, was collected for 30 seconds from the femoral artery. The methods are described in detail elsewhere (10). Cardiac output measured by an electromagnetic flow meter well correlated with the results of this radioactive microsphere method (r = 0.86, n=12, p<0.001).

Study Drugs and Statistical Analysis

L-Arg (Sigma Chemicals, St. Louis, MO) and L-N03-nitro-L-arginine (Aldrich Chemicals, Milwaukee, WI) were used. Data, expressed as mean ± SEM, from three groups of rats were compared by analysis of variance; for F ratios significant at 5% or less, differences between pairs of means were examined by Duncan’s new multiple range test. Data from only two groups were compared by using Student’s t-test.

Results

Blood Pressure and Pulse Rate

Systolic blood pressure (SBP) on both the 3rd and 6th days was significantly elevated in LNNA-treated rats, as compared with the L-Arg group and the vehicle group (Fig. 1). Blood pressure was not influenced by L-Arg treatment. Pulse rate was significantly decreased on the 3rd day of the LNNA treatment, and returned to the control level on the 6th day. It was not influenced in groups receiving L-Arg or the vehicle. These effects were not related to changes in body weight because there was no difference between any groups.

Urine Volume Urinary Excretions of Nitrate Ions (NO3−)

Urine volume was comparable in the three groups of rats on all four days of measurement.

The concentration of NO3− in urine was extremely low, and the level was under the detection limit of the HPLC system (Fig. 2). Since it is thought that NO2− is converted exclusively into NO3−, in this study, we measured only the concentration of NO3−. As shown in Fig. 2, urinary NO3− levels were markedly lower in the LNNA group than in

the water group and L-Arg group throughout the study period.

**Serum Levels of NO$_3^-$**

Serum NO$_3^-$ concentrations were measured on the 3rd and the 7th days in different groups of rats (Fig. 3). On the third day, serum NO$_3^-$ concentrations were measured only in two groups. Serum NO$_3^-$ concentration was markedly lower (about 1/3 that of control) in the LNNA group than in the vehicle control group. On the 7th day, a similar reduction was seen in the LNNA group. Although the mean serum NO$_3^-$ concentration was slightly higher in the L-Arg group than in the vehicle group, the difference was not significant.

**Urinary Catecholamines**

All three measured catecholamines tended to be increased in rats treated with L-Arg, but statistical significance was not reached (Fig. 4). The noradrenaline level in the LNNA group on the 2nd day of the treatment was significantly lower than those in the other two groups.

**Hemodynamic Alterations Caused by Chronic Inhibition of Nitric Oxide Production**

Mean arterial pressure (MAP), measured directly through the femoral catheter, was significantly elevated in anesthetized rats treated with LNNA, but was not influenced by L-Arg (Fig. 5, 6). Heart rate was not influenced by either LNNA or L-Arg. On the other hand, cardiac output was significantly decreased by treatment with LNNA. Therefore, the calculated total vascular resistance index was markedly increased in rats treated with LNNA.

The regional arterial resistance index significantly increased in many organs and tissues, except the
brain and the heart. On the other hand, regional blood flow was well preserved, even after arterial vasoconstriction because the perfusion pressure was markedly elevated. A significant decrease in blood flow occurred only in the liver and skin.

**Discussion**

NO binds to hemoglobin (Hb) in erythrocytes to form NOHb where it is immediately converted to NO$_2^-$, and finally to NO$_3^-$ (5, 6). NO is exclusively excreted into the urine in the form of NO$_3^-$ . Therefore, the NO$_3^-$ measured in serum and urine in the present study may have been derived from a number of sources, such as food, drinking water, inspired air, Kupffer cells, macrophages, neutrophils, autonomic nerve terminals, and endothelial cells. However, because the amount of NO$_3^-$ excreted into the urine was markedly reduced by a NO synthase inhibitor in this study, the measured NO$_3^-$ was apparently derived exclusively from endogenously formed NO. Furthermore, since endothelium is in direct contact with the blood stream and has an enormous surface area, the major part of circulating and urinary NO$_3^-$ is likely to be of endothelial cell origin. Assuming this is true, this would be the first report to show by measuring NO$_3^-$ in biological fluid that a NO synthase inhibitor decreases NO formation in vivo.

