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Aminoguanidine reverses aortic hyporeactivity to noradrenaline in portal vein-ligated rats

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Abstract

To evaluate the role of the inducible and endothelial constitutive nitric oxide synthase in vascular hyporeactivity to vasopressors in portal hypertension, in vitro experiments were performed on intact and endothelium-denuded isolated thoracic aortic rings from portal vein-ligated and sham-operated rats in control conditions, in the presence of aminoguanidine alone, considered to be a selective inhibitor of the inducible nitric oxide synthase, and of aminoguanidine and the nonselective nitric oxide synthase inhibitor N^G-nitro-L-arginine. In control conditions, hyporeactivity to noradrenaline was observed in both rings with and without endothelium from portal hypertensive versus sham-operated rats. In the rings with endothelium, aminoguanidine reverted this hyporeactivity in portal hypertensive rats. N^G-Nitro-L-arginine caused an additional shift to the left of the concentration–response curves to noradrenaline in portal hypertensive and a similar shift in sham-operated rats. In the endothelium-denuded rings, aminoguanidine caused no significant changes in portal hypertensive rats, whereas a significant shift to the right in the sham-operated rats was noted, however similar as the shift in the time controls not preincubated with aminoguanidine. No significant further changes were observed after preincubation with the two inhibitors. The endothelium-dependent relaxations to acetylcholine were attenuated in portal hypertensive versus sham-operated rats; addition of aminoguanidine shifted the relaxation curves to the left in portal hypertensive but not in sham-operated rats. These results provide indirect evidence for an increased activity of the inducible nitric oxide synthase in the intact aortic rings but not in the endothelium-denuded rings from portal vein-ligated rats, where other factors seem to be responsible for the observed hyporeactivity to noradrenaline. The endothelial constitutive nitric oxide synthase in rings from portal vein-ligated rats shows a reduced activity which is alleviated after inhibition of the inducible enzyme by aminoguanidine. © 1997 Elsevier Science B.V.

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

1. Introduction

In experimental animals with portal hypertension, splanchnic vasodilatation is considered to be of major importance in the pathogenesis of the hyperdynamic circulation in this syndrome (Vorobioff et al., 1983, 1984). The low splanchnic vascular resistance observed in portal hypertension depends mostly on mesenteric resistance arteries (Benoit and Granger, 1988). On the other hand, hyperkinetic blood flow is now known to be a systemic syndrome with multiple organ involvement: splanchnic, pulmonary, brain and systemic circulation (Groszmann, 1994). Physiological regulatory systems may differ between regional vascular beds and between small and large arteries (Mulvany and Halpern, 1977). In the portal vein-ligated rat, in vitro studies have demonstrated hyporesponsiveness to vasoconstrictors in isolated mesenteric bed (Sieber and Groszmann, 1992a), mesenteric resistance arteries (Sogni et al., 1996) and thoracic aortic rings (Bomzon and Blendis, 1987; Michielsen et al., 1995a,b). Also in cirrhotic portal hypertensive rats, thoracic aorta has been studied (Castro et al., 1993). All these data indicate a general arterial phenomenon.

Previously, we provided indirect evidence for increased nitric oxide production in isolated aortic rings from short-

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term (3 weeks postoperatively) and long-term (6 months postoperatively) portal vein-ligated rats (Michielsen et al., 1995a,b) by using N^G-nitro-L-arginine, an inhibitor of both the constitutive and inducible nitric oxide synthase (Joly et al., 1994). Also other recent investigations using nonselective nitric oxide synthase inhibitors have suggested that increased nitric oxide synthase activity could be involved in the systemic hemodynamic disturbances occurring in cirrhotic portal hypertensive (Pizcueta et al., 1992b; Clària et al., 1992; Castro et al., 1993; Sieber et al., 1993) and portal vein-ligated rats (Pizcueta et al., 1992a; Sieber and Groszmann, 1992a,b; Lee et al., 1992; Karatapanis et al., 1994).

