Mice with targeted deletion of eNOS develop hyperdynamic circulation associated with portal hypertension

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Iwakiri, Yasuko, Gregory Cadelina, William C. Sessa, and Roberto J. Groszmann Mice with targeted deletion of eNOS develop hyperdynamic circulation associated with portal hypertension. Am J Physiol Gastrointest Liver Physiol 283: G1074–G1081, 2002.—Systemic vasodilation is the initiating event of the hyperdynamic circulatory state, being most likely triggered by increased levels of vasodilators, primarily nitric oxide (NO). Endothelial NO synthase (eNOS) is responsible for this event. We tested the hypothesis that gene deletion of eNOS and inducible NOS (iNOS) may inhibit the development of the hyperdynamic circulatory state in portal hypertensive animals. To test this hypothesis, we used mice lacking eNOS (eNOS–/–) or eNOS/iNOS (eNOS/iNOS–/–) genes. A partial portal vein ligation (PVL) was used to induce portal hypertension. Sham-operated animals were used as a control. Hemodynamic characteristics were tested 2 wk after surgery. As opposed to our hypothesis, PVL also caused significant reduction in peripheral resistance in eNOS–/– compared with sham animals (0.33 ± 0.02 vs. 0.41 ± 0.03 mm Hg·min·kg body wt·ml–1, P = 0.04) and in eNOS/iNOS–/– animals with PVL compared with that of the sham-operated group (0.44 ± 0.02 vs. 0.54 ± 0.04; P = 0.03). This demonstrates that, despite gene deletion of eNOS, the knockout mice developed hyperdynamic circulatory state. Compensatory vasodilator molecule(s) are upregulated in place of NO in the systemic and splanchnic circulation in portal hypertensive animals.

No diseases; nitric oxide; vasodilatation; and portal vein ligation.

Hypodynamic circulatory state is known as a hallmark of portal hypertension in liver cirrhosis. It is characterized by such hemodynamic abnormalities as generalized systemic vasodilation with lowered mean arterial pressure and systemic vascular resistance and subsequent blood volume expansion with elevated cardiac index and regional blood flows (28). Systemic vasodilation is the initiating event of this hyperdynamic circulatory state and is most likely triggered by increased levels of vasodilators, primarily nitric oxide (NO) (13, 14, 21–23). Inhibition of NO production has been shown to ameliorate the hyperdynamic circulatory state in patients with cirrhosis (12) as well as in animal models of cirrhosis and/or portal hypertension (14).

NO is produced by three distinct isoforms of NO synthase (NOS), two of which are constitutively expressed (endothelial NOS (eNOS), which is involved in regulating basal vasodilation, and neuronal NOS (nNOS)) and one that is inducible (iNOS) and produces NO in response to such stimuli as LPS and cytokines. It is known that eNOS is the major isoform responsible for excessive NO production in the systemic and splanchnic circulation of cirrhotic animals and humans (4, 5, 15, 27, 28).

The goal of the present study was to elucidate further the role of eNOS as well as iNOS in the development of the hyperdynamic circulatory state. To achieve this goal we used eNOS knockout mice as well as both eNOS and iNOS knockout mice. The utilization of these mice lacking eNOS and iNOS genes allowed us to investigate the effects of NO, or the lack thereof, without administering exogenous inhibitors that may not be specific enzymatically and may induce other unwanted hemodynamic effects that may obscure the sole effect of a deficient NOS. Furthermore, this is the first study to use mice for the study of the hyperdynamic circulatory syndrome in portal hypertension. Because the vast majority of gene deletion studies have been achieved in murine models, this study will expand the possibility of the usage of mice in the study of portal hypertension.

We found that knockout mice lacking eNOS and both eNOS and iNOS still developed similar hemodynamic characteristics to those seen in wild-type animals with portal hypertension. Our results strongly suggest that other vasodilator molecule(s) may compensate for the lack of NO. Additionally, this finding may suggest that the long-term inhibition of NO may not be enough to treat the vasodilatory syndrome observed in cirrhosis.

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The blockade of other vasodilator(s), besides NO, would be necessary for the effective long-term treatment of the hyperdynamic syndrome of chronic liver disease.

