ELSEVIER

European Journal of Pharmacology 273 (1995) 167–174

ejp

Role of nitric oxide in hyporeactivity to noradrenaline of isolated aortic rings in portal hypertensive rats

Peter P. Michielsen [a,*], Guy E. Boeckxstaens [a], Stanislas U. Sys [b], Arnold G. Herman [c], Paul A. Pelckmans [a]

[a] *Division of Gastroenterology, Faculty of Medicine, University of Antwerp (UIA), B-2610 Antwerpen-Wilrijk, Belgium*
[b] *Department of Physiology and Medicine, University of Antwerp (UCA), B-2020 Antwerpen, Belgium*
[c] *Division of Pharmacology, Faculty of Medicine, University of Antwerp (UIA), B-2610 Antwerpen-Wilrijk, Belgium*

Received 21 July 1994; revised MS received 2 November 1994; accepted 8 November 1994

Abstract

To test the hypothesis that induction of nitric oxide synthase causes systemic vascular hyporesponsiveness to vasopressors in portal hypertension, we performed in vitro experiments on isolated thoracic aortic rings from partial portal vein ligated or sham operated rats at 3 weeks postoperatively. The concentration-response curves to noradrenaline of intact and endothelium-denuded aortic rings from portal hypertensive rats were significantly shifted to the right as compared to those from sham operated animals. Maximal contractions did not significantly differ. Addition of N^G-nitro-L-arginine, a specific inhibitor of nitric oxide synthase, shifted the curves to the left in both sham operated and portal hypertensive rats, so that in intact rings, the concentrations of noradrenaline producing half-maximal response did not significantly differ any more between sham operated and portal vein ligated rats. In endothelium-denuded rings, a hyporeactivity to noradrenaline persisted in portal vein ligated rats. Furthermore, N^G-nitro-L-arginine induced an additional significant increase in the maximal response to noradrenaline in sham operated as compared to portal hypertensive rats. The endothelium-dependent relaxations to acetylcholine were attenuated in portal hypertensive rats as compared to sham operated animals. From these results, it can be concluded that increased nitric oxide production in the vascular wall of thoracic aorta of portal hypertensive rats is involved in their hyporesponsiveness to noradrenaline. Our findings in endothelium-denuded rings indicate the involvement of the inducible nitric oxide synthase in the smooth muscle layer. Involvement of an inducible nitric oxide synthase in the endothelium cannot be excluded. The endothelial constitutive nitric oxide synthase, however, seems to be suppressed in portal vein ligated rats.

Keywords: Nitric oxide (NO); Nitric oxide synthase, inhibition; Portal vein ligation, rat; Portal hypertension

1. Introduction

In man (Lebrec et al., 1983) and experimental animals such as portal vein ligated rats (Vorobioff et al., 1983), chronic portal hypertension with significant portosystemic shunting is associated with a hyperdynamic circulation, characterized by increased blood flow and reduced vascular resistance in the splanchnic and systemic circulation. As a result of the splanchnic vasodilation, portal venous inflow is elevated, which will itself contribute to the generation and maintenance of portal hypertension (Benoit et al., 1985). The splanchnic vasodilation may reflect the reduced sensitivity of the vascular tissue to vasoconstrictor mediators as catecholamines, angiotensin II, vasopressin (Kiel et al., 1985; Finberg et al., 1985; Murray and Paller, 1985, 1986), or elevations in plasma levels of vasodilator substances as glucagon (Benoit et al., 1986; Kravetz et al., 1988), insufficiently cleared by the liver.

Nitric oxide is a potent vasodilator and plays an important role in the physiological regulation of blood flow and blood pressure (Rees et al., 1989). It is synthesized from L-arginine in vascular endothelial

* Corresponding author. Division of Gastroenterology, University Hospital of Antwerp, Wilrijkstraat 10, B-2650 Edegem, Belgium. Tel. 32 3 8291111, fax 32 3 8254678.

Portions of this work were presented at the Digestive Disease Week, Boston, MA, USA, May 16–19, 1993 and at the 28th Annual Meeting of the European Association for the Study of the Liver, Paris, France, September 1–4, 1993.

Elsevier Science B.V.
SSDI 0014-2999(94)00691-1

cells, macrophages and many other cells and tissues (Moncada et al., 1989; De Man et al., 1991). This pathway is stereospecifically inhibited by N^G-nitro-L-arginine (Mülsch and Busse, 1990). Vascular endothelial cells contain a constitutive nitric oxide synthase that is Ca^{2+} dependent. In addition, these cells as well as vascular smooth muscle cells express an inducible Ca^{2+} independent nitric oxide synthase, after activation with endotoxin or cytokines (Radomski et al., 1990; Knowles et al., 1990).