Suppression of NO$_3^-$ levels in serum was smaller than that in urine. The amount of LNNA given to rats, estimated on the basis of the urine volume, was approximately 10 mg/d. Since inhibition of NO synthase activity by this amount of LNNA was most likely incomplete, small amounts of NO may have been continuously produced. Because the molecular weight of NO$_3^-$ is very small and this ion is excreted rapidly into urine, serum levels are expected to be consistently low. Therefore, even if the pro-

**Fig. 4.** Effects of L-Arg and LNNA on urinary excretions of catecholamines on the 2nd (Day 2), 4th (Day 4), 5th (Day 5) and 6th (Day 6) days of treatment (*p < 0.05 vs. vehicle).

**Fig. 5.** Effects of L-Arg and LNNA on hemodynamic variables on the 7th day of treatment (**p < 0.01 vs. vehicle).
duction of $\text{NO}_3^-$ is drastically changed, the change in the serum level might be very small. The urinary excretion of $\text{NO}_3^-$ is therefore superior to the serum level as an index of endogenous production of NO.

The hypertension produced by chronic administration of LNNA was associated with sympathetic inhibition during the early part of treatment, as shown by the decrease in urinary noradrenaline excretion and the occurrence of bradycardia. This early effect is most likely caused by the baroreceptor reflex due to vasopressor responses of peripheral origin. This is supported by hemodynamic changes, which indicated that LNNA produced systemic arterial vasoconstriction in all but a few organs. Further support is provided by a study in humans by Hansen et al. (11), in which LMMA, injected intravenously, increased blood pressure and suppressed sympathetic activity. However, this sympathetic inhibition was seen only in the first few days of treatment in the present study, and sympathetic activity returned to the control level subsequently. This may mean that the baroreceptor threshold was reset to a higher level of blood pressure. Since it has been reported that a NO synthase exists in the central nervous system (12, 13), and that the inhibition of NO synthase in brain elevates blood pressure (14), LNNA might have affected brain NO synthase and increased sympathetic outflow to a level similar to that in the control rats. Although the exact mechanism of this baroreceptor resetting is unknown, this may play a major part in maintaining elevated blood pressure during NO synthase inhibition.

The main cause of LNNA-induced hypertension is obviously vasoconstriction. Hemodynamic studies by radioactive microsphere technique clearly indicated that the systemic arterial beds are constricted by NO synthase inhibition. All tissues and organs except the brain and several other organs were affected by NO synthase inhibition. This clearly contrasts with the vascular effects of vasopressor peptides such as angiotensin II (15) and endothelin (16), which predominantly cause constriction of splanchnic vascular beds. Because cardiac output was decreased by LNNA, the vasopressor response is due solely to the increased vascular resistance. Similar results have been reported by Bank et al. (17). The decreased cardiac output is due to increased afterload on the heart. Vascular beds in the brain and the heart were beyond the range of effects of NO synthase inhibition. Since a NO donor, nitroglycerin, dilates coronary arteries, it is used for the treatment of angina pectoris. One of the adverse effects of nitroglycerin is headache due to vasodilation of the cerebral arteries. Therefore, vascular beds in the heart and the brain are apparently sensitive to NO, but NO synthase inhibition had no effect on these organs in this experiment. Since it is known that the heart (18) and the brain can produce NO, a compensatory, vasodilator mechanism may occur in response to the vasoconstriction caused by NO synthase inhibition.

LNNA was found to elevate blood pressure by decreasing the production of NO and by constricting peripheral arteries. The decreased cardiac output and the increased total peripheral resistance evoked by LNNA resembles the hemodynamic pattern seen in patients with established hypertension (19). Therefore, endothelial functions to produce NO may be deficient in patients with established hypertension.
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References