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Haemodynamic and hormonal responses to long-term inhibition of nitric oxide synthesis in rats with portal hypertension

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Abstract

In portal hypertension, the role of the vasorelaxant nitric oxide (NO) in long-term splanchnic and systemic vascular tone regulation is unclear. This study examined the effects of long-term administration of a NO synthesis inhibitor on haemodynamics in portal hypertensive rats. Rats were randomly assigned to receive either water (placebo) or 100 mg/kg · day of oral *N*-nitro-L-arginine methylester (L-NAME) for 28 days. At 14 days, the portal vein was ligated in 10 rats from each group. At 28 days, splanchnic and systemic blood flows were measured in 20 normal and 20 portal vein stenosed rats. Plasma atrial natriuretic peptide (ANP) concentrations as well as plasma and urinary cyclic guanosine monophosphate (cGMP) levels were also measured. Porto-systemic shunts were measured in other portal vein stenosed animals that had or had not received L-NAME. Portal vein stenosed rats that received L-NAME had significantly lower portal tributary blood flow and percentages of portal-systemic shunting (7.3 ± 0.5 versus 3.7 ± 0.2 ml/min · 100 g and 96 ± 1 versus $68 \pm 5\%$, respectively) and higher hepatocollateral vascular resistance (147 ± 10 versus 295 ± 30 dyn · s · cm^{-5} · 100 g · 10^3, respectively) than placebo portal vein stenosed rats. Portal pressure, ANP and cGMP levels did not differ between the groups. Arterial pressure was significantly higher and cardiac index lower after L-NAME than after placebo. Normal rats had similar but less marked L-NAME-induced responses than portal hypertensive rats. The presence of a long-term L-NAME-induced vasoconstriction in collateral vessels and splanchnic and systemic arterioles in portal vein stenosed rats indicates that a NO-mediated vasodilator tone may contribute to the development and the maintenance of collateral circulation as well as splanchnic and systemic vasodilation in portal hypertension. Moreover, the NO-mediated vasodilator tone in portal hypertensive animals seems to be increased.

Keywords: Portal hypertension; Baseline vascular tone; EDRF (endothelium-derived relaxing factor)

1. Introduction

In animals with portal hypertension, splanchnic vasodilation occurs, which increases portal tributary blood flow (Lee et al., 1985). In addition, portal hypertension is responsible for the development of a portal-systemic collateral circulation (Lee et al., 1985). Finally, portal hypertension is associated with systemic vasodilation and a high cardiac output (Lee et al., 1985). Most studies performed in animals with portal hypertension have shown that acute inhibition of nitric oxide (NO) synthesis causes vasoconstriction in splanchnic and systemic territories and arterial hypertension (Claria et al., 1992; Pizcueta et al., 1992; Sieber et al., 1993; Wu et al., 1993; Sogni et al., 1992, 1995). These findings indicate that NO plays a role in the short-term regulation of the circulation in portal hypertension by inducing a basal vasodilator (and vasodepressor) tone (Sogni et al., 1992). In contrast, studies of chronic pharmacological NO inhibition in portal hypertension are controversial (Lee et al., 1993a,b; Garcia-Pagan et al., 1994; Niederberger et al., 1995). Results of certain studies have shown that chronic NO inhibition caused sustained splanchnic and systemic vasoconstriction (Lee et al., 1993a,b; Niederberger et al., 1995), while another study reported a sustained vasoconstriction of systemic vessels but only transient vasoconstriction of splanchnic circulation (Garcia-Pagan et al., 1994). Therefore, more

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information on the role of NO in the long-term regulation of vascular tone in portal hypertension is needed. Thus, the aim of the present study was to examine the effects of chronic NO inhibition on splanchnic and systemic haemodynamics in rats with portal vein stenosis. Since NO induces a renal vasodilator tone and inhibits sodium reabsorption (De Nicola et al., 1992; Stoss et al., 1992; Gardes et al., 1994; Ros et al., 1995), chronic NO inhibition may induce renal hypoperfusion and sodium retention. Thus, this study also examined the effects of NO inhibition on renal haemodynamics and various substances whose concentrations are increased by hypervolemia, i.e., plasma atrial natriuretic peptide (ANP) concentrations, cyclic guanosine monophosphate (cGMP) plasma and urinary cGMP concentrations (Chinkers and Garbers, 1991; Lauster et al., 1993).