MATERIALS AND METHODS

Animals

Knockout mice lacking the gene for eNOS (eNOS−/−) and their corresponding wild-type controls of the strain C57BL/6 (eNOS+/+) were purchased from Jackson Laboratories (Bar Harbor, ME) as breeders. Double knockout mice lacking both genes for eNOS and iNOS (eNOS/iNOS−/−) were obtained from Dr. V. Laubach (Department of Surgery, University of Virginia) as breeders. The genotype of the breeders was confirmed by Southern blotting and RT-PCR. Wild-type breeders of the strain C57BL/6x129J for double knockout control animals (eNOS/iNOS−/−) were purchased from Jackson Laboratories. Mice were maintained in a temperature-controlled environment with a 12:12-h light-dark cycle and allowed free access to water and rodent chow (Harlan Teklad, Indianapolis, IN) until the time of experimentation. All experiments were conducted in strict accordance with the guidelines specified by the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. Animals used were 6- to 8-wk-old male offspring of these breeders.

Induction of Portal Hypertension

A prehepatic portal hypertensive animal model extensively studied in our laboratory (6) was used. Portal vein ligation (PVL) to induce portal hypertension in mice was performed (16). In brief, the animals were anesthetized with ketamine hydrochloride (Ketalar, 100 mg/kg body wt; Parke-Davis, Avon, CT) and xylazine (10 mg/kg body wt; Phoenix Pharmaceutical, St. Joseph, MO). After a midline abdominal incision, the portal vein was freed from surrounding tissue. A ligature (silk gut 6-0) was placed around a 27-gauge blunt-tipped needle lying along the portal vein. Subsequent removal of the needle yielded a calibrated stenosis of the portal vein. In sham-operated mice, the same operation was performed, with the exception that, after the portal vein was isolated, no ligature was placed. After the operation, the animals were housed in plastic cages and allowed free access to food and water. Studies were performed 14 days after operation.

Western Blotting

To confirm the absence of eNOS and iNOS protein in knockout mice, aorta from eNOS−/− or eNOS/iNOS−/− and their corresponding wild-type control were isolated. Also, to test the effect of PVL on eNOS protein expression in mice, superior mesenteric arterial beds (SMA) were harvested at 2 wk after PVL (n = 5) or sham operation (n = 5), immediately frozen in liquid nitrogen, and kept at −80°C until analyzed. SMA samples were homogenized in a lysis buffer containing (in mM) 50 Tris-HCl, 0.1 EGTA, 0.1 EDTA, 5 sodium fluoride, 1 sodium pyrophosphate, 1 sodium vanadate, and 1 mmol 4-(2-aminoethyl)-benzenesulfonyl fluoride; protease inhibitor cocktail tablet (Roche Diagnostics, Mannheim, Germany), 1% (vol/vol) Nonidet P-40, 0.1% SDS, and 0.1% deoxycholate, pH 7.5. Protein content in the supernatants was quantified using the Lowry method with bovine serum albumin as a standard. The supernatants were subjected to the SDS-PAGE gel electrophoresis of proteins (20 μg), and Western blotting was performed as described (28) using antibodies that recognize eNOS MAb, iNOS MAb (Transduction Laboratories, Lexington, KY), and β-actin MAb (Sigma, St. Louis, MO). Enhanced chemiluminescence was used for protein detection. Intensity of the bands corresponding to the protein of interest was measured using a densitometer.

Hemodynamic Studies

On the day of experimentation, animals were weighed and anesthetized with ketamine hydrochloride (100 mg/kg) and fastened to a surgical board. Mean arterial pressure (MAP) was measured by cannulating the exposed left femoral artery with a polyethylene (PE)-10 (intravascular) catheter joined to a PE-50 (extravascular) tubing (Clay Adams; Becton Dickinson, Parsippany, NJ) connected to a pressure transducer (Hewlett-Packard, Andover, MA). The external zero reference was placed at the midportion of the mice. Cardiac output was then measured by the thermodilution technique. Briefly, a thermistor (F.1) was placed in the aortic arch by way of the left carotid artery, and the thermal indicator (20 μl of normal saline maintained at a temperature of 15° or more below body temperature) was injected into the right atrium through a PE-10 catheter placed into the right jugular vein. The aortic thermistor was connected to a cardiac output computer (Columbus Instruments, Columbus, OH). Body temperature was maintained at 37.0 ± 0.2°C. At least three thermodilution curves were obtained for each cardiac output measurement, discarding those curves with unusual morphology. A typical curve had a rapid upstroke and a smooth decay. The final cardiac output value was obtained from the arithmetic mean of at least three cardiac output measurements. Cardiac index was calculated as cardiac output per 100 g body wt. Systemic vascular resistance was calculated from MAP divided by cardiac index. Right atrial pressure was regarded as negligible. Portal pressure (PP) was measured by inserting the tip of a 30-gauge needle into the superior mesenteric vein. The needle length was joined to a short length of PE-10 tubing, which in turn was joined to PE-50 tubing and connected to a Hewlett-Packard pressure transducer. All hemodynamic readings were monitored and saved on a computer using the analog-to-digital MacLab system (ADInstruments, Milford, MA).

