Nitric oxide mediates hepatic arterial vascular escape from norepinephrine-induced constriction

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Ming, Zhi, Chao Han, and W. Wayne Lautt. Nitric oxide mediates hepatic arterial vascular escape from norepinephrine-induced constriction. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G1200–G1206, 1999.—The involvement of nitric oxide (NO) in the vascular escape from norepinephrine (NE)-induced vasoconstriction was investigated in the hepatic arterial vasculature of anesthetized cats. The hepatic artery was perfused by free blood flow or pump-controlled constant-flow, and NE (0.15 and 0.3 µg·kg⁻¹·min⁻¹, respectively) was infused through the portal vein. In the free-flow perfusion model, the NE-induced hepatic vasoconstriction recovered from the maximum point of the constriction, resulting in 36.6 ± 5.9% vascular escape. Blockade of NO formation with N^ω-nitro-L-arginine methyl ester (L-NAME, 2.5 mg/kg ivp) potentiated NE-induced maximum vasoconstriction, and the potentiation was reversed by L-arginine (75 mg/kg ivp). Furthermore, NE-induced vasoconstriction became more stable after L-NAME, resulting in an inhibition of vascular escape (7.5 ± 3.3%), and the inhibition was reversed by L-arginine (23.0 ± 6.4%). Similar potentiation of NE-induced vasoconstriction and inhibition of hepatic vascular escape by L-NAME (40.4 ± 4.3% control vs. 10.2 ± 3.7% post-L-NAME escape) and the reversal by L-arginine were also observed in the constant-flow perfusion model. The data suggest that NO is the major endogenous mediator involved in the hepatic vascular escape from NE-induced vasoconstriction.

VASCULAR ESCAPE, characterized by the recovery of initial decreased blood flow and vascular conductance toward the control level during continued vasoconstrictive stimulation, is a unique vascular defense mechanism, preventing tissue from insufficient blood supply during prolonged vasoconstrictive challenge (7). The secondary vasodilation during vascular escape is due to the relaxation of the smooth muscle of the same vessels that were originally constricted (8). It has long been suggested that a vasodilative substance(s) released after the vasoconstrictive stimuli may be the primary mechanism responsible for the vascular escape. So far, the possible involvement of endogenous vasodilators such as adenosine, prostaglandins, histamine, β-adrenergic agonist, and acetylecholine has been tested and eliminated (reviewed in Refs. 7 and 13). Chen and Sheppherd (3) suggested that the reduced pH that occurred during reduction of blood flow induced by norepinephrine (NE) resulted in a selective inhibition of postfunctional α₂-adrenergic receptors in the canine intestine. However, hepatic venous pH was unaltered at the point of full escape in response to sympathetic nerve stimulation in the cat (10). The hypoxia induced by decreased blood flow during vasoconstriction also does not appear to cause vascular escape because a shift to anaerobic metabolism had no significant influence on vascular escape in rat skeletal microvascular circulation (23). Previous studies have shown an inhibitory effect of glucagon on vascular escape in cat hepatic artery (HA) (4) and of insulin in rabbit renal arterial circulation (6). However, the lack of a demonstrated local stimulation-release cascade and the supraphysiological doses required make it difficult to evaluate the contribution of insulin and glucagon in physiological situations.

Nitric oxide (NO) is a powerful vasodilator endogenously released from vascular endothelium in response to many vasoconstrictive stimuli, including NE and sympathetic nerve stimulation, through both a direct receptor-operated mechanism (27) and induction by increased shear stress caused by vasoconstriction (9). Increasing evidence suggests that NO released during vasoconstriction attenuates the initial constriction and probably mediates the vascular escape. Blockade of NO formation with NO synthase inhibitor potentiated the vasoconstriction induced by NE or sympathetic nerve stimulation (17, 19–21). In contrast, NO donors or endogenous activation of NO synthase inhibited NE-induced vasoconstriction (18, 28). Methylene blue, an inhibitor of guanylate cyclase, which is the key enzyme in NO-induced vasodilation, diminished vascular escape during NE infusion in rat mesenteric artery (24).

The purpose of the present study was to test the hypothesis that NO is the major mediator for the induction of vascular escape in the HA. The HA vascular escape from NE-induced vasoconstriction was compared before and after inhibition of NO formation in both free-flow and pump-controlled constant-flow perfusion of the HA. The results suggest that blockade of NO formation inhibited vascular escape and that this inhibition could be reversed by reactivation of NO synthase with L-arginine in cat HA.

