Effect of \( \text{N}^\text{G}\)-Nitro-\text{l}-Arginine on Effective Vascular Stiffness in Dogs

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ABSTRACT—It has been reported that there is a continuous release of nitric oxide (NO) that contributes to the regulation of vascular tone in the arterial system. In contrast, the role of NO on vascular tone in the venous system is controversial. We examined the effect of IVY-nitro-L-arginine (LNNA), an NO synthase inhibitor, on venous tone in dogs. Venous tone was evaluated by effective vascular stiffness (EVS), which was calculated from the changes in central venous pressure recorded simultaneously with changes in blood volume. LNNA (10 mg/kg, i.v.) increased EVS from 0.21 ± 0.04 to 0.30 ± 0.03 mmHg/kg/ml as well as increasing the systemic vascular resistance. N-Nitro-D-arginine had no effect on EVS. The LNNA-induced increase in EVS was partly reversed by L-arginine, but not by D-arginine, indicating that the increase in EVS was attributable to a blockade of NO synthesis. Since the present study was conducted under ganglion blockade, nitroxidergic nerve terminals do not seem to be the source of NO in this case. These findings suggest that NO (endothelium-derived relaxing factor) is constantly released in the venous system and contributes to the regulation of total systemic venous tone.

Keywords: \( \text{N}^\text{G}\)-Nitro-\text{l}-arginine, Nitric oxide, Venous tone, Effective vascular stiffness

It has been suggested that there is a continuous release of nitric oxide (NO) that contributes to the regulation of vascular tone in the arterial system. This thesis is based on data showing that the blockade of NO synthesis induced a constriction in rabbit aorta ring (1), an increase in perfusion pressure in the isolated perfused heart (2), and an increase in blood pressure in vivo (3). In contrast, there does not appear to be a continuous release of NO in the venous system (4). In humans, the blockade of NO synthesis induced a vasoconstriction in the brachial artery but not in the hand veins (5, 6). In hamsters, it caused a reduction in the diameter of the arterioles but not in the venules of the cheek pouch (7). However, these studies are confined to a small part of the venous system. Since the venous system may play an important role in the regulation of cardiac output by affecting the filling of the heart, one must know the effect of the blockade of NO synthesis on the vascular tone of the systemic venous system (8).

The purpose of this study was to investigate whether blockade of NO synthesis with \( \text{N}^\text{G}\)-nitro-\text{l}-arginine (LNNA) alters systemic venous tone in dogs. One common method to monitor systemic venous tone is the mean circulatory filling pressure (9), but it requires a cessation of cardiac output and may considerably affect the basal conditions of the cardiovascular system. In contrast, effective vascular stiffness (EVS) reported by Münzel et al. (10) was calculated from the changes in central venous pressure recorded simultaneously with artificial changes in blood volume and does not require the cessation of cardiac output. By using this method, we evaluated the role of NO in the regulation of the basal vascular tone in systemic capacitance vessels.

MATERIALS AND METHODS

Animal preparation

Mongrel dogs of either sex were anesthetized with pentobarbital sodium (30 mg/kg initial and 3 mg/kg/hr maintenance, i.v.). Dogs were intubated and ventilated mechanically with a respirator (model 607; Harvard Apparatus, South Natick, MA, USA), 18 times/min with a tidal volume of 15 ml/kg. The left brachial artery and vein were cannulated for monitoring blood pressure (BP) and heart rate and for drug infusion, respectively. A catheter was introduced into the abdominal aorta via the left femoral artery for infusion and withdrawal of blood. A Swan-Ganz catheter was inserted via the left femoral vein to the pulmonary artery to monitor pulmonary arteri-
al pressure (PAP), pulmonary capillary wedge pressure (PCWP), and central venous pressure (CVP). Cardiac output (CO) was measured by the thermal dilution method. Systemic vascular resistance (SVR) was calculated as follows:

$$SVR = \frac{BP - CVP}{CO}$$

Blood pressure, pulmonary arterial pressure, pulmonary capillary wedge pressure, and central venous pressure were measured with pressure transducers (model TP-400T; Nihon Kohden, Tokyo) and amplifiers (model AP-601, Nihon Kohden). Heart rate was measured with an amplifier (model AT-601G, Nihon Kohden). Cardiac output was measured with a cardiac output computer (model MTC-6210, Nihon Kohden).