Nitric oxide is synthesized from L-arginine by the enzyme nitric oxide synthase. It has recently become apparent that three isoforms of nitric oxide synthase exist, including a Ca^{2+}-dependent constitutive neural and vascular endothelial isoenzyme and a Ca^{2+}-independent inducible form (Moncada et al., 1991). The constitutive enzyme is normally present in the vascular endothelium, whereas the synthesis of the inducible enzyme is triggered by endotoxin and cytokines in several cell types including vascular endothelium, vascular smooth muscle cells, hepatocytes and macrophages. The synthesis of nitric oxide can stereospecifically be inhibited by L-arginine analogues such as N^G-nitro-L-arginine (Mülsch and Busse, 1990). Several reports indicate that aminoguanidine, another L-arginine analogue, is a selective inhibitor of the inducible nitric oxide synthase in rat pulmonary artery (Griffiths et al., 1993), rat mesenteric resistance arteries (Hasan et al., 1993) and rat aorta (Misko et al., 1993; Joly et al., 1994).

Controversy exists whether in portal hypertension the increased nitric oxide biosynthesis can be attributed to induction of the inducible isoform or to an upregulation of the constitutive enzyme by an increase in shear stress due to the increased blood flow itself (Rubanyi et al., 1986; Buga et al., 1991). Direct measurement of both Ca^{2+}-dependent and independent nitric oxide synthase activity in splanchnic tissues from portal vein-ligated and cirrhotic rats caused by bile duct ligation showed no significant differences with control animals; moreover, inducible nitric oxide synthase activity was close to the detection limit of the assay used (Fernández et al., 1995). Similar measurements in thoracic aorta and superior mesenteric artery from portal vein-ligated rabbits showed increased Ca^{2+}-dependent nitric oxide synthase activity as compared to controls; using specific antisera no inducible nitric oxide synthase was detected (Cahill et al., 1995). Aminoguanidine did not restore the hyporesponsiveness to the α agonist methoxamine of mesenteric arterial bed from portal vein-ligated rats, suggesting that the inducible isoenzyme is not involved (Heinemann and Stauber, 1995). On the other hand, transcriptional activation of inducible nitric oxide synthase was detected in aorta and superior mesenteric artery from cirrhotic rats with ascites caused by CCl_4 (Morales et al., 1995). Furthermore, aminoguanidine treatment improves the in vitro vascular hyporeactivity to methoxamine in the superior mesenteric bed (Atucha et al., 1994) and ameliorates the hyperdynamic syndrome in vivo (Lopez-Talavera and Groszmann, 1994) in portal vein-ligated rats, suggesting induction of the inducible nitric oxide synthase in portal hypertension. Treatment with anti-tumour necrosis factor-α antibodies has recently been shown to prevent the development of the hyperdynamic circulation in portal vein-ligated rats (Lopez-Talavera et al., 1995). As tumour necrosis factor-α has been shown to cause vasodilatation and a hyperdynamic state in mammals by activating nitric oxide biosynthesis (Kilbourn et al., 1990), these findings suggest that tumour necrosis factor-α could be the signal that triggers the induction of nitric oxide biosynthesis leading to the systemic and splanchnic hyperdynamic syndrome in portal hypertension.

In order to further clarify the potential role of the inducible nitric oxide synthase in portal hypertension, we performed in vitro experiments on isolated thoracic aortic rings from portal vein-ligated rats in control conditions, in the presence of aminoguanidine and in the presence of both aminoguanidine and the nonselective nitric oxide synthase inhibitor N^G-nitro-L-arginine.

2. Materials and methods

2.1. Animal preparation

The study was performed in 15 male Wistar rats weighing 433 ± 9 g, that had either undergone a sham operation or a portal vein ligation to induce portal hypertension, as previously described (Michielsen et al., 1995a). Briefly, using ether anaesthesia, the abdominal cavity was opened through a midline incision. After the portal vein was isolated, a ligature (silk gut 4-0) was placed around a 20-gauge blunt tipped needle lying alongside the portal vein. The needle was then removed and the portal vein allowed to reexpand, to yield a constant stenosis of the portal vein. In the sham-operated rats, the portal vein was similarly isolated but no ligation was used. After surgery, the rats were allowed to recover from anaesthesia and were returned to the vivarium, where they had free access to water and food. In preliminary experiments, the portal venous pressure in 10 portal vein-ligated rats was shown to be almost twice that in 14 sham-operated rats 2 weeks after surgery (18.3 ± 1.1 and 9.4 ± 0.4 mm Hg respectively, $P < 0.001$) (Michielsen et al., 1995a). Treatment of the animals during the experiments was conducted according to the rules of good animal care in conformity with the European legislation and was approved by the Ethical Committee of the University.