MATERIALS AND METHODS

Surgical Preparation

Free-flow infusion of HA and portal vein. The surgery and instrumentation were previously described (11). Briefly, cats of either sex (n = 9, 3.6 ± 0.1 kg body wt) were fasted overnight and anesthetized with pentobarbital sodium (32.5 mg/kg) via an intraperitoneal injection. Anesthesia was maintained using a continuous intravenous infusion (0.78 mg/ml saline) adjusted as required via a brachial vein cannula. Body

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Hepatic arterial vascular escape from NE stimulation. Intraportal infusion of NE (n = 9; 0.15 µg·kg⁻¹·min⁻¹ for 3 min) was performed to initiate HA vasoconstriction, and the vascular escape was measured. The dose of NE was chosen to achieve an adequate vasoconstriction in the HA vasculature without significant influence on portal venous blood flow, to rule out the potential impact of the change in portal venous blood flow on the reactivity of the HA due to the HA buffer response (12). Only one dose of NE was used because previous studies had shown that the HA escape during NE stimulation is dose independent in the dose range from 0.125–1.25 µg·kg⁻¹·min⁻¹ (4).

Blockade of NO formation on vascular escape. In the same group of animals, endogenous NO formation was blocked with N'-nitro-L-arginine methyl ester (L-NAME, 2.5 mg/kg iv). Fifteen minutes later, when steady-state basal hemodynamics were achieved, intraportal infusion of NE was repeated.

Reversal effect of L-arginine. In eight of nine cats in this group, an intravenous bolus injection of 75 mg/kg L-arginine was administered to restore the activity of NO synthase after L-NAME. NE infusion was repeated 10 min thereafter.

Pump-served constant-flow model. All of the above protocols were performed in the pump-controlled constant-flow condition in another group of seven cats. For this group, the dose of NE was 0.3 µg·kg⁻¹·min⁻¹. The high dose of NE in the constant-flow model could be used because NE-induced changes in portal flow were prevented by use of the perfusion circuit.

Calculations

Hemodynamic variables were determined before NE infusion (control) at maximum vasoconstriction during NE infusion judged from the minimal HA flow level or, in the pump-controlled constant-flow model, from the maximum hepatic arterial pressure (HAP) attained (peak response ~30 s after starting NE infusion) and at the end of a 3-min infusion period (plateau). Hepatic arterial conductance (HAC) was calculated as the ratio of HABF to (HAP – PVP) (ml·min⁻¹·kg⁻¹·mmHg⁻¹). The vascular escape was calculated as [(HAC₂ – HAC₁)/(HAC₁ – HAC₀)] × 100%, where HAC₀ is control conductance, HAC₁ is conductance at the peak constriction, and HAC₂ is conductance at the end of the 3-min NE infusion period. In the situation of constant-flow perfusion, vascular escape was directly calculated from HAP by [(HAP₂ – HAP₁)/(HAP₀ – HAP₁)] × 100%. Superior mesenteric arterial conductance (SMAC) was calculated as the ratio of SMABF to (AP – PVP).

All data are expressed as means ± SE. A one-way ANOVA followed by Tukey’s test was employed when the multiple means from different groups were compared. The paired t-test was employed when two means within the group were compared. In appropriate conditions, an unpaired Student’s t-test was applied. P < 0.05 was selected for acceptance of statistical significance.

All chemicals were purchased from Sigma (St. Louis, MO) and were freshly prepared daily. NE, L-NAME, and L-arginine were dissolved in saline. The experimental procedures were approved by the Ethics Committee on Animal Care at the University of Manitoba and performed in accordance with The Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care.

RESULTS

Free-Flow Model

General hemodynamics. Baseline hemodynamics in control, after L-NAME, and after L-arginine are re-
Table 1. Baseline hemodynamics in control, after L-NAME, and after L-NAME + L-arginine in free-flow perfusion model in cats

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>L-NAME (n = 9)</th>
<th>L-Arginine (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP, mmHg</td>
<td>101.6 ± 4.8</td>
<td>114.6 ± 4.9*</td>
<td>84.8 ± 3.1†</td>
</tr>
<tr>
<td>PVP, mmHg</td>
<td>7.4 ± 0.4</td>
<td>7.8 ± 0.4</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>3.3 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>HABF, ml·min⁻¹·kg⁻¹</td>
<td>18.2 ± 2.6</td>
<td>18.4 ± 2.2</td>
<td>15.6 ± 2.3</td>
</tr>
<tr>
<td>SMABF, ml·min⁻¹·kg⁻¹</td>
<td>19.9 ± 2.8</td>
<td>18.8 ± 3.0</td>
<td>19.4 ± 2.3</td>
</tr>
<tr>
<td>HAC, ml·min⁻¹·kg⁻¹·mmHg⁻¹</td>
<td>0.196 ± 0.036</td>
<td>0.178 ± 0.029</td>
<td>0.199 ± 0.024</td>
</tr>
<tr>
<td>SMAC, ml·min⁻¹·kg⁻¹·mmHg⁻¹</td>
<td>0.227 ± 0.042</td>
<td>0.179 ± 0.043*</td>
<td>0.256 ± 0.032†</td>
</tr>
</tbody>
</table>

Values are means ± SE. AP, carotid arterial blood pressure; PVP, portal venous blood pressure; CVP, central venous blood pressure; HABF, hepatic arterial blood flow; SMABF, superior mesenteric arterial blood flow; HAC, hepatic arterial conductance; SMAC, superior mesenteric arterial conductance. *P < 0.05 vs. control; †P < 0.05 vs. N-nitro-L-arginine methyl ester (L-NAME).