To further confirm the absence of the eNOS gene in the double knockout mice, an injection of Nω-nitro-L-arginine (1-NNa) (Sigma) dissolved in normal saline (12 mg/kg body wt) was made into the jugular vein catheter before death of the knockout mice and control. The subsequent effect of NNA on MAP was noted.

Additionally, to exclude the possibility of nNOS induction in eNOS−/− or eNOS/iNOS−/− with PVL surgery, 1-NNa was also injected in those animals. The changes in MAP were determined after 1-NNa injection.

Portal-Systemic Shunting

Portal-systemic shunting was estimated in at least 10 animals in each group by the splenic pulp injection of ~30,000 141Ce-labeled microspheres (15.5 ± 0.1 μm; New England Nuclear, Boston, MA), mixed in 0.1 ml of normal saline as previously described (6).

Statistics

Results were expressed as means ± SE. Statistical analyses were performed using the unpaired Student’s t-test and the Bonferroni-Dunn ANOVA.
RESULTS

A total of 32 wild-type control mice (eNOS+/+), 31 eNOS−/− knockout mice, 28 wild-type controls for eNOS/iNOS−/− mice (eNOS/iNOS+/+), and 35 eNOS/iNOS−/− mice were studied. Besides genotyping, Western blot analysis for eNOS or iNOS confirmed the knockout genotypes of the breeders that bred the mice used in the experiments (Fig. 1). Mean body weight of the wild-type control mice was no different from that of the eNOS−/− mice (30.2 ± 0.75 vs. 30.2 ± 2.58 g). Similarly, mean body weight of eNOS/iNOS−/− mice (27.1 ± 1.17 g) was no different from that of the wild-type control mice (27.4 ± 1.29 g).

We also tested the hemodynamic characteristics of wild-type control and knockout mice. Both types of knockout mice were hypertensive with elevated MAP, as also reported by others (3, 10, 20): MAP of wild-type control (eNOS+/+) and knockout eNOS−/− mice were 77.27 ± 1.4 and 124.3 ± 5.6 mmHg (P = 0.0001), respectively; MAP of wild-type control (eNOS/iNOS+/+) and knockout eNOS/iNOS−/− mice were 117.2 ± 4.4 and 151.9 ± 7.1 mmHg (P = 0.001), respectively. Perfusion of the NOS inhibitor (L-NNA) raised the MAP of all the wild-type animals tested, whereas that of knockout mice did not change, confirming the lack of NO production in knockout animals.

Expression of eNOS Protein in PVL Animals

It has been shown that eNOS protein expression is upregulated in the splanchnic circulation in portal hypertensive rats and rabbits (4, 5, 15, 27, 28). It is not known whether this is also the case for a mouse model of PVL. Western blot analysis indicated that PVL significantly increased eNOS protein expression in the SMA compared with that of sham-operated mice (P < 0.05). Similar to SMA of rats, eNOS protein is significantly upregulated in the splanchnic circulation in mice with chronic portal hypertension (Fig. 1).

