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1Institute of Health Sciences, Quinta da Granja, Monte da Caparica, 2825 Portugal, Spain; and 2Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

Macedo, M. Paula, and W. Wayne Lautt. Shear-induced modulation of vasoconstriction in the hepatic artery and portal vein by nitric oxide. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G253–G260, 1998.—The effect of shear stress on nitric oxide (NO)-mediated suppression of sympathetic nerve (2–6 Hz) and norepinephrine (0.5 μg·kg–1·min–1)-induced vasoconstriction in the hepatic artery (HA) and portal vein (PV) was studied using a perfusion circuit to regulate blood pressure and flow in the cat liver in situ. Holding flow constant resulted in increased shear stress during constriction; holding pressure steady prevented changes in shear stress. When shear stress was allowed to rise, the vasoconstriction (indicated by elevation in perfusion pressure) in response to nerve stimulation and norepinephrine was significantly potentiated after NO synthase blockade using Nω-nitro-l-arginine methyl ester (l-NAME, 2.5 mg/kg iv) in both the HA and PV (response to nerves: HA control 28.8 ± 6.5 mmHg, l-NAME 62.7 ± 14.6 mmHg; PV control 1.5 ± 0.5 mmHg, l-NAME 3.3 ± 0.5 mmHg; response to norepinephrine: HA control 32.4 ± 9.0 mmHg, l-NAME 60.3 ± 8.0 mmHg; PV control 1.3 ± 0.3 mmHg, l-NAME 3.4 ± 0.7 mmHg). The potentiation was reversed by l-arginine (75 mg/kg). When shear stress was held constant by maintaining constant perfusion pressure, l-NAME did not cause potentiation of vasoconstriction. The data are consistent with the hypothesis that elevated shear stress in the hepatic blood vessels leads to NO-dependent postjunctional modulation of vasoconstriction.

Shear stress; neuromodulation; blood flow; portal pressure

VASCULAR REGULATION by nitric oxide (NO) is dependent on the activation of the NO synthase III present in endothelial cells either by hormonal or physical stimuli such as shear stress (23, 25). Although the modulation of peripheral resistance by NO has been implicated to be due to relaxation of the vascular smooth muscle, it has also been shown that NO modulates sympathetic nerve actions in perfused pulmonary vessels and superior mesenteric artery (SMA), as well as in isolated segments of both rabbit carotid artery and rat tail artery (5, 6, 16, 30). The NO influence on prejunctional vs. postjunctional effects on sympathetic neurotransmission appears to be organ specific. In the canine pulmonary arteries and veins and SMA, NO was suggested to modulate sympathetic nerve activity via a prejunctional mechanism (6, 16), leading to suppression of transmitter release. On the other hand, in the dog temporal artery, the rat caudal artery, and the rat tail artery, a postjunctional effect seems to be the responsible mechanism (3, 31, 32). Even though different studies showed a neuromodulatory role for NO, only recently was the trigger mechanism responsible for this effect demonstrated. Shear stress resulting from vasoconstriction is the stimulus responsible for the NO release that regulates sympathetic nerve effects in the SMA so that in the absence of an increase in shear stress no modulation occurs (16). We showed that if vasoconstriction is induced in a situation where blood flow is allowed to decline and perfusion pressure is not elevated, shear stress does not increase. In contrast, if flow is held steady, shear stress increases at the site of the constriction and leads to NO release (16). Under normal conditions, a local vasoconstriction will lead to reduced flow with minimal or no increase in shear stress. In the liver, in contrast, although this situation holds for the hepatic artery (HA), constriction of the portal vessels results in increased portal pressure with no decrease in portal flow.

Thus it is important to determine whether NO modulates vasoconstriction in the liver and, if so, by what mechanism. To achieve this objective, an in situ blood-perfused feline liver was used as a model with a perfusing circuit incorporated to obtain situations of varying shear stress separately in the HA and portal vein (PV). Our results are consistent with the hypothesis that the NO suppression of sympathetic nerve activity is dependent on the shear stress in the vessel and that the site of modulation for both the HA and PV is postjunctional, in contrast to the SMA, where the modulation is prejunctional. The data are consistent with previous reports of organ-selective mechanisms of NO-mediated vascular regulation in the liver and intestine (18).