2. Materials and methods

2.1. Animal preparation

Forty-eight male Sprague-Dawley rats (Charles River Laboratories, Saint-Aubin-Lès-Elbeuf, France) weighing 280–300 g were divided into two groups. One group was composed of normal rats. A second group had portal hypertension as a result of portal vein ligation as previously described (Lee et al., 1985). Under either anaesthesia, the abdomen was opened and the portal vein was exposed. A polyethylene catheter of 0.96-mm external diameter was passed along the portal vein, and 3-0 silk was used to ligate both the catheter and the portal vein. All rats were allowed free access to food and water up to 14–16 h before the study, when food was withdrawn. Protocols performed in this laboratory were approved by the French Agricultural Office in conformity with European legislation for research involving animals.

2.2. Haemodynamic measurements

Four hours before haemodynamic measurements, catheters were inserted under light ether anaesthesia. Arterial pressure and heart rate were measured using a catheter inserted into a femoral artery. Portal pressure was measured using a catheter inserted into the portal vein. Briefly, the abdomen was opened and a polypropylene catheter (0.7 mm diameter) was inserted into a small ileal vein and gently advanced to the bifurcation of the superior mesenteric and the splenic veins. The abdominal incision was closed with catgut. The left ventricle was cannulated via the right carotid artery. All catheters were fixed to the external vascular walls and then tunneled subcutaneously to the back of the neck. Haemodynamic studies were performed in conscious unrestrained rats. Cardiac and regional blood flows were measured by the radioactive microsphere method and the reference sample method as previously described (Lee et al., 1985; Sogni et al., 1992). a precounted aliquot of approximately 60000, 16 ± 1-μm diameter, ^{113}Sn-labelled microspheres (specific activity 10 mCi/g; New England Nuclear, Boston, MA, USA), suspended in Ficoll 70 (10% Pharmacia Fine Chemicals AB, Uppsala, Sweden) and Tween 80 (0.01%) and ultrasonically agitated, was injected into the ventricular catheter and flushed through with 1 ml of isotonic saline for 45 s. During microsphere injection, a reference blood sample was drawn from the catheter in the femoral artery into a motor-driven syringe at 0.8 ml/min for 1 min. The animal was then killed with an overdose of pentobarbitone sodium. Individual organs were dissected and placed in individual tubes for counting with a gamma counter (MINAXI gamma, Atogamma 5000 series; Packard Instrument Co., Downers Grove, IL, USA) at energy settings of 115–165 KeV. Errors due to the spillover of the ^{113}Sn channel were corrected using ^{113}Sn standards. Adequate microsphere mixing was assumed with a difference $< 10\%$ between the left and right kidneys. Cardiac index (CI) was calculated by the following formula: CI (ml/min · 100 g) = [radioactivity injected (cpm)/reference blood sample radioactivity (cpm)] × [100/body weight (g)] · 0.8 (ml/min). Systemic vascular resistance (SVR) was calculted by using the following formula: SVR [(dyn · s · cm^{-5} · 100 g) · 10^3] = mean arterial pressure (mm Hg) · 80/CI (ml/min · 100 g). Regional blood flows were calculated by using the following formula: organ blood flow (ml/min · 100 g) = [organ radioactivity (cpm)/radioactivity injected (cpm)] · CI (ml/min · 100 g). Portal tributary blood flow was calculated as the sum of stomach, intestine, colon, spleen, and mesenteric-pancreas flows. Portal territory vascular resistance (PTVR) was calculated using the following formula: PTVR [(dyn · s · cm^{-5} · 100 g) · 10^3] = [mean arterial pressure (mm Hg) − portal pressure (mm Hg)] · 80/portal tributary blood flow (ml/min · 100 g). Hepatocollateral vascular resistance (HVR) was calculated by using the following formula: HVR [(dyn · s · cm^{-5} · 100 g) · 10^3] = portal pressure (mm Hg) · 80/portal tributary blood flow (ml/min · 100 g). Renal vascular resistance (RVR) was calculated by using the following formula: RVR [(dyn · s · cm^{-5}.100 g) · 10^3] = mean arterial pressure (mm Hg) · 80/renal blood flow (ml/min · 100 g).