Effect of PVL on Hemodynamic Characteristics

Wild-type control for eNOS−/− mice. Portal-systemic shunting increased in the PVL group compared with the sham-operated group (91.8 ± 4.3 vs. 0.2 ± 0.1%, P = 0.0001). The PVL resulted in significantly increased PP compared with the sham-operated group (8.1 ± 0.6 vs. 4.7 ± 0.3 mmHg, P = 0.003). Similarly, the PVL group showed a significantly increased cardiac index (CI) compared with that of the sham-operated group (534.0 ± 25.9 vs. 407.5 ± 33.9 mmHg; P = 0.008). The systemic vascular resistance (SVR) of the PVL group significantly decreased compared with the sham-operated group (0.133 ± 0.008 vs. 0.192 ± 0.03 mmHg·min·kg body wt⁻¹·m⁻¹, P = 0.03). These data strongly suggest that, similar to rats (7, 26), mice developed the hyperdynamic circulatory state as a result of PVL (Fig. 2).

eNOS knockout mice. Portal-systemic shunting significantly increased in the PVL group compared with the sham-operated group (58.6 ± 15.7 vs. 0.3 ± 0.17%, P = 0.03). We then compared factors associated with the hyperdynamic circulatory state, including MAP, CI, SVR, and PP, between sham-operated and PVL groups (Fig. 3). PVL caused a significant twofold increase in PP (P = 0.0001) compared with that of the sham-operated group (8.1 ± 0.5 vs. 4.6 ± 0.1 mmHg). CI of the PVL group significantly increased compared with that of the sham-operated group (369.3 ± 16.9 vs. 311.4 ± 11.4 ml·min⁻¹·kg body wt⁻¹, P = 0.01). The SVR of the PVL group was significantly lower than that of sham-operated eNOS−/− group (0.33 ± 0.02 vs. 0.41 ± 0.03 mmHg·min·kg body wt·m⁻¹, P = 0.04). Collectively, these data demonstrate that eNOS−/− mice also developed the hyperdynamic circulatory state as a result of PVL.

Wild-type control for eNOS/iNOS−/− mice. Portal-systemic shunting increased in the PVL group compared with the sham-operated group (72.0 ± 18.4 vs. 0.4 ± 0.13%, P = 0.0046). The PVL resulted in significantly increased PP compared with the sham-operated group (7.1 ± 0.8 vs. 4.9 ± 0.2 mmHg, P = 0.015).
Similarly, the PVL group showed a significantly increased CI compared with that of the sham-operated group (474.1 ± 21.9 vs. 416.4 ± 14.3 mmHg, \(P = 0.04\)). The SVR of the PVL group significantly decreased compared with the sham-operated group (0.242 ± 0.009 vs. 0.284 ± 0.009 mmHg·min·kg body wt·ml\(^{-1}, P = 0.005\)). These data strongly suggest that, similar to rats (7, 26), these wild-type mice also developed the hyperdynamic circulatory state as a result of PVL (Fig. 4).

\textit{eNOS/iNOS} \(-/-\) mice. As also seen in eNOS \(-/-\) mice, PVL resulted in the developed hyperdynamic circulatory state in eNOS/iNOS \(-/-\) mice (Fig. 5). Increased portal-systemic shunting was observed in the PVL group.
compared with the sham-operated group (84.8 ± 12.1 vs. 1.1 ± 0.7%, P = 0.002). PVL resulted in a significant increase in PP compared with that of the sham-operated group (6.3 ± 0.3 vs. 5.2 ± 0.3 mmHg, P = 0.016). PVL significantly increased CI compared with the sham group (370.2 ± 22.8 vs. 287.4 ± 11.0 ml·min⁻¹·kg body wt⁻¹, P = 0.004). The peripheral resistance of the PVL group significantly decreased compared with the sham group (0.44 ± 0.02 vs. 0.54 ± 0.04 mmHg·min·kg body wt·ml⁻¹, P = 0.03). Collectively, these data suggest that PVL also resulted in the development of the hyperdynamic circulatory state in eNOS/iNOS−/− mice.

Fig. 4. PVL caused the development of a hyperdynamic circulatory state in the wild-type controls for the eNOS/iNOS−/− mice. n = 12 Sham-operated mice; n = 13 PVL mice.

Fig. 5. PVL caused the development of a hyperdynamic circulatory state in eNOS/iNOS−/− knockout mice. n = 11 Sham-operated mice; n = 15 PVL mice.
Fig. 6. NOS inhibition by l-NNA caused a slight decrease in MAP of CI, PP, and SVR after the PVL operation, which were out mice, we determined the percent changes in MAP, the wild-type controls and their corresponding knockout mice (see Table 2). To compare the levels of the line levels are different between wild-type and knockout mice. These data strongly suggest that mice lacking eNOS and iNOS still developed the hyperdynamic circulatory state.