There is evidence that nitric oxide production is increased in certain pathological conditions. Decreased responsiveness to catecholamines, angiotensin II and vasopressin has been observed in animal models of sepsis and endotoxemia (Miller et al., 1973; Schaller et al., 1985), and persists in vessels removed from these animals (Wakabayashi et al., 1987; Bigaud et al., 1990). It has been demonstrated that activation of the L-arginine pathway is involved in this vascular hyporeactivity in endotoxemia (Julou-Schaeffer et al., 1990).

It has recently been postulated that in portal hypertension with collateral circulation, low-grade endotoxemia causes induction of nitic oxide synthase, and is responsible for the hyperdynamic circulation (Vallance and Moncada, 1991). Inhibition of nitric oxide synthase has been shown to result in attenuation or reversal of the hemodynamic disturbances and splanchnic vasodilation in vivo in portal vein ligated (Pizcueta et al., 1992a; Lee et al., 1992) and cirrhotic rats (Claria et al., 1992; Pizcueta et al., 1992b). Also the hyporeactivity to vasopressors in mesenteric vessels in vitro in portal vein ligated (Sieber and Groszmann, 1992a,b) and cirrhotic rats (Sieber et al., 1993) is reverted by nitric oxide synthase inhibition, which seems to confirm this hypothesis. Furthermore, endothelial nitric oxide has been demonstrated to play a role in the portal-collateral resistance of portal hypertensive rats (Mosca et al., 1992). In contrast, other authors failed to demonstrate an excessive nitric oxide synthesis in cirrhotic rats (Sogni et al., 1992) or a significant role for nitric oxide on the hyperdynamic circulation in portal vein ligated rats (Iwata et al., 1992). In addition, the experiments of Mehta et al. (1990) failed to demonstrate a major pathogenic role for endotoxin in the hyperdynamic circulation in the portal vein ligated rat model.

If a circulating factor is responsible for induction of nitric oxide synthase, the effect should not be limited to resistance vessels of the splanchnic circulation, but should also involve large conductance vessels. To test this hypothesis, we performed in vitro experiments on isolated aortic rings from partial portal vein ligated rats, a well known animal model for portal hypertension (Mesh et al., 1988). The advantage of an in vitro system is the lack of circulating vasodilators and their possible interactions with the vascular responsiveness to endogenous vasopressors. Furthermore, intact and endothelium-denuded preparations can be studied separately.

2. Materials and methods

2.1. Animal preparation

For this study, male Wistar rats weighing 422 ± 8 g ($n = 14$) were used, that either had undergone a sham operation or a portal vein ligation to induce portal hypertension.

Partial portal vein ligation was performed as previously described (Chojkier and Groszmann, 1981). Briefly, using ether anesthesia, the abdominal cavity was opened through a midline incision under strict sterile conditions. The omentum and part of the intestine were gently lifted out of the abdomen and kept moist with saline moistened gauze. After the portal vein was isolated, a 20-gauge blunt-end needle was placed alongside the length of the portal vein, and one ligature of 4-0 surgical silk placed proximal to the bifurcation of the vein was tied around both the needle and the portal vein. The needle was then removed and the portal vein was allowed to reexpand, yielding a consistent stenosis of the portal vein. The abdominal viscera were placed back into the abdomen and the operative incision was then closed in a double layer.

In the sham operated rats, the portal vein was similarly isolated but no ligature was applied and the abdomen was similarly closed.

After surgery, the rats were allowed to recover from anesthesia and were returned to the vivarium, where they had free access to water and food.

In preliminary experiments, presence or absence of portal hypertension was assessed after 2 weeks. Under anesthesia with sodium pentobarbital (60 mg/kg intraperitoneally), the abdomen was opened, and the ileocolic vein was cannulated with a 24-gauge catheter connected to a Statham P-23-id strain transducer for pressure registration with a carrier amplifier HP 8805 B. Portal venous pressure in 10 portal vein ligated animals was almost twice that of 14 sham operated rats (18.3 ± 1.1 and 9.4 ± 0.4 mm Hg respectively, $P < 0.001$).

Treatment of the animals during the experiments was conducted according to the rules of good animal care in conformity with the European legislation.

2.2. Tissue preparation

Since in the used animal model, vascular hyporeactivity in aortic strips to noradrenaline has been demonstrated to occur at 21, but not at 10 days postoperatively (Bomzon and Blendis, 1987), experiments were performed after 3 weeks.

Under ether anesthesia, the animals were killed by opening of the thorax and cardiectomy. The thoracic aorta was removed, cleared of adherent tissue, and cut into rings approximately 2 mm in length. In some rings, the vascular endothelium was mechanically removed by rubbing gently with blunt forceps. The aortic rings were mounted horizontally between two stainless-steel stirrups in organ chambers filled with 25 ml Krebs-Ringer solution (composition in mM: NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1), maintained at 37°C and bubbled with 95% O_2-5% CO_2. One of the stirrups was anchored to the organ chamber, and one was connected to a strain gauge (Statham UC2), for recording of isometric tension. To prevent interference from in vitro induction of nitric oxide synthase in the rings, due to endotoxin present in the Krebs buffer, dexamethasone (10^{-7} M) was added to the Krebs-Ringer solution from the isolation of the vessels and throughout the whole experiment. Dexamethasone was chosen as it was previously shown to prevent this de novo synthesis (Rees et al., 1990a), without affecting the activity of the enzyme already present (Radomski et al., 1990).