The extent of portal-systemic shunting was determined as previously described (Lee et al., 1985). In brief, a catheter was placed in the trunk of the superior mesenteric vein just before the portal vein, under light ether anaesthesia. Rats were permitted to recover and radioactive microspheres (60000) were injected via the catheter. Animals were then killed and the organs were counted in a gamma counter. Almost all radioactivity was found in the liver or the lungs. The percentage of portal-systemic shunting (PPS) was calculated according to the following formula: PPS (%) = [lung radioactivity (cpm)/lung and liver radioactivities (cpm)] · 100.

2.3. Measurements of plasma cGMP concentrations

Blood was taken from a femoral artery before haemodynamic measurements. Blood samples (600 μl) were drawn into EDTA K_3 (7.5%)-treated tubes. After centrifugation (4400 · *g*), the plasma obtained was stored at −20°C until analysis. For cGMP plasma extraction, 1 ml of absolute ethanol was added to 250 μl of plasma. After centrifugation for 15 min, supernatants were collected and the pellets were dissolved in 500 μl of absolute ethanol and centrifuged for 15 min. The supernatants were then combined with the first supernatant and dried at 57°C under a stream of nitrogen. Extracted samples were reconstituted in 500 μl of assay buffer, and 100 μl was used for analysis. Recovery of this extraction step was greater than 90%. Concentrations of immunoreactive cGMP were determined using a radioimmunoassay (cGMP assay RPA 525; Amersham International, Les Ulis, France). Interassay and intraassay coefficients of variation were 11.4% and 8.5%, respectively. The sensitivity limit of the assay was 0.5 pmol/ml.

2.4. Measurements of urinary cGMP concentrations

Urine was collected on chlorhydric acid during the 24 h preceding the haemodynamic study. Ten milliliters of urine were taken from the 24-h collected urine and then were centrifuged for 15 min (4400 · *g*). Urinary cGMP concentrations were measured using the same procedure as that used for plasma cGMP determination.

2.5. Measurement of plasma ANP concentrations

Blood was withdrawn from a femoral artery before the haemodynamic study to measure plasma ANP concentrations using a radioimmunoassay technique. Blood samples (5 ml) were collected in polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (500 kallikrein-inhibiting units/ml) and were immediately centrifuged at 4°C for 10 min at 2000 · *g*. Plasma samples were stored at −80°C until assay. Two-milliliter aliquots of plasma were extracted on C 18 octadecylsilane cartridges (Sep Pak, Water Associates, Milford, MA, USA) and the peptide was eluted in 4 ml of 80% ethanol and 4% acetic acid (v/v). Extracts were reconstituted in 0.6 to 1 ml of assay buffer. Plasma concentrations of immunoreactive ANP were determined using a radioimmunoassay kit (Amersham, Les Ulis, France). Recovery of cold ANP added in the range of 20–250 pg/ml was 78 ± 6%. The sensitivity limit, defined as the amount of ANP needed to reduce zero-dose binding by two standard deviations, was less than 3 pg/ml and was equivalent to a sample concentration of 12 pg/ml. The intraassay variation was 6.3% and the interassay variation was 11.2%. Antibodies used to perform the assay were specific for ANP and no cross-reaction occurred between other natriuretic peptides i.e., C-type and brain natriuretic peptides.