Reported in Table 1, L-NAME increased baseline AP and decreased SMAC but had no significant influence on other hemodynamics. The changes in baseline AP and SMAC caused by L-NAME were reversed by L-arginine. Furthermore, L-NAME potentiated NE-induced vasoconstriction in both systemic and portal venous circulation, as demonstrated by the greater increases in both AP and PVP, and the potentiated effects of L-NAME were reversed by L-arginine (Fig. 1). Intraportal infusion of NE did not alter SMABF in any of the conditions tested. The data indicate an adequate blockade of NO formation by L-NAME and the efficiency of L-arginine to reverse the formation of NO in hepatic circulation in our model.

Hepatic arterial vascular escape from NE stimulation. Intraportal infusion of NE induced an intense initial HA vasoconstriction, which reached a maximum ~30 s after the start of NE infusion (Fig. 2). The maximum vasoconstriction resulted in a 26.0 ± 5.7% decrease in HABF and a 40.9 ± 5.0% decrease in HAC. Thereafter, the HABF and HAC underwent a partial return toward baseline despite continued NE infusion and reached a plateau within 3 min (vasoconstriction). The calculated flow escape was 92.8 ± 16.8%, and the conductance escape was 36.6 ± 5.9%, as shown in Fig. 2.

Blockade of NO formation on vascular escape. Administration of L-NAME had no significant influence on basal HA vascular tone but enhanced NE-induced HA vasoconstriction. The HABF decreased by 7.5 ± 1.2 ml·min⁻¹·kg⁻¹, and HAC decreased by 0.097 ± 0.018 ml·min⁻¹·kg⁻¹·mmHg⁻¹ at the point of maximum constriction, resulting in a 40.0 ± 4.2% decrease in HABF and a 52 ± 3.7% decrease in HAC (both P < 0.05). However, vascular escape was inhibited by L-NAME. The return of HABF at the end of the NE infusion only reached 12.2 ± 1.4 ml·min⁻¹·kg⁻¹, and HAC only reached 0.092 ± 0.018 ml·min⁻¹·kg⁻¹·mmHg⁻¹, representing a significant decrease both in flow escape (22.1 ± 11.8%) and conductance escape (7.5 ± 3.3%)
compared with before L-NAME (both P < 0.001), as reported in Figs. 3 and 4. The data suggested that a NO component was involved in counteracting NE-induced hepatic vasoconstriction and resulted in vascular escape thereafter.

Reversal effect of L-arginine. To further confirm that the inhibitory effect of L-NAME on hepatic vascular escape was due to the inhibition of NO formation, L-arginine, a NO synthesis precursor, was used after L-NAME to reverse the inhibition of NO synthase. Administration of L-arginine had no significant influence on basal HABF or HAC but partially reversed the potentiating effect of L-NAME on NE-induced vasoconstriction of the HA (Fig. 4). Furthermore, L-arginine reversed the inhibitory effect of L-NAME on hepatic vascular escape after NE stimulation, as reported in Figs. 3 and 4.

Constant-Flow Model

Overall, comparisons of baseline HAP, PVP, HABF, and SMABF revealed no significant differences between the control after L-NAME and after L-arginine, as shown in Table 2. Intraportal infusion of NE increased HAP by 44.4 ± 7.2 from 88.9 ± 3.5 mmHg at the initial maximum point of constriction and by 27.1 ± 5.6 mmHg at the plateau, resulting in 40.4 ± 4.3% vascular escape. After L-NAME, NE-induced HAP increased to 78.6 ± 12.4 mmHg at the maximum point and remained at 72.7 ± 13.1 mmHg at the plateau, representing a significant inhibition of vascular escape (10.2 ± 3.7%) compared with before L-NAME. In five of seven cats in this group, L-arginine was used after L-NAME. L-Arginine totally reversed the potentiated effect on HA vasoconstriction and the inhibitory effect on vascular escape by L-NAME, as reported in Figs. 5 and 6.