The aortic rings were stretched to the point of their optimal length-tension relationship (4 g, determined in preliminary experiments by repeated exposure to 50 mM KCl). The rings were allowed to equilibrate for 60 min.

2.3. Experimental design

In a first set of experiments, the rings were contracted with noradrenaline, in a concentration resulting in a stable, submaximal contraction (3×10^{-7} M or 10^{-6} M). Cumulative relaxation curves to the endothelium-dependent dilator acetylcholine (10^{-9} to 10^{-4} M) were obtained in each ring, to assess the integrity of the endothelium. Rings showing $<60\%$ relaxation of the noradrenaline-induced contraction were discarded. The failure of acetylcholine to induce relaxation of rubbed rings was taken as an indication of endothelium removal.

In a second set of experiments, contractile responses to cumulative concentrations of noradrenaline (10^{-10} to 10^{-5} M) were studied.

In a third set of experiments, contractile responses to cumulative concentrations of noradrenaline (10^{-10} to 10^{-5} M) were repeated after 10-min preincubation with N^G-nitro-L-arginine (3×10^{-5} M).

In a fourth set of experiments, the effect of consecutively added concentrations of nitric oxide (10^{-7} to 10^{-5} M) on rings maximally precontracted with noradrenaline (10^{-5} M), was assessed. As the relaxations to nitric oxide were transient and nitric oxide had certainly disappeared at the time the next concentration was added, the concentrations of nitric oxide were considered as noncumulative.

2.4. Drugs

The drugs used were acetylcholine hydrochloride and dexamethasone 21-phosphate (Sigma Chemical Co., St. Louis, MO, USA), noradrenaline hydrogentartrate (Fluka, Buchs, SG, Switzerland), N^G-nitro-L-arginine (Janssen Chimica, Belgium). All solutions were prepared on the day of experimentation and were administered as aqueous solutions except N^G-nitro-L-arginine, which was dissolved in 0.065 M HCl. Ascorbic acid (5.7×10^{-4} M) was added to the stock solutions of noradrenaline. All drugs were administered in volumes not exceeding 0.5% of the bath volume. Standards of aqueous nitric oxide solutions were prepared by saturation with purified nitric oxide gas of argon-degassed and deoxygenated water, and further diluted (Kelm et al., 1988).

2.5. Presentation of results and statistical analysis

The force of contraction is expressed as gram contraction. The relaxations are expressed as percent decrease of the noradrenaline-induced contraction. The results are shown as mean ± standard error of mean. To fit the sigmoidal noradrenaline concentration-response data, simultaneous nonlinear regression analysis (SPSS/PC + Advanced Statistics 4.0, SPSS, Chicago, IL, USA) was performed as a two-factor analysis with repeated measures on one factor, absence or presence of N^G-nitro-L-arginine (i.e. 2 levels), and with sham or portal hypertension as the nonrepeated-measures factor (i.e. 2 levels). Rings with endothelium and rings without endothelium were treated in a separate analysis. Results for the negative logarithm of the concentration of agonist that produced a half-maximal response (pD_2) and for the maximal response (T^m) from a given analysis were similarly treated. Different rings in sham and portal hypertensive groups and the two experimental factors were encoded by implementation of dummy variables using effects coding rather than reference coding (Glantz and Slinker, 1990). The use of dummy variables for the different rings resulted in a significantly better fit quality as demonstrated by Fisher's F-test on the sum of square residuals of the fits with or without encoding for different rings (Meddings et al., 1989). Because of a major interest in comparison among treatment means and because of significant interaction between the two experimental factors (except for pD_2 of noradrenaline response in rings without endothelium), multiple comparisons among treatment means were performed in each analysis, based on the computations given by Winer (1971), by using differences between means and standard er-

rors of the estimates. The reported standard error in Table 1 is the weighted average of two standard errors, used for the multiple comparisons; the standard error of subjects within groups was estimated from the coefficients associated with the dummy variables associated with the dummy variables for the different rings. The concentration-response data of acetylcholine were similarly compared. The possible differences in animal weight between sham operation and portal vein ligation, in the portal venous pressure, in the starting contractions to noradrenaline before acetylcholine addition, and the relaxations to nitric oxide were analyzed by unpaired Student's t-test. P values of less than 0.05 were considered as significant.