2.6. Protocols

For 28 days, all rats drank either normal water ($n = 24$) or water containing the NO synthesis inhibitor, *N*-nitro-L-arginine methylester (L-NAME, 1 mg/ml drinking water, changed daily) ($n = 24$). L-NAME was chosen because it is water-soluble and orally active, and induces a prolonged vasoconstrictor effect for many hours when given to rats in the drinking water (Baylis et al., 1992). Since normal and portal vein stenosed rats drink about 100 ml water/kg per 24 h (Baylis et al., 1992), the dose of L-NAME was approximately 100 mg/kg per 24 h.

At 14 days, portal vein stenosis was performed in 14 rats drinking normal water and in 14 rats drinking water with L-NAME. In other words, four groups of animals were created, i.e., normal rats receiving normal water (group 1, $n = 10$), normal rats receiving L-NAME (group 2, $n = 10$), portal vein stenosed rats receiving normal water (group 3, $n = 14$), portal vein stenosed rats receiving L-NAME (group 4, $n = 14$). Two sets of experiments were

Table 1
Haemodynamic responses to long-term administration of *N*-nitro L-arginine methylester (L-NAME) or placebo in normal rats and portal vein stenosed rats

	Normal		Portal vein stenosis	
	Placebo ($n = 10$)	L-NAME ($n = 10$)	Placebo ($n = 10$)	L-NAME ($n = 10$)
Mean arterial pressure (mm Hg)	111 ± 2 [a]	150 ± 3 [b]	103 ± 1 [c]	152 ± 3 [a]
Heart rate (beats/min)	381 ± 10	366 ± 9	378 ± 10	393 ± 5
Cardiac index (ml/min · 100 g)	27.9 ± 0.7	20.7 ± 0.9 [b]	38.9 ± 0.2 [c]	22.2 ± 0.2 [b]
Systemic vascular resistance (dyn · s · cm^{-5} · 100 g · 10^3)	328 ± 15	578 ± 35 [b]	213 ± 4 [c]	586 ± 54 [b]
Portal pressure (mm Hg)	7.6 ± 0.2	7.6 ± 0.2	13.1 ± 0.2 [c]	13.0 ± 0.4 [c]
Portal tributary blood flow (ml/min · 100 g)	4.2 ± 0.2	3.0 ± 0.1 [b]	7.3 ± 0.5 [b]	3.0 ± 0.2 [b]
Portal territory vascular resistance (dyn · s · cm^{-5} · 100 g · 10^3)	2067 ± 157	3743 ± 224 [b]	1027 ± 76 [c]	3170 ± 297 [b]
Hepatocollateral vascular resistance (dyn · s · cm^{-5} · 100 g · 10^3)	151 ± 10	204 ± 11 [b]	147 ± 10	295 ± 30 [b]
Hepatic arterial blood flow (ml/min · 100 g)	0.9 ± 0.2	0.4 ± 0.1 [b]	1.4 ± 0.1 [c]	0.5 ± 0.1 [b]
Renal blood flow (ml/min · 100 g)	4.4 ± 0.2	3.3 ± 0.3 [b]	5.8 ± 0.4 [c]	4.1 ± 0.5 [b]
Renal vascular resistance (dyn · s · cm^{-5} · 100 g · 10^3)	1082 ± 167	3805 ± 290 [b]	1463 ± 95 [c]	3461 ± 517 [b]

[a] Means ± S.E.M. [b] Significantly different from corresponding placebo ($P < 0.05$). [c] Significantly different from corresponding normals ($P < 0.05$).

performed on day 28. In the first set, haemodynamics were measured in all rats in groups 1 and 2, in 10 rats in group 3 and in 10 rats in group 4. Urine was collected during the 24-h period prior to haemodynamic studies and urinary cGMP concentrations were measured in all these rats. Moreover, when rats were killed following haemodynamic studies, blood was taken to measure plasma cGMP and ANP concentrations. The second set of experiments measured the percentage of portal-systemic shunting in the remaining rats in group 3 ($n = 4$) and group 4 ($n = 4$).

2.7. Statistical analysis

Values are means ± S.E.M. Analysis of variance, paired and unpaired t-tests were performed where appropriate.