MATERIALS AND METHODS

Cats of either sex (3.3 ± 0.2 kg) were fasted overnight and were anesthetized with pentobarbital sodium (32.5 mg/kg) administered intraperitoneally. During and after the surgical procedure, anesthesia was maintained using a drip bag (390 mg pentobarbital sodium/500 ml saline). The use of continuous diluted doses of anesthetic minimizes secondary effects of large bolus doses on the splanchnic vasculature. To verify that the animal was in deep anesthesia, limb reflexes, ear and eye movements, and jaw tone were checked frequently. Body temperature was maintained at 37.5°C by the use of a rectal probe and a thermal control unit (Yellow Springs Instruments model 72) operating heating rods in the table. All cannulas were heparinized. Heparin (3,000 IU) was administered before the vascular circuits were established.

The vascular circuit consisted of two cannulas in the femoral arteries from which blood was pumped by a variable speed pump (Master-flex; Cole Parmer, Chicago, IL) to control the blood flow rate through the circuit to the HA. The blood passed through a silk filter and a windkessel chamber to trap air bubbles as well as to buffer pressure fluctuations gener-
ated by the pump. A Y branch was placed in the circuit with an electrical ground for the flow probe in one branch and the electromagnetic flow probe (Carolina Medical Electronics 300AP-1/8) in the other branch to allow blood flow to be diverted around the flow probe to obtain a zero baseline. A cannula for monitoring the circuit pressure and a multilobe infusion cannula were situated proximal to the catheter that delivered blood to the HA. The circuit was sterilized and filled with saline 1 day before use.

**Surgical Methodology**

Blood pressures were monitored via catheters using Gould and Statham pressure transducers, and all parameters were recorded on a Sensor Medic R611 Dynograph. Transducers were set to zero reference level relative to the midpoint of the inferior vena cava at the hepatic outlet. The zero reference was checked for drifting periodically throughout the experiment. Systemic arterial pressure and central venous pressure were monitored via a right carotid arterial catheter and a left femoral venous catheter, respectively. The trachea was cannulated, and ventilation was controlled by the use of a respirator. A recovery time of 45–60 min was allowed before the first experimental maneuver.

Via laparotomy, the inferior mesenteric artery was ligated and the spleen was removed. The periaortic nerve bundle of the SMA was gently separated, ligated, and cut. An electromagnetic flow probe (Carolina Medical Electronics EP408) and a micrometer-controlled screw clamp were placed on the artery. J just before the HA circuit was established, the celiac artery was ligated. Anastomotic connections to the SMA provided adequate blood supply to areas normally supplied by the ligated vessels. This methodology assures that all blood supplying the PV derives from the SMA, and thus SMA flow was synonymous with portal blood flow as previously described (14). The HA was isolated, and the gastroduodenal artery was isolated and ligated. The nerve bundle around the HA was gently separated, ligated, and cut, and the peripheral end was placed in a circular bipolar stimulating electrode. The vascular long circuit was connected between the femoral arteries and the HA. HA pressure was monitored from a catheter incorporated into the circuit, and drugs were administered through a separate infusion line.

**Nonsurgical Methodology**

The circuit allowed control and measurement of blood flow. Shear stress was increased or maintained constant by controlling the circuit blood flow. Increase in shear stress was achieved by maintaining the flow delivered to the HA constant when vasoconstriction was induced. Shear stress was prevented from rising by decreasing blood flow delivered to the vein during vasoconstriction to maintain a constant portal pressure.

The PV was cannulated via the cecal vein for drug administration and by puncture with a 24-gauge 1/2-in. J oiv intravenous catheter to monitor portal pressure. Intravenous infusions were made directly into the inferior vena cava through a cannula placed in the femoral vein.