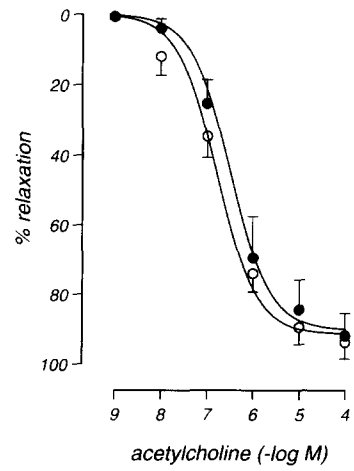

Fig. 1. Relaxation curves to acetylcholine (10^{-9} to 10^{-4} M) on intact aortic rings submaximally precontracted with noradrenaline (3×10^{-7} M) from sham operated (○) and portal vein ligated (●) rats. Data are presented as means ± S.E.M. in percent decrease of the noradrenaline-induced contraction from 7 experiments in portal vein ligated and 6 in sham operated rats.

3. Results

3.1. Body weights

The body weights on the day of the experiment (449 ± 16 g ($n = 7$) and 403 ± 19 g ($n = 7$) in respectively sham operated and portal vein ligated rats) were not significantly different.

3.2. Concentration-response curves to acetylcholine

Experiments were performed on aortic rings submaximally precontracted with noradrenaline (3×10^{-7} or 10^{-6} M). As expected, in the endothelium-denuded rings, no acetylcholine-induced relaxations were noted. In the intact rings, the starting contractions were 1.87 ± 0.12 g ($n = 7$) in sham operated and 1.80 ± 0.19 g ($n = 6$) in portal vein ligated rats (n.s.) In these intact rings, acetylcholine caused a dose-dependent relaxation of the precontracted rings. The relaxation curves were significantly shifted to the right in portal vein ligated rats as shown by the significant increase in pD_2 in portal vein ligated rats compared to sham operated rats (6.49 ± 0.09 versus 6.78 ± 0.09 respectively, $P < 0.05$). The maximal relaxations (91.3 ± 4.6% versus 91.5 ± 4.6% respectively), however, were not significantly different (Fig. 1).

3.3. Concentration-response curves to noradrenaline

The concentration-response curves to noradrenaline in intact and endothelium-denuded rings of portal vein ligated rats were significantly shifted to the right as compared to those of sham operated rats as was shown by the significant decrease of pD_2 value of noradrenaline in portal vein ligated versus sham operated rats. The maximal contractions were not significantly different (Table 1; Fig. 2).

In the intact aortic rings, N^G-nitro-L-arginine significantly shifted the dose-response curves to the left,

Table 1
pD_2 and T^m values of noradrenaline in rat aortic rings with or without endothelium in the absence or presence of N^G-nitro-L-arginine (3×10^{-5} M)

	pD_2			T^m		
	Control		N^G-Nitro-L-arginine	Control		N^G-Nitro-L-arginine
With endothelium						
Sham operation	7.02 ± 0.08	a	7.82 ± 0.08	2.72 ± 0.14	a	3.70 ± 0.14
	| a		| ns	| ns		| a
Portal hypertension	6.67 ± 0.08	a	7.97 ± 0.08	2.46 ± 0.14	ns	2.44 ± 0.14
Without endothelium						
Sham operation	7.93 ± 0.09	a	8.30 ± 0.09	2.32 ± 0.13	a	2.91 ± 0.13
	| a		| b	| ns		| a
Portal hypertension	7.54 ± 0.09	a	8.08 ± 0.09	2.40 ± 0.13	ns	2.38 ± 0.13

Results are shown as means ± S.E.M. for 6 or 7 experiments. Maximal contraction (T^m) is expressed in g contraction.
ns Non-significant; a $P < 0.005$, b $P < 0.05$, portal hypertension compared to sham operation or after N^G-nitro-L-arginine versus control.

both in sham operated and portal vein ligated rats, as illustrated by the significant increase in pD_2 (Table 1; Fig. 3A). In the sham operated rats, but not in the portal vein ligated rats, this was also accompanied by a significant increase in the maximal contraction to noradrenaline (Table 1; Fig. 3A).

In the endothelium-denuded rings, N^G-nitro-L-arginine significantly shifted the dose-response curves to noradrenaline to the left, as shown by the significant increase in pD_2 in both sham operated and portal vein ligated rats; as in intact rings, the maximal contractions to noradrenaline were only significantly increased by N^G-nitro-L-arginine in sham operated, but not in portal vein ligated rats (Table 1; Fig. 3B).

After N^G-nitro-L-arginine, the pD_2 values to noradrenaline were not significantly different any more between sham operated and portal vein ligated rats in intact rings. In the endothelium-denuded rings, however, a significant hyporeactivity for noradrenaline persisted in portal vein ligated rats (Table 1). After blockade of nitric oxide synthesis by N^G-nitro-L-arginine, the maximal responses in portal vein ligated as compared to those in sham operated rats were significantly lower in both intact and endothelium-denuded aortic rings (Table 1).

3.4. Response curves to noncumulative concentrations of nitric oxide

Addition of nitric oxide on aortic rings maximally contracted with noradrenaline (10^{-5} M) resulted in immediate but transient dose-dependent relaxations. There were no significant differences between the relaxations to the individual concentrations of nitric oxide in rings from sham operated or portal vein ligated rats with or without endothelium (Fig. 4).