3. Results

In animals receiving a placebo, portal pressure, regional blood flows (in portal tributaries, hepatic artery and kidneys) and cardiac index were significantly higher while arterial pressure and vascular resistance in the portal, hepatocollateral, renal and systemic territories were significantly lower in portal vein stenosed rats than in normal rats (Table 1).

In portal vein stenosed rats, regional blood flow (in portal tributaries, hepatic artery and kidneys) and cardiac index were significantly lower while arterial pressure and vascular resistance in the portal, hepatocollateral, renal and systemic territories were significantly higher after L-NAME than after placebo. Portal pressure did not significantly differ between portal vein stenosed rats receiving L-NAME and those receiving placebo (Table 1). In portal vein stenosed rats, the percentage of portal-systemic shunting was significantly lower in animals that received L-NAME than in those that received placebo (68 ± 5% versus 96 ± 1%, respectively) (Fig. 1).

In normal rats, regional blood flow (in portal tributaries, hepatic artery and kidneys) and cardiac index were significantly lower while arterial pressure and vascular resistance in the portal, hepatocollateral, renal and systemic territories were significantly higher after L-NAME than after placebo (Table 1). The L-NAME-induced decreases in portal tributary blood flow and cardiac index were significantly higher in portal vein stenosed rats than in normal rats (−48 ± 4% versus −23 ± 7% and −44 ± 3% versus −26 ± 2%, respectively). The L-NAME-induced increases in portal territory vascular resistance and systemic vascular resistance were significantly higher in portal vein stenosed rats than in normal rats (210 ± 22% versus 89 ± 16% and 173 ± 21% versus 76 ± 6%, respectively). The L-NAME-induced changes in renal blood flow and renal vascular resistance did not differ between portal vein stenosed rats and normal rats (−29 ± 7% and 136 ± 32% versus −20 ± 10% and 91 ± 17%, respectively).

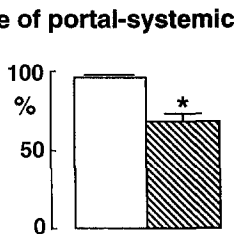

Fig. 1. Percentage of portal-systemic shunting following placebo (empty column) or 100 mg/kg·day of oral N-nitro-L-arginine methylester (L-NAME, hatched column) for 28 days, in portal vein stenosed rats ($n = 4$ per group). * $P < 0.05$ vs. placebo.

Plasma ANP and cGMP concentrations as well as urinary cGMP concentrations were not significantly different after L-NAME and after placebo in either group of rats (normal or portal vein stenosed animals) (Table 2). Urinary excretion of cGMP did not differ between the different groups of animals.

4. Discussion

This study compared the haemodynamic changes induced by chronic L-NAME administration or placebo in portal vein ligated animals. As expected, portal vein ligation without L-NAME induced portal hypertension and splanchnic and systemic hyperdynamic circulation (Lee et al., 1985). In rats receiving L-NAME, portal vein ligation caused portal hypertension, but did not cause splanchnic or systemic hyperdynamic circulation since these animals had vasoconstriction in these territories. Since L-NAME inhibits NO production (Moncada et al., 1991) and since NO induces vasorelaxant mechanisms (Moncada et al., 1991),

Table 2
Plasma atrial natriuretic peptide (ANP) concentrations, plasma and urinary cyclic GMP (cGMP) concentrations following long-term administration of N-nitro-L-arginine methylester (L-NAME) or placebo in normal rats and portal vein stenosed rats

	Normal		Portal vein stenosis	
	Placebo ($n = 10$)	L-NAME ($n = 10$)	Placebo ($n = 10$)	L-NAME ($n = 10$)
Plasma ANP concentrations (pg/ml)	98 ± 12	91 ± 15	137 ± 19	113 ± 10
Plasma cGMP concentrations (pmol/ml)	5.3 ± 1.0	5.3 ± 1.0	5.3 ± 1.0	6.4 ± 2.0
Urinary cGMP concentrations (nmol/ml)	2.7 ± 0.3	2.5 ± 0.2	2.2 ± 0.1	2.4 ± 0.1

[a] Means ± S.E.M.

this suggests that NO-related vasodilator tone is involved in the development and long-term maintenance of vasodilation in the portal and systemic territories in this model of portal hypertension.