2.2. Tissue preparation

The experiments were performed 3 weeks postoperatively. Under ether anaesthesia the animals were killed by

opening the thorax and cardiectomy. The thoracic aorta was removed, cleared from 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. Dexamethasone (10^{-7} M) was added to the Krebs-Ringer solution from the isolation of the vessels and throughout the whole experiment, in order to prevent in vitro induction of nitric oxide synthase in the rings by endotoxins present in the organ baths (Rees et al., 1990; Radomski et al., 1990). One of the stirrups was anchored to the organ chamber, one was connected to a strain gauge (Statham UC2), for recording of isometric tension. The aortic rings were stretched to the point of their optimal length-tension relationship (4 g, determined in similar preliminary experiments using repeated exposure to 50 mM KCl) and allowed to equilibrate for 60 min.

The following interventions on intact and endothelium-denuded thoracic aortic rings were performed:

– In a first intervention, the rings were contracted with noradrenaline in a concentration resulting in a stable, submaximal contraction (3×10^{-7} M or 3×10^{-6} M). Cumulative relaxation curves to the endothelium-dependent vasodilator acetylcholine (10^{-9}–10^{-4} M) were obtained in each ring, in control conditions. Rings showing < 60% relaxation of the noradrenaline-induced contraction were discarded (Rees et al., 1990). The failure of acetylcholine to induce relaxation of rubbed rings was taken as an indication of endothelium removal.

– In a second intervention, contractile responses to cumulative concentrations of noradrenaline (10^{-10}–10^{-5} M) were studied in control conditions.

– In a third intervention, the rings were contracted with noradrenaline as in the first set of experiments, after preincubation with aminoguanidine (3×10^{-4} M) for 15 min. Cumulative relaxation curves to acetylcholine (10^{-9}–10^{-4} M) were repeated as in the first set of experiments.

– In a fourth intervention, contractile responses to cumulative concentrations of noradrenaline (10^{-10}–10^{-5} M) were repeated after preincubation with aminoguanidine (3×10^{-4} M) for 15 min.

– In a fifth intervention, contractile responses to cumulative concentrations of noradrenaline (10^{-10}–10^{-5} M) were repeated after incubation with both aminoguanidine (3×10^{-4} M) and the nonselective nitric oxide synthase inhibitor N^G-nitro-L-arginine (3×10^{-5} M) for 15 min.

– In a sixth intervention, the effect of consecutively added concentrations of nitric oxide (10^{-7}–10^{-5} M) on rings maximally contracted 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.

– In a separate set of rings with endothelium from control rats submaximally contracted with noradrenaline and showing > 60% relaxation during a response to cumulative concentrations of acetylcholine (10^{-9}–10^{-4} M), the effect of preincubation during 10 min with N^G-nitro-L-arginine (3×10^{-5} M) on these relaxations was assessed.

From each animal, two rings with and two rings without endothelium were prepared: one set was used to perform the experiments, the other set was studied in the absence of nitric oxide inhibitors and was taken as time control.

The experiments were consecutively performed as follows. After equilibration of the rings in Krebs-Ringer solution for 60 min at basal tension, acetylcholine-induced relaxation curves of noradrenaline-contracted rings were performed. After washout, returning the residual tension to baseline, a first concentration–response curve to noradrenaline was constructed. The obtained maximal tension was washed out, afterwards the rings were incubated with aminoguanidine for at least 15 min; then a second concentration–response curve to noradrenaline was repeated. The tension of this curve was then washed out, followed by reincubation with aminoguanidine for at least 15 min and performance of a second acetylcholine-induced relaxation curve of noradrenaline-contracted rings. After washout, the rings were incubated with both N^G-nitro-L-arginine and aminoguanidine for at least 15 min, followed by construction of a third concentration–response curve to noradrenaline. In the time control rings, repetitive concentration–response curves to acetylcholine and noradrenaline were obtained in the absence of nitric oxide synthase inhibitors.