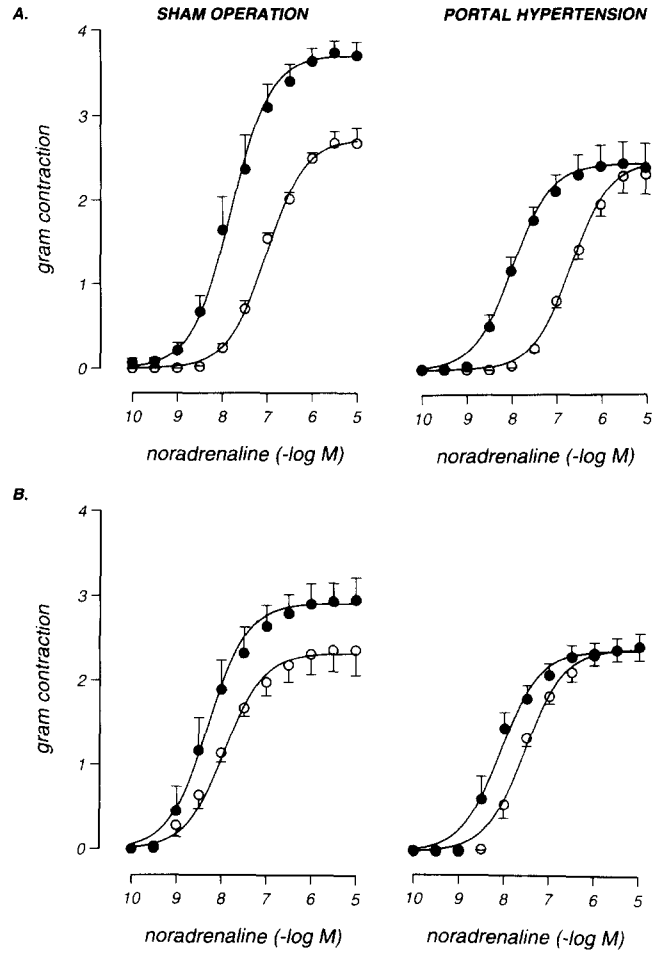

Fig. 3. Concentration-response curves to noradrenaline (10^{-10} to 10^{-5} M) in aortic rings with (A) and without endothelium (B) from sham operated and portal vein ligated rats, before (○) and after (●) preincubation with N^G-nitro-L-arginine. Data are presented as means ± S.E.M. in g contraction from 6 or 7 experiments.

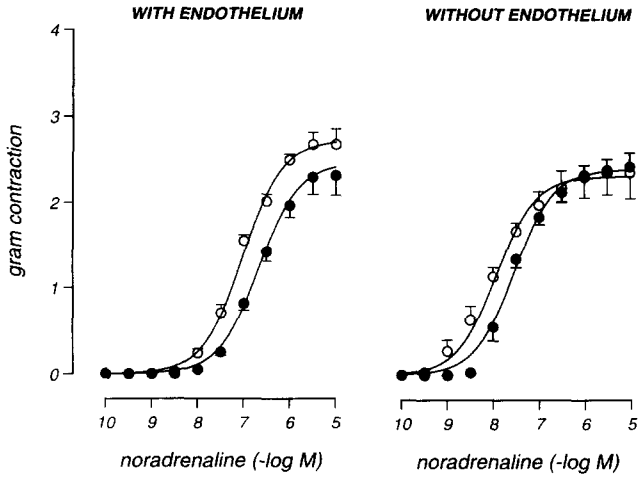

Fig. 2. Concentration-response curves to noradrenaline (10^{-10} to 10^{-5} M) in aortic rings with or without endothelium, of sham operated (○) and portal vein ligated (●) rats. Data are presented as means ± S.E.M. in g contraction from 6 or 7 experiments.

4. Discussion

Controversy still exists regarding the mechanisms involved in the systemic and splanchnic vasodilation in portal hypertension. An increase in circulating levels of vasodilators such as glucagon is only responsible for part of the observed splanchnic vasodilation in portal hypertensive rats (Benoit et al., 1986). In analogy with the recognized role of nitric oxide synthase induction in septic shock (Julou-Schaeffer et al., 1990), a similar induction by circulating endotoxin was postulated to be involved in the hemodynamic disturbances of portal hypertension (Vallance and Moncada, 1991). Because nitric oxide acts locally, the vasodilatory state in portal hypertension should be initiated by a systemic activation of nitric oxide synthesis. In the present study, we