This study shows that long-term L-NAME administration decreased portal-systemic shunting in portal vein stenosed rats, and significantly increased hepatocollateral vascular resistance. This suggests that L-NAME prevented the development of the collateral circulation in these rats. This L-NAME-elicited prevention was not a result of a decrease in portal hypertension since the degree of portal hypertension was similar in rats that received L-NAME and in those that did not, but may be due to a reduced amount of blood entering the collateral circulation since L-NAME decreased portal tributary blood flow. However, this mechanism does not explain all the reduction in portal-systemic shunting since the increase in portocollateral vascular resistance was greater than the reduction in portal tributary blood flow (100% versus 40%, respectively). Thus, direct effects of L-NAME on the portal-collateral vascular bed may also help to prevent the development of collateral circulation. In fact, previous results obtained in an isolated portal-collateral perfused model have shown that NO inhibitors increase vascular resistance in the portal-collateral vascular bed of portal hypertensive rats (Mosca et al., 1992). Although an undefined action of L-NAME cannot be ruled out, the present and previous results were probably due to a L-NAME-induced inhibition of NO synthesis in the walls of portal-collateral vessels. Indeed, the finding that L-arginine decreases the vasoconstrictor action of a NO inhibitor in an isolated portal-collateral perfused model from portal hypertensive rats (Mosca et al., 1992) shows that the effects of NO inhibitors on collateral vessels result from a competition between these inhibitors and L-arginine as substrates of NO synthase. Thus, NO produced in the portal-collateral vascular bed seems to be involved in the development of the collateral circulation in portal hypertensive animals.

Although an L-NAME-induced decrease in portal tributary blood flow should lower portal pressure, this did not occur in portal hypertensive rats (see above). This lack of portal hypotensive action was probably a result of the increase in vascular resistance in collateral vessels. Other studies have shown that short- and long-term inhibition of NO synthesis does not decrease portal pressure in portal hypertensive animals (Sogni et al., 1992; Lee et al., 1993b).

The results of the present study did not confirm findings by Garcia-Pagan et al. (1994). These authors showed only a transient reduction in splanchnic hyperdynamic circulation following chronic L-NAME administration in portal vein stenosed rats (Garcia-Pagan et al., 1994). These discrepancies may be due to methodological differences. In the present study, portal vein ligation was performed in animals which had already received a 14-day L-NAME treatment. In addition, haemodynamics were measured in conscious animals 14 days after surgery (i.e., 28 days after the onset of L-NAME). In contrast, Garcia-Pagan et al. (1994) began L-NAME the same day as portal ligation and haemodynamics were measured in anaesthetized animals at day 4 and day 8 following surgery. Finally, the daily dose of L-NAME differed in the studies [100 versus 45 mg/kg per 24 h in the present versus the study of Garcia-Pagan et al. (1994), respectively].

In portal vein stenosed rats treated with L-NAME, the increased systemic vascular tone induced an arterial vasopressor effect which, in turn, elicited a reflex reduction in cardiac index. These findings confirm previous results obtained with acute and chronic NO inhibition in portal hypertensive rats (Sogni et al., 1992; Lee et al., 1993a).

In this study, portal vein stenosed rats tended to have higher baseline plasma ANP concentrations than normal rats. Since sodium retention occurs in portal vein stenosed rats (Albillos et al., 1992), this alteration may explain the increase in plasma ANP concentrations in portal hypertensive rats. However, since the main cellular source of plasma and urinary cGMP is particulate guanylyl cyclase (which is stimulated by ANP) but not soluble guanylyl cyclase (which is stimulated by NO) (Stasch et al., 1989), it may seem surprising that concentrations of these nucleotides were not elevated in portal vein stenosed rats. The reasons for this dissociation between cGMP and ANP levels are unclear but may be related to a downregulation of ANP receptors.