Circuit pressure-flow relationships were determined at the end of each experiment to account for resistance to flow in the circuit. All reported pressures were corrected for circuit resistance.

**Protocol 1.** Sympathetic nerve stimulation (2–6 Hz) for HA and PV (15 V square pulse, 1-ms duration) was performed under conditions of constant flow or constant pressure. The responses are reported for nerve frequencies of 2–6 Hz because the frequency used in each cat was determined from a series of stimulations at the beginning of the experiment to select a frequency that produced ~50% of the maximal vascular response (H$_{50}$). Analogous to pharmacodynamics, using a 50% effective dose, the H$_{50}$ zone of the frequency response curve is best suited to detect modulation of nerve-induced responses. Responses to nerve stimulation were determined in a control condition, after intravenous bolus administration of N$^\text{N}$-nitro-$\text{L}$-arginine methyl ester (L-NAME, 2.5 mg/kg) and after an intravenous bolus administration of $\text{L}$-arginine (75 mg/kg) to reverse the action of L-NAME. This dose of L-NAME was previously shown to block shear stress-induced production of NO in the SMA and to induce systemic hypertension. L-arginine dose reversed these effects in cats in the same preparation (16, 17).

**Protocol 2.** In separate sets of experiments, a similar approach to protocol 1 was used but sympathetic nerve stimulation was replaced by norepinephrine infusion (0.50 μg·kg$^{-1}$·min$^{-1}$) into the PV. Intraportal venous administration of norepinephrine for studies related to both the HA and PV was selected for several reasons. Ideally one would prefer to have the concentration of norepinephrine remain constant throughout the period of infusion. In situations where blood flow changes, this is not possible; however, all comparisons were made during the peak of the constrictor response. Blood flow remained constant in the PV in conditions of increased shear stress, thereby eliminating changes in drug concentration as a confounding factor. In situations of constant portal pressure, portal blood flow was manually reduced during the norepinephrine infusion, but because shear stress was prevented from occurring during the vasoconstriction, L-NAME did not cause potentiation and therefore the blood flow attained in both situations was similar. It was unnecessary to carry out studies on the HA using separate arterial infusions because the HA is well known to respond to vasoactive substances administered through the PV, as verified in this study.

All drugs were obtained from Sigma Chemical (St. Louis, MO). L-NAME and L-arginine were dissolved in saline. Norepinephrine was dissolved in saline and diluted in a pH 5.6 ascorbic acid solution. All drugs were dissolved with the dose adjusted for the weight of the animal.

The results were expressed as means ± SE. Statistical analysis between groups was made using paired t-test or, when applicable, analysis of variance (ANOVA) for multiple comparisons followed by Tukey's honestly significant difference test. Data compared followed normal distribution. Differences were accepted as statistically significant at P < 0.05.
The cats were treated according to the Guidelines of the Canadian Council on Animal Care, and all protocols were approved by the Animal Use Committee of the University of Manitoba.

RESULTS

Mean arterial pressure, as measured from the carotid artery, was 103.8 ± 7.6 mmHg before and 144.3 ± 9.9 mmHg 20 min after L-NAME (P < 0.001, n = 12). With HA flow held constant using the circuit (9.4 ± 0.9 and 9.8 ± 1.2 ml·kg⁻¹·min⁻¹ before and after L-NAME), HA pressure (isolated from systemic pressure by the vascular circuit) was not altered by L-NAME (108.5 ± 6.6 and 112.7 ± 6.8 mmHg during the control state and L-NAME, respectively). The drug had no significant effect on central venous pressure, portal venous pressure, or portal flow. Thus basal tone in the HA and PV was not altered by L-NAME. Mean absolute pressures and flows for the HA and PV are shown in each of the figures in the control state, after L-NAME, and after L-arginine.