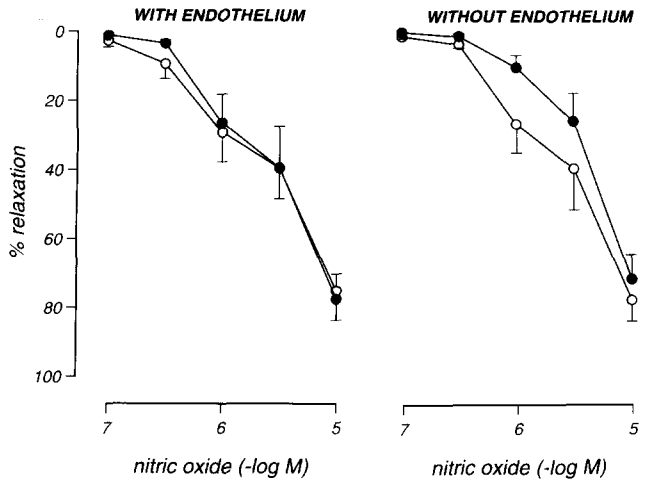

Fig. 4. Relaxation curves to nitric oxide (10^{-7} to 10^{-5} M) on aortic rings maximally precontracted with noradrenaline (10^{-5} M) from sham operated (○) and portal vein ligated (●) rats. Data are presented as means ± S.E.M. in percent decrease of the noradrenaline-induced contraction from 6 or 7 experiments.

provide evidence suggesting that this activation is indeed not limited to the resistance vessels and/or the mesenteric vascular bed, but can also be demonstrated in the aorta of partial portal vein ligated animals.

In the partial portal vein ligation model, the hyperdynamic state is fully developed 4 days postoperatively. At 8 days, maximal portosystemic shunting is achieved (Sikuler et al., 1985). In this model, in vivo splanchnic and systemic hemodynamics and in vitro mesenteric vessels perfusions were performed 8–14 days postoperatively (Lee et al., 1992; Sieber and Groszmann, 1992a,b). The hyporesponsiveness to noradrenaline in thoracic aortic strips, however, was only demonstrated at 3 weeks postoperatively, not at 2 or 10 days (Bomzon and Blendis, 1987). Therefore, experiments were performed on aortic rings of partial portal vein ligated and sham operated rats at 3 weeks postoperatively.

pD_2 values to noradrenaline in both intact and endothelium-denuded aortic rings were significantly lower in portal vein ligated as compared to sham operated rats, indicating vascular hyporesponsiveness to noradrenaline in portal vein ligated rats. Inhibition of nitric oxide biosynthesis by N^G-nitro-L-arginine (Ishii et al., 1990; Fineman et al., 1991) increased the pD_2 in endothelium-denuded and intact rings, reducing and even completely reverting the hyporesponsiveness to noradrenaline respectively. In contrast, the vasodilating effect of nitric oxide was not significantly different in sham operated and portal vein ligated rats, illustrating that the sensitivity of the vascular smooth muscle to nitric oxide does not differ. Therefore, our data provide indirect evidence for increased nitric oxide synthase activity in aortic rings from portal vein ligated rats.

In sham operated rats, blockade of the nitric oxide biosynthesis significantly increased the maximal response to noradrenaline, a finding previously described by Rees et al. (1990b). Interestingly, this effect was not observed in rings obtained from portal vein ligated rats. This difference can not be explained by failure of N^G-nitro-L-arginine to block nitric oxide synthase as the pD_2 to noradrenaline was significantly increased. Furthermore, it was demonstrated that N^G-nitro-L-arginine at the concentration used in our experiments is able to abolish acetylcholine-induced relaxations (Martin et al., 1992) and nitric oxide production from cultured bovine aortic endothelial cells (Ishii et al., 1992). Alternatively, structural changes in the aortic rings from portal vein ligated rats may be responsible for this observation. This could also explain the finding that the perfusion pressure of mesenteric vessel preparations from cirrhotic rats (Sieber et al., 1993) or from prehepatic portal hypertensive rats (Sieber and Groszmann, 1992) remained lower compared to sham operated animals even after nitric oxide synthesis blockade. Furthermore, in the in vivo study of Lee et al. (1992) in partial portal vein ligated rats, nitric oxide synthase inhibition also failed to completely reverse the hemodynamic abnormalities in portal hypertensive rats. Incomplete nitric oxide was considered improbable as the cause of this phenomenon by the authors, as raising the concentration of inhibitor had no additional effect (Lee et al., 1992).

In order to investigate the role of the endothelial constitutive nitric oxide synthase, we also studied the effect of portal hypertension on the endothelium-dependent acetylcholine-induced relaxations. These relaxations in the rat aorta are entirely mediated by nitric oxide, as they are abolished by inhibition of nitric oxide biosynthesis (Martin at al., 1992), a finding we confirmed in preliminary experiments using 3×10^{-5} M N^G-nitro-L-arginine (data not shown). The relaxations to acetylcholine were significantly reduced in portal vein ligated rats, indicating a reduced activity of the endothelial constitutive nitric oxide synthase. This is in analogy with the reduced vasodilatory potency of acetylcholine in aortic rings from endotoxin-pretreated rats (Julou-Schaeffer et al., 1990), and may be explained by the knowledge that nitric oxide has a negative feedback effect on the nitric oxide synthase activity (Buga et al., 1993): nitric oxide produced by the induced nitric oxide synthase will reduce the activity of the constitutive endothelial nitric oxide synthase.