Portal vein stenosed rats that did not receive L-NAME had renal hyperdynamic circulation (Lee et al., 1985; Moreau et al., 1992), while portal vein stenosed animals receiving chronic L-NAME administration did not. This suggests that NO-mediated vasodilator tone contributes to the long-term regulation of renal vascular tone in portal vein stenosed rats. Since NO seems to inhibit tubular sodium reabsorption in normal kidneys (Stoss et al., 1992), this study examined whether NO inhibition decreases sodium excretion and thus causes hypervolemia. In fact, this hypothesis is unlikely since urinary and plasma cGMP levels as well as plasma ANP concentrations (which all are dependent on plasma volume) (Chinkers and Garbers, 1991; Lauster et al., 1993) did not increase following L-NAME administration.

The finding that plasma and urinary cGMP concentrations did not decrease following L-NAME administration in portal hypertensive and normal rats confirms that the main cellular source of plasma and urinary cGMP is not the NO-activated guanylyl cyclase (see above) (Stasch et al., 1989).

In this study, L-NAME-induced vasoconstriction in systemic and portal territories was more marked in portal vein stenosed rats than in normal animals. In contrast, L-NAME induced similar increases in renal vascular resistance in both groups. Therefore, the NO-mediated splanchnic and systemic (but not renal) vasodilator tone seems to be more marked in portal hypertension than in normal conditions. This may be due to a greater NO production in animals

with portal hypertension than in normal animals. In fact, a chronic increase in blood flow (and shear stress) has been shown to enhance NO production and endothelial cell NO synthase (ecNOS) gene expression in normal animals (Sessa et al., 1994). Studies on ecNOS gene expression are needed in portal hypertensive animals.

In conclusion, L-NAME-induced vasoconstriction occurred in splanchnic and systemic arterioles in portal vein stenosed rats. In these animals, L-NAME decreased portosystemic shunts, increased vascular resistance in collateral vessels and did not change portal pressure. Therefore, NO-mediated vasodilator tone may contribute to the development and maintenance of splanchnic and systemic vasodilation as well as collateral circulation in portal hypertension. Moreover, portal hypertensive animals seem to have a more marked splanchnic and systemic NO-mediated vasodilator response than normal animals.

References

Albillos, A., L.A. Colombato and R.J. Groszmann, 1992, Vasodilation and sodium retention in prehepatic portal hypertension, Gastroenterology 102, 931.

Baylis, C., B. Mitruka and A. Deng, 1985, Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage, J. Clin. Invest. 90, 278.

Chinkers, M. and D.L. Garbers, 1991, Signal transduction by guanylyl cyclases, Annu. Rev. Biochem. 60, 553.

Claria, J., W. Jimenez, J. Ros, M. Asbert, A. Castro, V. Arroyo, F. Rivera and J. Rodes, 1992, Pathogenesis of arterial hypotension in cirrhotic rats with ascites: role of endogenous nitric oxide, Hepatology 15, 343.

De Nicola, L., R.C. Blantz and F.B. Gabbai, 1992, Nitric oxide and angiotensin II, J. Clin. Invest. 89, 1248.

Garcia-Pagan, J.C., M. Fernandez, C. Bernadich, P. Pizcueta, J.M. Pique, J. Bosch and J. Rodes, 1994, Effects of continued NO inhibition on portal hypertensive syndrome after portal vein stenosis in rat, Am. J. Physiol. 267, G984.

Gardes, J., M.F. Gonzales, F. Alhenc-Gelas and J. Menard, 1994, Influence of sodium diet on L-NAME effects on renin release and renal vasoconstriction, Am. J. Physiol. 267, F798.

Lauster, F., J.M. Heim, C. Drummer, H.J. Fülle, R. Gerzer and H. Schiffl, 1993, Plasma cGMP level aa a marker of the hydration state in renal replacement therapy, Kidney Int. 43 (Suppl. 41), S57.