Effects of a NOS Inhibitor on MAP

To test the involvement of nNOS in knockout mice with PVL, l-NNA (a nonspecific NOS inhibitor) was injected, and MAP was measured. l-NNA did not increase MAP in either eNOS−/− or eNOS/iNOS−/− knockout mice with PVL. This result ruled out the involvement of nNOS in the development of hyperdynamic circulation in the PVL knockout mice (Fig. 6).

DISCUSSION

We found that mice lacking eNOS or eNOS/iNOS genes still developed the hyperdynamic circulatory state after partial PVL. Our results may suggest that involvement of another vasodilator(s), besides NO, are involved in the development of the hyperdynamic circulatory state, a hallmark of portal hypertension. Furthermore, when NO is lacking, this vasodilator(s) plays a significant role in enhanced vasodilatation in the systemic and splanchnic circulation in portal hypertensive animals.

Table 1. Percent changes in hemodynamic indicators due to the portal vein ligation

<table>
<thead>
<tr>
<th></th>
<th>Wild-type control</th>
<th>eNOS−/−</th>
<th>P value*</th>
<th>Wild-type control</th>
<th>eNOS/iNOS−/−</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, %</td>
<td>–6.3</td>
<td>–0.9</td>
<td>0.60</td>
<td>–7.8</td>
<td>–3.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Cardiac index, %</td>
<td>+18.4</td>
<td>+37.6</td>
<td>0.31</td>
<td>+16.0</td>
<td>+28.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Portal pressure, %</td>
<td>+126.9</td>
<td>+72.9</td>
<td>0.13</td>
<td>+36.0</td>
<td>+37.7</td>
<td>0.89</td>
</tr>
<tr>
<td>Systemic vascular resistance, %</td>
<td>–15.7</td>
<td>–13.7</td>
<td>0.91</td>
<td>–11.7</td>
<td>–19.8</td>
<td>0.47</td>
</tr>
</tbody>
</table>

%Changes in hemodynamic indicators as a result of portal vein ligation were determined in each group. *Statistical comparison between wild-type control and eNOS−/− mice; †Statistical comparison between wild-type control and eNOS/iNOS−/− mice. eNOS, endothelial nitric oxide synthase; iNOS, inducible NOS.

Magnitude of Development of Hyperdynamic Circulation

Values of hemodynamic characteristics at their baseline levels are different between wild-type and knockout mice (see Table 2). To compare the levels of the development of the hyperdynamic circulation between the wild-type controls and their corresponding knockout mice, we determined the percent changes in MAP, CI, PP, and SVR after the PVL operation, which were then compared between the wild-type and knockout mice (Table 1). The percent changes in those factors were no different between wild-type and knockout animals, suggesting that knockout mice developed the hyperdynamic circulatory state to an extent similar to that observed in wild-type control mice. Collectively, these data strongly suggest that mice lacking eNOS and iNOS still developed the hyperdynamic circulatory state.

Table 2. Physiological and hemodynamic characteristics of wild-type control and eNOS/iNOS−/− knockout mice

<table>
<thead>
<tr>
<th></th>
<th>Wild-type control</th>
<th>Knockout eNOS/iNOS−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>27.1 ± 1.7</td>
<td>27.4 ± 1.29</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>117.2 ± 4.4</td>
<td>151.9 ± 7.1</td>
</tr>
<tr>
<td>Cardiac index, ml/min·kg body wt−1</td>
<td>416.4 ± 14.3</td>
<td>287.4 ± 11.0</td>
</tr>
<tr>
<td>Portal pressure, mmHg</td>
<td>4.9 ± 0.1</td>
<td>5.22 ± 0.2</td>
</tr>
<tr>
<td>Systemic vascular resistance*</td>
<td>0.284 ± 0.009</td>
<td>0.54 ± 0.04</td>
</tr>
</tbody>
</table>