In conclusion, our results indicate that activation of nitric oxide synthase in the vascular wall of the aorta of portal vein ligated rats is involved in the hyporesponsiveness of these vessels to the endogenous vasopressor noradrenaline. As the findings persist in endothelium-denuded rings, induction of nitric oxide synthase in the smooth muscle layer has to be implicated. The involve-

ment of endothelial inducible nitric oxide synthase cannot be excluded. As such, this activation seems not to be limited to the splanchnic circulation. The factors involved in increasing nitric oxide synthase activity in portal hypertension need to be further investigated.

Acknowledgements

This work was supported by the Belgian Fund for Medical Research (F.G.W.O. Grant 3.0077.94).

The authors gratefully acknowledge F.H. Jordaens for his technical assistance.

References

Benoit, J.N., W.A. Womack, L. Hernandez and D.N. Granger, 1985, 'Forward' and 'backward' flow mechanisms of portal hypertension. Relative contributions in the rat model of portal vein stenosis, Gastroenterology 89, 1092.

Benoit, J.N., B. Zimmerman, A.J. Premen, V.L.W. Go and D.N. Granger, 1986, Role of glucagon in splanchnic hyperemia of chronic portal hypertension, Am. J. Physiol. 251, G674.

Bigaud, M., G. Julou-Schaeffer, J.R. Parratt and J.-C. Stoclet, 1990, Endotoxin-induced impairment of vascular smooth muscle contractions elicited by different mechanisms, Eur. J. Pharmacol. 190, 185.

Bomzon, A. and L.M. Blendis, 1987, Vascular reactivity in experimental portal hypertension, Am. J. Physiol. 252, G158.

Buga, G.M., J.M. Griscavage, N.E. Rogers and L.J. Ignarro, 1993, Negative feedback regulation of endothelial cell function by nitric oxide, Circ. Res. 73, 808.

Chojkier, M. and R.J. Groszmann, 1981, Measurement of portal-systemic shunting in the rat by using γ-labeled microspheres, Am. J. Physiol. 240, G371.

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

De Man, J.G., P.A. Pelckmans, G.E. Boeckxstaens, H. Bult, L. Oosterbosch, A.G. Herman and Y.M. Van Maercke, 1991, The role of nitric oxide in inhibitory non-adrenergic non-cholinergic neurotransmission in the canine lower oesophageal sphincter, Br. J. Pharmacol. 103, 1092.

Finberg, J.P.M., H.A. Syrop and O.S. Better, 1985, Blunted pressor response to angiotensin and sympaticomimetic amines in bile-duct ligated dogs, Clin. Sci. 61, 535.

Fineman, J.R., M.A. Heymann and S.J. Soifer, 1991, N^{ω}-Nitro-L-arginine attenuates endothelium-dependent pulmonary vasodilation in lambs, Am. J. Physiol. 260, H1299.

Glantz, S.A. and B.K. Slinker, 1990, Primer of Applied Regression and Analysis of Variance (McGraw-Hill, New York) p. 404.

Ishii, K., B. Chang, J.F. Kerwin, Z.J. Huang and F. Murad, 1990, N^{ω}-Nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation, Eur. J. Pharmacol. 176, 219.

Iwata, F., T. Joh, T. Kawai and M. Itoh, 1992, Role of EDRF in splanchnic blood flow of normal and chronic portal hypertensive rats, Am. J. Physiol. 263, G149.

Julou-Schaeffer, G., G.A. Gray, I. Fleming, C. Schott, J.R. Parratt and J.-C. Stoclet, 1990, Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway, Am. J. Physiol. 259, H1038.

Kelm, M., M. Feelisch, R. Spahr, H.M. Piper, E. Noack and J. Schrader, 1988, Quantitative and kinetic characterization of nitric oxide and EDRF released from cultured endothelial cells, Biochem. Biophys. Res. Commun. 154, 236.

Kiel, J.W., V. Pitts, J.N. Benoit, D.N. Granger and A.P. Shepherd, 1985, Reduced vascular sensitivity to norepinephrine in portal hypertensive rats, Am. J. Physiol. 248, G192.

Knowles, R.G., M. Salter, S.L. Brooks and S. Moncada, 1990, Anti-inflammatory glucocorticoid inhibit the induction by endotoxin of nitric oxide synthase in the lung, liver and aorta of the rat, Biochem. Biophys. Res. Commun. 172, 1042.

Kravetz, D., J. Bosch, M.T. Arderiu, M.P. Pizcueta, R. Casmitjana, F. Rivera and J. Rodés, 1988, Effects of somatostatin on splanchnic hemodynamics and plasma glucagon in portal hypertensive rats, Am. J. Physiol. 254, G322.