Sympathetic Nerve Stimulation

**HA responses.** Figure 1 represents the mean response of six cats to nerve stimulation under the state of constant flow. Vasoconstriction under constant flow results in elevated shear stress, and the constriction is assessed from the rise in perfusion pressure. Nerve stimulation in the control state caused the perfusion pressure to rise by 28.8 ± 6.5 mmHg. In the presence of L-NAME, the pressure rose by 62.7 ± 14.6 mmHg, which was greater than the control response (P < 0.01).

The observed potentiation was able to be reversed by L-arginine, as the increase in pressure in response to nerve stimulation was only 6.3 ± 3.0 mmHg (P < 0.001).

To determine if the constriction was increased in a situation where the shear stress was maintained constant, the sympathetic nerves were stimulated under constant pressure by reducing flow using the vascular circuit. The calculated mean values for the decrease in flow during nerve stimulation in the control state (2.5 ± 0.8 ml·kg⁻¹·min⁻¹), after L-NAME (3.8 ± 0.9 ml·kg⁻¹·min⁻¹), and after L-arginine (1.2 ± 1.1 ml·kg⁻¹·min⁻¹) were similar (Fig. 2). Thus L-NAME potentiated nerve-induced constriction in the HA if shear stress was allowed to rise but not if shear stress was held steady.

**PV responses.** Figure 3 represents the mean responses to nerve stimulation under constant flow (n = 6, elevated shear stress). The pressure increase in response to control nerve stimulation at constant flow was 15.7 ± 6.1 ml·kg⁻¹·min⁻¹, after L-NAME (12.3 ± 3.5 ml·kg⁻¹·min⁻¹), and after L-arginine (7.7 ± 1.8 ml·kg⁻¹·min⁻¹) as measured by a similar decrease in blood flow (Fig. 4).

Thus both the HA and PV showed potentiated responses to nerve stimulation after L-NAME if shear stress was allowed to rise but not if it was held steady.
Norepinephrine Infusion Experiments

HA responses. Responses to norepinephrine measured before (32.4 ± 9.0 mmHg), after L-NAME (60.3 ± 8.0 mmHg), and after L-arginine (23.8 ± 9.1 mmHg) at a constant flow (increased shear stress) are shown in Fig. 5 (P < 0.01). The reduction in flow, as the index of vasoconstriction, observed during the effect of norepinephrine infusion at a constant pressure (shear stress not increased) was 3.2 ± 2.6 and 4.1 ± 0.6 ml·kg⁻¹·min⁻¹ for control and after L-NAME and 3.4 ± 0.3 ml·kg⁻¹·min⁻¹ after L-arginine as represented in Fig. 6 (no significant differences).
PV responses. The index of vasoconstriction, represented by the increase in perfusion circuit pressure with portal flow held constant (shear stress increased), due to norepinephrine infusion was $1.3 \pm 0.3$ mmHg for the control, $3.4 \pm 0.7$ mmHg after L-NAME, and $1.4 \pm 0.2$ after L-arginine ($P < 0.05$, Fig. 7).

Flow decreased during norepinephrine infusion under constant pressure (shear stress not increased) by $32.9 \pm 9.4$ ml·kg$^{-1}$·min$^{-1}$ in the control state, $28.5 \pm 6.3$ ml·kg$^{-1}$·min$^{-1}$ after L-NAME, and $21.6 \pm 10.4$ ml·kg$^{-1}$·min$^{-1}$ after L-arginine, and there was no statistical difference among the three responses (Fig. 8).

Thus norepinephrine-induced constriction was potentiated by L-NAME in both the PV and HA only when shear stress was allowed to increase.

**DISCUSSION**

These studies provide evidence that NO plays an important role in modulating the sympathetic nervous system when sympathetic nerve activation results in increased vascular shear stress and that the mechanism of regulation is organ specific. After inhibition of NO synthase activity with L-NAME, systemic arterial pressure, but not HA or portal venous basal tone, was increased. In the liver, NO released due to shear stress modulated vasoconstriction promoted by both the sympathetic nervous system and exogenous norepinephrine. Because the amplitude of the potentiation is similar for sympathetic nerves and norepinephrine, it is suggested that in the liver NO acts postjunctionally. To the best of our knowledge, this is the first in vivo study performed in the liver circulation showing endogenous NO as a modulator of vasoconstriction in a shear stress-dependent manner.