2.3. Drugs

The drugs used were acetylcholine hydrochloride, aminoguanidine bicarbonate salt and dexamethasone 21-phosphate (Sigma, St. Louis, MO, USA), noradrenaline hydrogentatrate (Fluka, Buchs, Switzerland), N^G-nitro-L-arginine (Janssen Chimica, Beerse, Belgium). All solutions were prepared on the day of experimentation and were administered as aqueous solutions, except N^G-nitro-L-arginine and aminoguanidine bicarbonate salt, which were dissolved in 6.5×10^{-2} 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).

2.4. 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 the 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 (A) and nonrepeated measures on the second factor (B). Repeated measures on one factor (A) consisted in three levels representing control, presence of aminoguanidine alone and presence of both aminoguanidine and N^G-nitro-L-arginine. The nonrepeated measures factor (B) had two levels, i.e., sham or portal hypertension. Different rings in different animal groups and the two experimental factors (A and B) 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 versus without encoding for different rings (Meddings et al., 1989). 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) were similarly treated. Because of a major interest in comparison among treatment means and because of significant interactions between experimental factors, multiple comparisons among treatment means were performed in each analysis, based on the computations given by Winer, by using differences between means and standard errors of the estimates (Winer, 1971). The standard error used for these comparisons, that is reported in the results, is the weighted average of two standard errors; the standard error of subjects within groups was estimated from the coefficients associated with the dummy variables for the different rings.

Rings with endothelium and rings without endothelium were treated in separate analyses.

The concentration–response data of acetylcholine were similarly analysed.

The possible differences in animal weight and portal venous pressure between sham-operated and portal vein-ligated rats, in the starting contractions to noradrenaline before acetylcholine and in the relaxations to nitric oxide were analysed by unpaired Student's t-test.

The number of experiments equals the number of animals used. P values of less than 0.05 were considered as significant.

3. Results

3.1. Body weights

The body weights of the rats on the day of the experiment were 432 ± 13 g ($n = 8$) in the sham-operated rats and 434 ± 13 g ($n = 7$) in the portal vein-ligated rats and were not significantly different.

3.2. Relaxation curves to acetylcholine

Experiments were performed on aortic rings submaximally contracted with noradrenaline from 8 sham-operated and 7 portal hypertensive rats. One ring in each group was discarded as the relaxation to cumulative concentrations of acetylcholine was less than 60%.

As expected, in the endothelium-denuded rings no acetylcholine-induced relaxations were noted. In the intact rings, the noradrenaline-induced contractions were 2.31 ± 0.13 g and 2.51 ± 0.15 g in 7 sham-operated and 6 portal vein-ligated rats respectively (not significantly different). The aortic rings from portal vein-ligated rats were less reactive to acetylcholine as indicated by the significantly lower pD_2 values as compared to sham-operated rats; the maximal relaxations (T^m) were not significantly different (Table 1).

After preincubation with N^G-nitro-L-arginine (3×10^{-5} M), the relaxations to acetylcholine in 14 aortic rings submaximally contracted with noradrenaline were entirely abolished.

Aminoguanidine (3×10^{-4} M) had no effect on the noradrenaline-induced contractions (data not shown). Furthermore, it did not influence pD_2 values to acetylcholine in sham-operated rats. In portal hypertensive rats, however, the relaxation curves significantly shifted to the left as compared to control conditions (Table 1; Fig. 1). After aminoguanidine, pD_2 values to acetylcholine were significantly lower in sham-operated as compared with portal vein-ligated rats. The maximal relaxations in both sham-

Table 1
pD_2 and T^m values of acetylcholine in rat aortic rings with endothelium submaximally precontracted with noradrenaline, in the absence and presence of aminoguanidine (3×10^{-4} M)

	pD_2 (S.E.M. = 0.10)			T^m (S.E.M. = 5.2)		
	Control		Aminoguanidine	Control		Aminoguanidine
Sham operation ($n = 7$)	6.79	← ns →	6.67	82.9	← a →	75.8
	↕ a		↕ a	↕ ns		↕ ns
Portal hypertension ($n = 6$)	6.34	← a →	6.96	85.7	← a →	65.5

Results are shown as means and S.E.M. for 6 or 7 experiments. Maximal relaxation (T^m) is expressed as percent decrease of the noradrenaline-induced contraction. [ns] Not significant; [a] $P < 0.05$, portal hypertension versus sham operation or after aminoguanidine versus control.