Lebrec, D., C. Bataille, E. Bercoff and D. Valla, 1983, Hemodynamic changes in patients with portal venous obstruction, Hepatology 3, 550.

Lee, F.-Y., A. Albillos, L.A. Colombato and R.J. Groszmann, 1992, The role of nitric oxide in the vascular hyporesponsiveness to methoxamine in portal hypertensive rats, Hepatology 16, 1043.

Martin, G.R., M.L. Bolofo and H. Giles, 1992, Inhibition of endothelium-dependent vasorelaxation by arginine analogues: a pharmacological analysis of agonist and tissue dependence, Br. J. Pharmacol. 105, 643.

Meddings, J.B., R.B. Scott and G.H. Fick, 1989, Analysis and comparison of sigmoidal curves: application to dose-response data, Am. J. Physiol. 257, G982.

Mehta, R., J. Gottstein, W.P. Zeller, R. Lichtenberg and A.T. Blei, 1990, Endotoxin and the hyperdynamic circulation of portal vein-ligated rats, Hepatology 12, 1152.

Mesh, C.L., J.N. Benoit and R.J. Korthuis, 1988, The rat portal vein stenosis model of prehepatic portal hypertension, in: Animal Models of Portal Hypertension, eds. S.K. Sarin and N.C. Nayak (Radha Press, New Delhi) p. 1.

Miller, R.L., R.P. Forsyth, B.I. Hoffbrand and K.L. Melmon, 1973, Cardiovascular effects of hemorrhage during endotoxemia in unanesthetized monkeys, Am. J. Physiol. 224, 1087.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1989, Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication, Biochem. Pharmacol. 38, 1709.

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

Mülsch, A. and R. Busse, 1990, N^{G}-Nitro-L-arginine (N5-[imino(nitroamino)methyl]-L-ornithine) impairs endothelium-dependent dilations by inhibiting cytosolic nitric oxide synthesis from L-arginine, Naunyn-Schmied. Arch. Pharmacol. 341, 143.

Murray, B.M. and M.S. Paller, 1985, Decreased pressor reactivity to angiotensin II in cirrhotic rats, Circ. Res. 57, 424.

Murray, B.M. and M.S. Paller, 1986, Pressor resistance to vasopressin in sodium depletion, potassium depletion, and cirrhosis, Am. J. Physiol. 251, R525.

Pizcueta, M.P., J.M. Piqué, J. Bosch, B.J.R. Whittle and S. Moncada, 1992a, Effects of inhibiting nitric oxide biosynthesis on the systemic and splanchnic circulation of rats with portal hypertension, Br. J. Pharmacol. 105, 184.

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

Radomski, M.W., R.M.J. Palmer and S. Moncada, 1990, Anti-inflammatory glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells, Proc. Natl. Acad. Sci. USA 87, 10043.

Rees, D.D., R.M.J. Palmer and S. Moncada, 1989, Role of endothe-

lium-derived nitric oxide in the regulation of blood pressure, Proc. Natl. Acad. Sci. USA 86, 3375.

Rees, D.D., S. Allec, R.M.J. Palmer and S. Moncada, 1990a, Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: an insight into endotoxin shock, Biochem. Biophys. Res. Commun. 173, 541.

Rees, D.D., R.M.J. Palmer, R. Schulz, H.F. Hodson and S. Moncada, 1990b, Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo, Br. J. Pharmacol. 101, 746.

Schaller, M.D., B. Waeber, J. Nussberger and H.R. Brunner, 1985, Angiotensin II, vasopressin, and sympathetic activity in conscious rats with endotoxemia, Am. J. Physiol. 249, H1086.

Sieber, C.C. and R.J. Groszmann, 1992a, In vitro hyporeactivity to methoxamine in portal hypertensive rats: reversal by nitric oxide blockade, Am. J. Physiol. 262, G996.

Sieber, C.C. and R.J. Groszmann, 1992b, Nitric oxide mediates hyporeactivity to vasopressors in mesenteric vessels of portal hypertensive rats, Gastroenterology 103, 235.

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.

Sikuler, E., D. Kravetz and R.J. Groszmann, 1985, Evolution of portal hypertension and its maintenance in a rat model, Am. J. Physiol. 248, G618.

Sogni, P., M. Moreau, 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.

Vallance, P. and S. Moncada, 1991, Hyperdynamic circulation in cirrhosis: a role for nitric oxide?, Lancet 337, 776.

Vorobioff, J., J.E. Bredfeldt and R.J. Groszmann, 1983, Hyperdynamic circulation in a portal hypertensive rat model: a primary factor for maintenance of chronic portal hypertension, Am. J. Physiol. 244, G52.

Wakabayashi, I., K. Hatake, E. Kakishita and K. Nagai, 1987, Diminution of contractile response of the aorta from endotoxin-injected rats, Eur. J. Pharmacol. 141, 117.

Winer, B.J., 1971, Statistical Principles in Experimental Design (McGraw-Hill, New York) p. 528.