Reflexes were minimized by hexamethonium chloride (10 mg/kg and 10 mg/kg/hr, i.v.). This dose of hexamethonium abolished the reflex tachycardia produced by intravenous injection of 10 µg/kg of nitroglycerin (increase in heart rate by 49 ± 12 beats/min during the control period and 3 ± 2 beats/min following hexamethonium infusion, n=4). Heparin was administered intravenously (500 units/kg and 250 units/kg/hr, i.v.). To reverse the hypotension and venous collapse induced by hexamethonium, saline and dextran 40 (average molecular weight of 40 K dalton, 10% w/v) were both infused continuously at a rate of 2.5 ml/kg/hr after an initial infusion of 10 ml/kg during the equilibration time.

Measurement of effective vascular stiffness

EVS was measured according to Münzel et al. (10). Dextran (4 ml/kg) was given to replace an equal volume of blood, which was stored in a water bath at 37°C. The stored blood was infused into the abdominal aorta through the femoral artery catheter at a rate of 2 ml/kg/min for 2 min with a Masterflex pump equipped with a quick loading head (model 7520-25 and 7021-20; Cole-Parmer Instrument Co., Chicago, IL, USA). After 1 min, an equal volume was withdrawn at the same rate for 2 min. After another min, the withdrawal procedure was repeated and followed by reinfusion in an identical manner. Thus, the total cycle time was 11 min and the blood volume was changed four times. The central venous pressure was plotted every min against the volume change, and a regression line was drawn (Fig. 1). The slope of this line was defined as EVS (unit: mmHg/kg/ml). Compliance is regarded as the inverse of EVS.

Protocol

Dogs were divided into three groups. In the first group (n=4) and second group (n=4), LNNA (total 10 mg/kg) was intravenously infused for 30 min after one hr of equilibration. Thirty minutes after cessation of LNNA infusion, 500 mg/kg of L-arginine was infused for another 30 min to the dogs in the first group. Dogs in the second group were given D-arginine instead of L-arginine. To examine if the action of LNNA is stereospecific, N⁶-nitro-D-arginine (DNNA) (total of 10 mg/kg) was infused instead of LNNA in the third group (n=4), and the effect of DNNA on EVS and other hemodynamic parameters were assessed. EVS was measured during the control period, 15 min after the cessation of L(D)NNA infusion and just after cessation of L(D)-arginine. Other parameters were continuously recorded. The same doses of LNNA and L-arginine were used for the inhibition and disinhibition of NO synthase in anesthetized dogs (11).

Statistics

Data were expressed as the mean ± S.E.M. One-way analysis of variance was used to compare the data obtained from multiple groups of dogs and a randomized blocked analysis of variance was used to analyze the data for the time course effects. Individual comparisons were made with the Duncan multiple comparison test. A value of P<0.05 was considered statistically significant.

Drugs

The following drugs were dissolved in 0.9% saline and administered intravenously: N⁶-nitro-L-arginine and N⁶-nitro-D-arginine (Peptide Institute, Inc., Osaka); L-arginine monohydrochloride and pentobarbital sodium (Tokyo Chemical Industry, Tokyo); D-arginine monohydrochloride, heparin sodium, and hexamethonium chloride (Wako Pure Chemical Industries, Ltd., Osaka). Dextran 40 was a kind gift from Green Cross, Osaka.

RESULTS

The control values of each measured parameter are shown for each group in Table 1. There were no significant differences in these values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td>144.0±12.7</td>
<td>125.3±7.5</td>
<td>121.8±10.4</td>
</tr>
<tr>
<td>CO (/min)</td>
<td>1.7±0.3</td>
<td>1.9±0.2</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>4.9±0.6</td>
<td>5.1±0.6</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>SVR (mmHg/min/l)</td>
<td>88.2±16.6</td>
<td>63.8±7.8</td>
<td>64.7±10.9</td>
</tr>
<tr>
<td>PVR (mmHg/min/l)</td>
<td>4.9±0.8</td>
<td>5.1±0.4</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>EVS (mmHg/kg/ml)</td>
<td>0.21±0.04</td>
<td>0.23±0.03</td>
<td>0.25±0.07</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M. BP, blood pressure; CO, cardiac output; CVP, central venous pressure; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; EVS, effective vascular stiffness.
Effect of LNNA and L-arginine on hemodynamic parameters (group 1)