Lee, F.Y., L.A. Colombato, A. Albillos and R.J. Groszmann, 1993a, Nω-Nitro-L-arginine administration corrects peripheral vasodilation and systemic capillary hypotension and ameliorates plasma volume expansion and sodium retention in portal hypertensive rats, Hepatology 17, 84.

Lee, F.Y., L.A. Colombato, A. Albillos and R.J. Groszmann, 1993b, Administration of Nω-nitro-L-arginine ameliorates portal-systemic shunting in portal-hypertensive rats, Gastroenterology 105, 1464.

Lee, S.S., C. Girod, D. Valla, P. Geoffroy and D. Lebrec, 1985, Effects of pentobarbital sodium anesthesia on splanchnic hemodynamics of normal and portal-hypertensive rats, Am. J. Physiol. 249, G528.

Moncada, S., R.J.M. Palmer and E.A. Higgs, 1991, Nitric oxide: physiology, pathophysiology, and pharmacology, Pharmacol. Rev. 43, 109.

Moreau, R., H. Komeichi, S. Cailmail and D. Lebrec, 1992, Blockade of ATP-sensitive K^+ channels by glibenclamide reduces portal pressure and hyperdynamic circulation in portal hypertensive rats, J. Hepatol. 16, 215.

Mosca, P., F.Y. Lee, A.J. Kaumann and R.J. Groszmann, 1992, Pharmacology of portal-systemic collaterals in portal hypertensive rats: role of the endothelium, Am. J. Physiol. 263, G544.

Niederberger, M., P. Gines, P. Tasi, P.Y. Martin, K. Morris, A. Weigert, I. McMurtry and R.W. Schrier, 1995, Increased aortic cyclic guanosine monophosphate concentration in experimental cirrhosis in rats: evidence for a role of nitric oxide in the pathogenesis of arterial vasodilation in cirrhosis, Hepatology 21, 1625.

Pizcueta, M.P., J.M. Piqué, M. Fernadez, J. Bosch, J. Rodes, B.J.R. Whittle and S. Moncada, 1992, Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxide inhibition, Gastroenterology 103, 1909.

Ros, J., J. Claria, W. Jimenez, M. Bosch-Marce, P. Angeli, V. Arroyo, F. Rivera and J. Rodes, 1995, Role of nitric oxide and prostacyclin in the control of renal perfusion in experimental cirrhosis, Hepatology 22, 915.

Sessa, W.C., K. Pritchard, N. Seyedi, J. Wang and T.H. Hintze, 1994, Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression, Circ. Res. 74, 349.

Sieber, C.C., J.C. Lopez-Talavera and R.J. Groszmann, 1993, Role of nitric oxide in the in vitro splanchnic vascular hyporeactivity in ascitic cirrhotic rats, Gastroenterology 104, 1750.

Sogni, P., R. Moreau, M. Ohsuga, S. Cailmail, F. Oberti, A. Hadengue, E. Pussard and D. Lebrec, 1992, Evidence for normal nitric oxide-mediated vasodilator tone in conscious rats with cirrhosis, Hepatology 16, 980.

Sogni, P., R. Moreau, A. Gadano and D. Lebrec, 1995, The role of nitric oxide in the hyperdynamic circulatory syndrome associated with portal hypertension, J. Hepatol. 23, 218.

Stasch, J.P., S. Kazda and D. Neuser, 1989, Different effects of ANP and nitroprusside on cGMP extrusion of isolated aorta, Eur. J. Pharmacol. 174, 279.

Stoss, B.A., O.A. Carretero, R.D. Farhy, G. Scicli and J.L. Garvin, 1992, Endothelium-derived relaxing factor inhibits transport and increases cGMP content in cultured mouse cortical collecting duct cells, J. Clin. Invest. 89, 761.

Wu, Y., R. Burns and J.V. Sitzmann, 1993, Effects of nitric oxide and cyclooxygenase inhibition on splanchnic hemodynamics in portal hypertension, Hepatology 18, 1416.