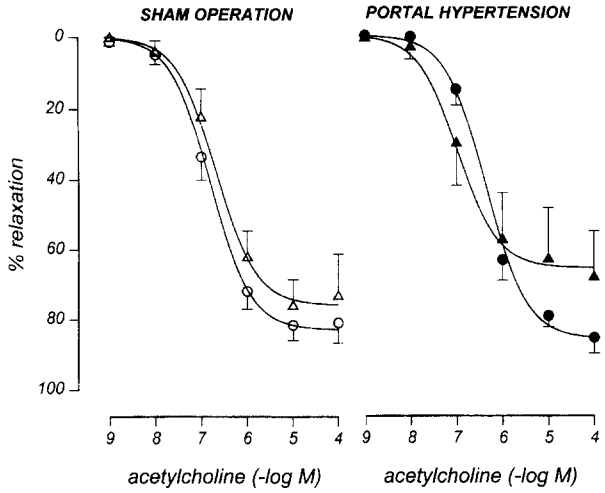

Fig. 1. Relaxation curves to acetylcholine (10^{-9}–10^{-4} M) on intact aortic rings from sham-operated and portal hypertensive rats before (circles) and after incubation with aminoguanidine (triangles). Data are presented as means ± S.E.M. in % decrease of the noradrenaline-induced contraction from 6 or 7 experiments.

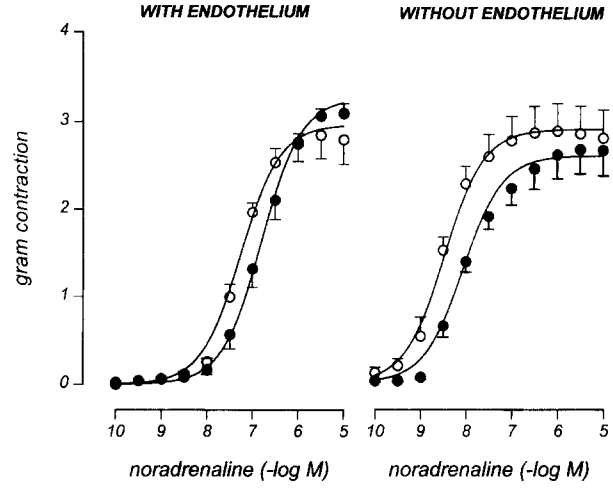

Fig. 2. Concentration–response curves to noradrenaline (10^{-10}–10^{-5} M) in aortic rings with or without endothelium in control conditions, of sham-operated (○) and portal hypertensive (●) rats. Data are presented as means ± S.E.M. in g contraction from 7 or 8 experiments.

operated and portal hypertensive rats were significantly reduced as compared to control conditions.

3.3. Concentration–response curves to noradrenaline

Experiments were performed on intact rings from 8 sham-operated and 7 portal hypertensive rats and in endothelium-denuded rings from 7 sham-operated and 7 portal hypertensive rats. The concentration–response curves to noradrenaline in both intact and endothelium-denuded rings from portal vein-ligated rats were significantly shifted to the right as compared to those of sham-operated rats as was shown by the significantly lower pD_2 values of noradrenaline in portal vein-ligated versus sham-operated rats; the maximal contractions were not significantly different (Table 2; Fig. 2).

In the intact rings, preincubation with aminoguanidine caused a significant shift of the concentration–response curve to the left in portal vein-ligated rats as illustrated by the significant increase in pD_2, but not in sham-operated animals (Table 2; Fig. 3A). In the sham-operated rats but not in the portal vein-ligated rats, the maximal contractions (T^m) significantly increased (Table 2; Fig. 3A).