**Methodological Considerations**

Shear stress is caused by the movement of fluid past the endothelial cells. A decrease in diameter at a constant arterial flow augments shear stress because of its direct relationship to flow and inverse relationship to the third power of the internal diameter of the blood vessel (10). Inability to quantitate the absolute level of shear stress was a limitation of this preparation. However, it was possible to quantitate the vascular responses to sympathetic nerve stimulation or norepinephrine infusion in the presence and absence of changes in shear stress by using a pump-controlled long circuit. In the case of constant flow during vasoconstriction, an increase in shear stress occurs (16). When vasoconstriction was induced under constant perfusion pressure, flow was reduced using the perfusion pump such that vascular shear stress was maintained constant (16). Thus, during increases in shear stress, the response was assessed as an increase in perfusion pressure. Conversely, when perfusion pressure and shear stress were maintained constant, the responses were assessed as a decrease in blood flow.

L-NAME was chosen as one of the more potent stereospecific inhibitors of NO biosynthesis (26). Although the effect of the same dose of L-NAME on the basal tone of the HA was dramatically different from other vascular beds, adequacy of the dose was supported by the effect on systemic arterial pressure and by the ability to block shear stress-induced inhibition of HA constriction. The effect of L-NAME on the HA basal tone was insignificant and confirms results obtained in other types of preparations (2, 8, 18–20). Greenblatt et al. (8) observed a decrease in HA resistance and an
increase in HA blood flow as PV blood flow decreased in conscious rats. The response of the HA was likely a consequence of the HA buffer response, whereby a decrease in portal blood flow produces an increase in HA blood flow via an adenosine-mediated mechanism previously described (14). Portal flow was not significantly affected in the present study. This lack of effect on the HA vascular tone could be because either there is less synthesis or basal release of NO, there is a lack of the specific NO synthase enzyme responsible for the basal tone, or the HA has less smooth muscle guanylate cyclase (9).

Effects of L-NAME and L-Arginine on Sympathetic Nerve-Induced Constriction

Vasoconstriction due to nerve stimulation was potentiated when shear-dependent NO release was inhibited in both the PV and HA (Figs. 1 and 3). This observation could be explained either by a shear-dependent NO release acting directly on the nerves or by the action of NO on the constricted vascular smooth muscle. These alternatives are resolved by the responses to norepinephrine.

Effects of L-NAME on Norepinephrine-Induced Constriction

The vasoconstriction induced by intraportal infusion of norepinephrine was also potentiated in the HA and PV after the NO synthase had been blocked by L-NAME. Such effects were observed only when shear stress was increased (Figs. 5 and 7). We conclude that the NO effect in these vessels was directly on the contracted smooth muscle because the extent of potentiation was similar with both nerve stimulation and norepinephrine infusion.

Related Studies

NO modulates vascular responses to nerve stimulation in an endothelium-dependent manner (5, 6, 16, 24, 28, 30, 34). The absence of the endothelial cells enhanced the responses to nerve stimulation in perfused pulmonary vessels and in isolated segments of both rabbit carotid artery and rat tail artery (5, 6, 30). Observations contrary to the previous studies were reported by Wennmalm et al. (33) and Thatikunta et al. (29) in the coronary artery and external anal sphincter, respectively. However, direct comparison is difficult because of a lack of control of shear stress. Thus many but not all of the studies with isolated vessels suggest a role for NO suppression of norepinephrine-induced constriction that is uncovered by blockade of NO synthase. This demonstration, combined with the in vitro (4, 11–13) and in vivo (1, 22, 34) demonstration of shear stress-dependent release of NO, makes the interpretation of contradictory results difficult.