All values were obtained from the sham-operated animals. *Systemic vascular resistance is in mmHg·min·kg body wt·ml−1. aP < 0.05; bP < 0.001.
NO is a vasodilator that regulates vascular tone (11). eNOS is the key NOS isoform that regulates blood pressure (10). Thus knockout mice lacking the eNOS gene demonstrate abnormally increased vascular resistance; thereby, they are arterial hypertensive (Table 1). In contrast, mice lacking the iNOS gene only do not develop hypertension. However, to further rule out the possibility of iNOS involvement in arterial vasodilation in portal hypertensive animals, we also studied the effects of PVL on mice lacking both eNOS and iNOS genes. The partial portal vein ligation is a commonly used method to study hemodynamic characteristics of portal hypertension and has been used by our laboratory and others in rats (7, 9, 13, 19, 22–24, 26, 28) and by us in mice (16). Animals with PVL produce similar hemodynamic characteristics to those seen in chronic liver diseases with portal hypertension. Those characteristics include reduced peripheral resistance due to increased vasodilation owing to an excessive NO production in the splanchnic and systemic circulation (1, 15). Of the three isoforms, eNOS, not iNOS, plays a central role in vasodilation in the splanchnic and systemic circulation and contributes to the development of the hyperdynamic circulatory state (4, 5, 15, 27, 28). Thus we hypothesized that knockout mice lacking eNOS could be resistant to the development of the hyperdynamic circulation due to the lack of vascular NO production. In contrast to our hypothesis, PVL still led to the development of the hyperdynamic circulatory state in knockout mice to a similar extent to that observed in wild-type control animals (Table 2).

We also tested the possibility of compensatory NO production by nNOS. l-NNA, known to produce arterial hypertensive responses in mice, did not cause any increase in arterial pressure in PVL knockout mice (Fig. 6). If nNOS had a significant role in the compensatory mechanism observed in PVL knockout mice, l-NNA would have induced an increase in arterial pressure. Therefore, a compensatory role of nNOS is unlikely to account for our results.

Until now, in experimental animals, studies about the hyperdynamic state have been done mainly in rats. To our knowledge, this is the first demonstration of a study of the hyperdynamic state using a mouse model. Utilization of murine models will enable us to explore the role of specific gene products in the hyperdynamic circulatory state. In rats with chronic portal hypertension, studies have shown an upregulation of eNOS protein expression in the splanchnic circulation. We demonstrated that in wild-type mice there is a significant upregulation of eNOS protein expression, similar to the rat model of portal hypertension, suggesting the similarity in the effects of PVL between rats and mice.

Baseline hemodynamic characteristics (such as MAP, CI, and resistance) as expected are significantly different between wild-type and knockout mice. Thus the percent changes in each factor between sham-operated and PVL may be a better indicator for the evaluation of the development of the hyperdynamic circulatory state (Table 2). The percent changes in each hemodynamic factor as a result of PVL were not different between wild-type and knockout animals, suggesting that knockout mice developed the hyperdynamic circulatory state to an extent similar to that observed in wild-type animals.

Our results suggest that, in the absence of NO, one or more compensatory vasodilator(s) seems to be upregulated and play a role in the reduction of peripheral resistance in portal hypertensive animals. The presence of a vasodilator(s) distinct from NO has been suggested in vessels of both wild-type and eNOS knockout mice (2, 8, 17, 18, 25). A compensatory role by the endothelium-dependent hyperpolarization factor (EDHF) in the deficiency of NO has been suggested in mesenteric vessels (17, 18) and mouse hindlimb (2). In coronary circulation and skeletal muscle arterioles, endothelial vasodilator prostaglandins are upregulated in the absence of eNOS (8, 25). Considering this evidence, it is highly possible that compensatory vasodilators, such as prostaglandins, EDHF, or another molecule, may be upregulated and consequently reduce the peripheral resistance in eNOS and eNOS/iNOS knockout mice with PVL in our study.

In conclusion, a compensatory vasodilator molecule seems to be upregulated in place of NO in the systemic and splanchnic circulation in portal hypertensive knockout animals. Although studies have shown the beneficial effects of NO inhibitors for the treatment of the hyperdynamic circulation in portal hypertension in both human and animal models (12, 14), it would be important to take into account this compensatory response by vasodilators that seems to replace the chronic absence of NO production and to lead to the development of the hyperdynamic circulation. Thus an inhibition of NO may not be enough for the chronic treatment of hyperdynamic circulatory syndrome observed in portal hypertension. This compensatory mechanism in an NO-depleted vasculature may need to be determined for the development of effective pharmacological agents that can reduce the occurrence of the hyperdynamic circulatory syndrome.

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REFERENCES