Figure 1 shows a typical recording of the central venous pressure during a cycle of volume change. The change in the central venous pressure during a volume change cycle was increased by LNNA, and the slope of the regression line was also increased, meaning an increase in EVS. Mean pressure-volume curves and the average EVS for each group are shown in Figs. 2 and 3. LNNA increased EVS from 0.21 ± 0.04 to 0.30 ± 0.03 mmHg·kg/ml. L-Arginine partially restored this increase. The central venous pressure rose from 3.7 ± 0.4 to 4.5 ± 0.4 mmHg with the LNNA infusion. The blood pressure also increased from 144.0 ± 12.7 to 163.3 ± 14.8 mmHg at 30 min. After cessation of the infusion, the pressure dropped gradually and returned to the baseline value at 60 min. The infusion of L-arginine further lowered the blood pressure to 127.0 ± 9.4 mmHg at 90 min (Fig. 4). The cardiac output was reduced by the infusion of LNNA from 1.66 ± 0.30 to 1.05 ± 0.12 l/min at 30 min and 0.82 ± 0.07 l/min at 60 min. L-Arginine had a tendency to restore the cardiac output which recovered to 0.99 ± 0.04 l/min at 90 min, but this change was not statistically significant compared to the value at 60 min (Fig. 4). Systemic vascular resistance was increased by LNNA from 88.2 ± 16.6 to 152.7 ± 23.5 mmHg·min/l at 30 min, and further increased to 172.6 ± 18.5 mmHg·min/l at 60 min, even though blood pressure was restored to the control level at this point. L-Arginine significantly decreased the systemic vascular resistance to 120.5 ± 9.5 mmHg·min/l at 90 min (Fig. 4).

Effect of LNNA and D-arginine on hemodynamic parameters (group 2)

The infusion of LNNA induced almost the same effect on blood pressure, cardiac output, systemic vascular resistance and EVS as in group 1. In contrast to L-arginine, however, the infusion of D-arginine did not restore the LNNA-induced changes of these parameters (Figs. 3 and 4).

Effect of DNNA on hemodynamic parameters (group 3)

DNNA had no significant effects on blood pressure, cardiac output, systemic vascular resistance or EVS (Figs. 3 and 4).
DISCUSSION

The present study demonstrated that LNNA increased EVS as well as systemic vascular resistance. DNNA did not show such effects. These changes lasted after the cessation of LNNA infusion and were significantly reversed by L-arginine infusion, but not by D-arginine infusion. These data, together with our previous finding that LNNA inhibits acetylcholine-induced vasodilation in dogs (12), indicate that these hemodynamic changes caused by LNNA are attributable to a blockade of NO synthesis.

EVS (10, 13) mainly reflects the total systemic venous tone, but several limitations must be considered: (1) The increase in systemic vascular resistance and the reduction in cardiac output induced by LNNA may diminish vascular compliance and hence increase EVS. However, previous studies (13, 14) have shown that these changes were independent of EVS. (2) LNNA-induced increase in central venous pressure may also alter EVS. This notion is unlikely, since the regression between volume change and central venous pressure during the control period compared to that obtained after LNNA infusion (Figs. 1 and 2) was,
for the most part, in the same range of central venous pressures; and in this pressure range, LNNA increased EVS at the same pressure level. (3) Redistribution of blood flow following LNNA administration also may affect the measured EVS value. For example, if LNNA selectively constricts arterioles to high capacitance (low stiffness) areas, such as the splanchnic region, blood will shift to other regions where the capacitance is low, and EVS will therefore increase. However, this does not seem to be the case, because LNNA was reported to reduce the blood flows of renal, mesenteric, femoral, and carotid arteries to the same extent (15). These findings, taken together, suggest that the LNNA-induced increase in EVS was mainly due to venoconstriction, and that NO is constantly released in capacitance vessels and regulates their basal tone.

A possible origin of constant NO release is the endothelium and/or nitroxidergic neurons. The synthesis of NO has been associated with stimulation of $\alpha_2$-adrenergic, muscarinic, and thrombin receptors on venous endothelial cells (16), indicating the presence of NO synthase, which may constantly release NO in capacitance vessels. Another possible candidate for the source of constant NO release is nitroxidergic nerve endings (17). The contribution of these neurons, however, may be minor in the present study, since ganglion blockade was performed with infusion of hexamethonium, which has been shown to abolish vasorelaxation induced by nitroxidergic nerve transmission (18). Thus, in this model, NO is probably released under basal conditions as endothelium-derived relaxing factor (EDRF).

Although systemic vascular resistance remained high after the cessation of LNNA infusion, blood pressure gradually declined and returned to the control levels at 60 min. This spontaneous decline of blood pressure was not found in rabbits, where the pressor effect of NO synthesis inhibition is long-lasting (7, 12). The mechanism underlying this difference is unclear, but may be attributable to cardiac depression induced by LNNA in dogs (19).

In conclusion, the present study demonstrated that (1) NO regulates the total systemic venous tone in dogs and that (2) the source of NO in this case was most probably EDRF, not nitroxidergic nerve terminals.

Acknowledgment

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