In the rings without endothelium, however, preincubation with aminoguanidine resulted in a significant decrease in pD_2 and increase in T^m in sham-operated rats, whereas no significant changes in portal vein-ligated rats were observed (Table 2; Fig. 3B).

After aminoguanidine, the concentration–response curves to noradrenaline were not significantly different any more between sham-operated and portal vein-ligated rats (Table 2).

In the intact rings, preincubation with aminoguanidine and N^G-nitro-L-arginine resulted in an additional shift of the concentration–response curve to noradrenaline to the left in portal vein-ligated rats and a similar shift in sham-

Table 2
pD_2 and T^m values of noradrenaline in rat aortic rings with or without endothelium in control conditions, after preincubation with aminoguanidine (AG; 3×10^{-4} M) and with aminoguanidine (AG; 3×10^{-4} M) and N^G-nitro-L-arginine (L-NOARG; 3×10^{-5} M)

	pD_2					T^m				
	Control		+AG		+AG and L-NOARG	Control		+AG		+AG and L-NOARG
	With endothelium (S.E.M. = 0.09)					With endothelium (S.E.M. = 0.14)				
Sham operation ($n = 8$)	7.26	← ns →	7.36	← a →	7.82	2.96	← a →	3.42	← ns →	3.45
	↕ a		↕ ns		↕ ns	↕ ns		↕ ns		↕ ns
Portal hypertension ($n = 7$)	6.80	← a →	7.15	← a →	7.61	3.26	← ns →	3.28	← ns →	3.31
	Without endothelium (S.E.M. = 0.08)					Without endothelium (S.E.M. = 0.20)				
Sham operation ($n = 7$)	8.47	← a →	8.06	← ns →	8.21	2.88	← a →	3.18	← ns →	3.11
	↕ a		↕ ns		↕ ns	↕ ns		↕ ns		↕ ns
Portal hypertension $n = 7$	8.06	← ns →	8.05	← ns →	8.05	2.58	← ns →	2.74	← ns →	2.69

Results are shown as means and S.E.M. for 7 or 8 experiments. Maximal contraction (T^m) is expressed in g contraction. [ns]Not significant; [a] $P < 0.05$, portal hypertension versus sham operation, after aminoguanidine versus control or after aminoguanidine and N^G-nitro-L-arginine versus after aminoguanidine alone.

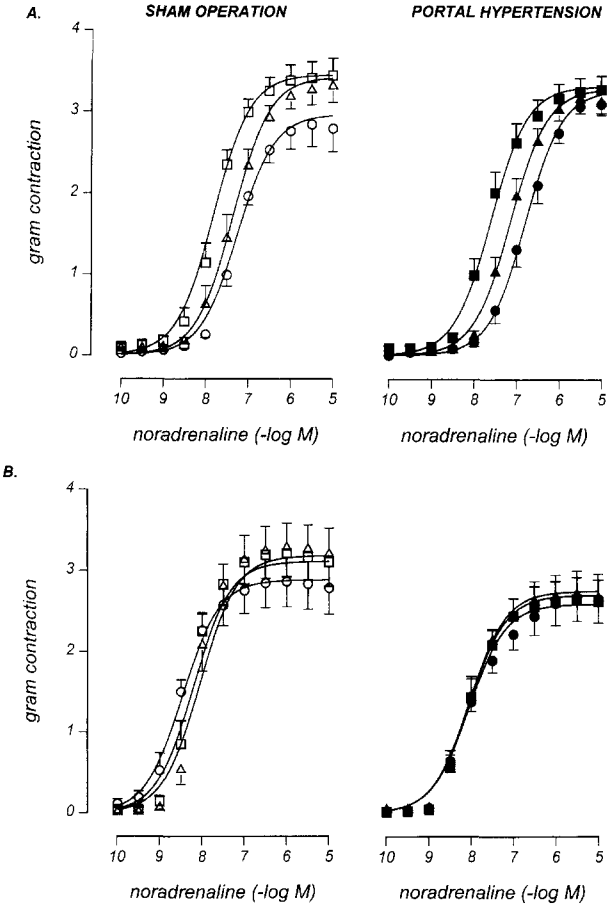

Fig. 3. Concentration–response curves to noradrenaline (10^{-10}–10^{-5} M) in aortic rings with (A) and without endothelium (B) from sham-operated and portal hypertensive rats, in control conditions (circles), after preincubation with aminoguanidine (triangles) and after preincubation with aminoguanidine and N^G-nitro-L-arginine (squares). Data are presented as means ± S.E.M. in g contraction from 7 or 8 experiments.