The prejunctional vs. postjunctional effects of NO on the modulation of vasoconstriction appear to be organ specific. In the canine pulmonary arteries and veins and in the feline mesenteric artery (6, 16) NO was shown to act prejunctionally. In the kidney, Yasuhiro et al. (34) have also shown that increments in renal sympathetic nerve activity as well as norepinephrine release occur in the presence of L-NAME, suggesting a prejunctional effect of NO. The prejunctional mechanism has been suggested to be an NO-induced decrease in norepinephrine release (7, 15, 16). Conversely, in the dog temporal artery and rat tail artery a postjunctional effect seems to be the responsible mechanism (3, 31), consistent with the observations in the liver.

Organ Selectivity

Organ selectivity clearly exists based on the different results in the HA and PV compared with the data obtained with the same protocol for the SMA (18). These studies may explain some of the discrepancy in the literature in that we show that NO-dependent inhibition of nerve-induced vasoconstriction occurs only in conditions where shear stress is increased, and in many previous studies shear stress cannot be evaluated. Both HA and PV appear to behave in a different fashion than other vessels in relation to the involvement of NO regulation of basal tone. In this study it was observed that NO is involved in a shear-dependent manner in the regulation of vasoconstriction but not in the basal tone in the HA and PV. We therefore propose independent mechanisms for the regulation of the basal tone and modulation of vasoconstriction. In the SMA NO produced by shear stress modulates nerve but not exogenous norepinephrine-induced constriction perhaps because the NO is released at the smallest resistance vessels, which are least sensitive to NO (27). In the liver NO is released because of shear stress to modulate vasoconstriction, but we speculate that the enzyme is not preponderantly present in the small vessels but at larger vessels and NO therefore regulates vasoconstriction at the smooth muscle preferentially.

The liver per se does not control portal blood flow. Local vasoconstriction in vivo leads to elevated portal pressure but unchanged portal flow. This phenomenon can lead to conditions of high shear stress in the PV. The HA blood flow is modulated locally secondary to changes in the portal venous blood flow by an adenosine-dependent mechanism (the hepatic arterial buffer response) (14). In this case, if the HA basal tone was dependent on NO levels, the buffer response would become dependent not only on the portal blood flow but also on the vascular tone of the PV and basal levels of NO. The lack of NO effect on the HA basal tone would appear to uniquely allow the HA to be under local regulation that has evolved to maintain a total hepatic flow as constant as possible. However, vasoconstriction is locally modulated both in the intestine and liver by NO to protect the delicate endothelial cells from excessive shear stress.

Other evidence of organ-selective vascular regulation by NO is seen in the liver and intestine. In the absence of NO there is a potentiation of adenosine vasodilatory properties in the intestine but not in the liver (18). Another dissimilarity between the two vascular beds is that NO antagonizes autoregulation in the intestine.
(17) but not in the liver (unpublished observations). All the discrepancies observed suggest that there are different mechanisms involved in the release and physiological function of NO, one that is shear stress-dependent and modulates vasoconstriction and another that modulates basal tone, autoregulation, and the vasodilatory effects of adenosine.

In conclusion, the L-NAME-induced potentiation of vasoconstriction induced by norepinephrine infusion, seen only in the constant flow (shear stress elevated) protocol, suggests that elevated shear stress results in release of NO that suppresses constriction by acting directly on the vascular smooth muscle in these blood vessels. From our study we cannot assess whether shear stress in one vessel is capable of modulating effects in the other vessel. In the liver, the release of NO might be at larger vessels rather than at the smallest vessels, which are less sensitive to NO and where NO could have closer contact with the nerve endings. Our findings have implications for both the physiological and pathophysiological control of vascular resistance in the liver. Impairment of endothelial cells would result in reduced ability of the vessels to defend themselves against increased shear stress, thereby aggravating endothelial damage. Hepatic endothelial dysfunction in pathological states known to be associated with endothelial and NO dysfunction has not been evaluated.

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REFERENCES