DISCUSSION

The purpose of the present study was to investigate the hypothesis that endogenous NO regulates vascular escape during sustained NE infusion in cat HA vasculature. This hypothesis was tested by examining the effects of blockade of NO formation with L-NAME on the responses of prolonged NE infusion in two different situations: free-flow and constant-flow perfusion of the HA. The main finding is that NO endogenously released after NE stimulation appears to...
be the major mediator responsible for inducing HA vascular escape from NE-induced vasoconstriction. Blockade of NO formation with L-NAME significantly inhibited vascular escape, and this inhibition was reversed by reactivation of NO synthase with L-arginine. To our knowledge, this is the first in vivo study performed in the HA vasculature showing endogenous NO as a major mediator in inducing vascular escape.

Methodological Considerations

The free-flow perfusion model is more physiological than the constant-flow model. However, three limitations exist with the free-flow preparation in assessing hepatic vascular escape: 1) The potential change in SMABF during NE infusion will impact the responsiveness of the HA to NE due to the HA buffer response, and this mechanism accounts for the observation that a change in portal flow leads to a rapid and opposite change in HA flow so that total hepatic flow tends to be maintained constant (14); 2) the administration of NE changes AP and HABF simultaneously, and this makes it difficult to determine the true maximal point of vasoconstriction, which is essential in analyzing the vascular escape response; and 3) HA blood pressure is estimated from AP. To avoid these limitations, we also performed the experiments in an in situ perfusion model that allowed only HA blood pressure to increase and at the same time held the HABF and portal flow constant during NE infusion. The responses of systemic and hepatic vascular beds to NE, L-NAME, or L-arginine are similar in these two models, indicating that both models are reliable in assessing hepatic hemodynamics.

Ideally, the same approach would be tested in the constant-flow condition in which shear stress is allowed to rise and in the constant-pressure situation in which the change in shear stress is avoided (17). We did not perform this test because of technical limitations. Even though the HA blood pressure may be held steady to avoid the increase in HA shear stress induced by vasoconstriction, the portal system also responds to constriction by elevated shear stress and NO release (16), so that both circuits would have to be precisely and rapidly controlled to avoid shear effects during the peak constriction and the escape. In this event, the required decrease in portal flow would have a dilation effect on the HA through the HA buffer response. Because of these limitations, we cannot answer whether the NO released during the vasoconstriction and leading to the escape is agonist dependent (1, 25) or shear stress dependent (2, 5, 9) or whether the site of NO release is from the HA or the portal vessels.

The dose of L-NAME or L-arginine used in the present study has previously been demonstrated to achieve an adequate blockade of NO formation or reactivation of NO synthase after L-NAME in hepatic circulation (17). The effectiveness of the drugs was verified in the present study, as indicated by the significant elevation in baseline AP and the more apparent increment of HAP and PVP following NE infusion after L-NAME and the reversal of these effects by L-arginine.

Hepatic Vascular Escape

The vascular escape from sympathetic nerve stimulation- and NE infusion-induced vasoconstriction is well established as a genuine physiological phenomenon in
cat HA in vivo and in vitro (7). Greenway et al. (8) observed that the vascular escape in the cat mesenteric vascular bed was not related to blood flow redistribution and concluded that the vasodilation during the vascular escape involves mainly the relaxation of the same vessels that originally constricted. The lack of flow redistribution has been further supported in the HA by the measurement of microsphere distribution as well as oxygen consumption (10) and lidocaine clear- ance (16). The present study demonstrates that NO is the major endogenous vasodilator involved in inducing the hepatic vascular escape from NE stimulation.

Our findings are consistent with other reports that suggest the involvement of NO in vascular escape. In rat systemic circulation, the vascular escape from angiotensin II-induced constriction was inhibited by NO synthase blockade, and L-arginine reversed this inhibition (26). In rat SMA, Remak et al. (24) observed that vascular escape from NE was inhibited by the inhibition of NO action with methylene blue. Interestingly, NO is also the major mediator involved in the vascular escape of hepatic portal circulation; blockade of NO formation significantly inhibited the portal venous vascular escape from ethanol-induced vasoconstriction in rat liver (22).

In conclusion, although NO was not involved in control of basal HA tone, the continuous release of NO during adrenergic stimulation plays an important role in modulating vascular tone in cat HA vasculature. In addition to preventing extreme constriction of arterial vessels by limiting maximum adrenergic vasoconstriction, the direct vasodilative effect of NO and its modulation of adrenergic vasoconstriction regulate the vascular tone during adrenergic stimulation toward the prestimulation level and cause the HA escape. This regulatory effect of NO is important in maintaining the blood supply to the liver in conditions of high adrenergic stress. In the liver, the blood from the HA is the main source of oxygen supply and the adequacy of blood supply is essential for maintaining hepatic metabolic function (15).

We thank Dallas Legare for excellent technical support.

This study was supported by operating grants from the Medical Research Council of Canada and the Heart and Stroke Foundation of Manitoba. Z. Ming is the recipient of a Manitoba Health Research Council Fellowship Award.

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Received 12 April 1999; accepted in final form 18 August 1999.

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