operated rats, as illustrated by the significant increase in pD_2 (Table 2; Fig. 3A).

In the endothelium-denuded rings, no significant further changes were observed (Table 2; Fig. 3B).

After preincubation with aminoguanidine and N^G-nitro-L-arginine, the concentration–response curves to noradrenaline showed no significant differences between sham-operated and portal vein-ligated rats (Table 2).

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 concentration-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).

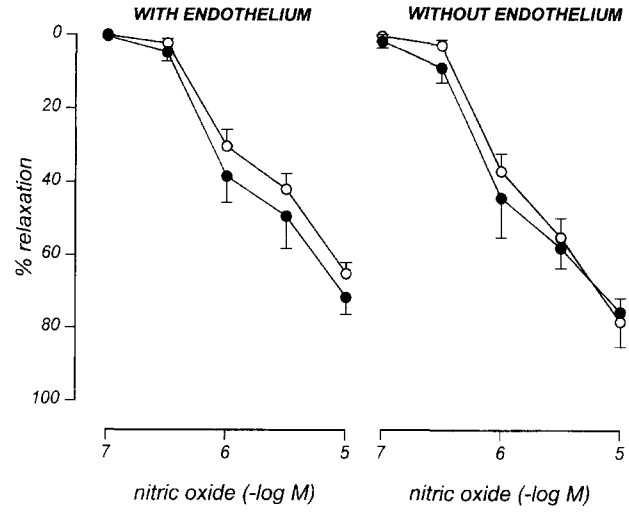

Fig. 4. Relaxation curves to nitric oxide (10^{-7}–10^{-5} M) on aortic rings maximally contracted with noradrenaline (10^{-5} M) from sham-operated (○) and portal vein-ligated (●) rats. Data are presented as means ± S.E.M. in % decrease of the noradrenaline-induced contraction from 7 or 8 experiments.

3.5. Evaluation of time controls

In order to evaluate possible time-related changes, the first and second concentration–response curves to acetylcholine were compared in 7 and 5 aortic rings with endothelium from respectively sham-operated and portal vein-ligated rats, which had shown at least 60% relaxation. There was a significant increase in T^m value in the second as compared to the first curve in both sham-operated and portal vein-ligated rats. Furthermore, the second curve was significantly shifted to the left in sham-operated rats as illustrated by the significant increase in pD_2 (Table 3).

The first and second concentration–response curves to noradrenaline were compared in 8 and 9 aortic rings with endothelium from respectively sham-operated and portal vein-ligated rats, which had shown at least 60% relaxation to acetylcholine. There was a significant increase in pD_2 from the first to the second curve in the sham-operated rats, indicating a sensitization to noradrenaline with time,

Table 3
Differences in pD_2 and T^m values of acetylcholine between the first and second concentration–response curves to acetylcholine in rat aortic rings with endothelium used as time controls

	ΔpD_2	ΔT^m
Sham operation ($n = 7$)	+0.30 ± 0.13 ($P < 0.05$)	+13.4 ± 3.8 ($P < 0.05$)
Portal hypertension ($n = 5$)	+0.23 ± 0.12 (ns)	+13.5 ± 3.5 ($P < 0.05$)

The results are shown as mean differences ± S.E.M. for 5 or 7 experiments. Maximal contraction (T^m) is expressed as percent decrease of the noradrenaline-induced contraction. ΔpD_2: pD_2 of the second curve minus pD_2 of the first curve. ΔT^m: T^m of the second curve minus T^m of the first curve. ns: not significant.

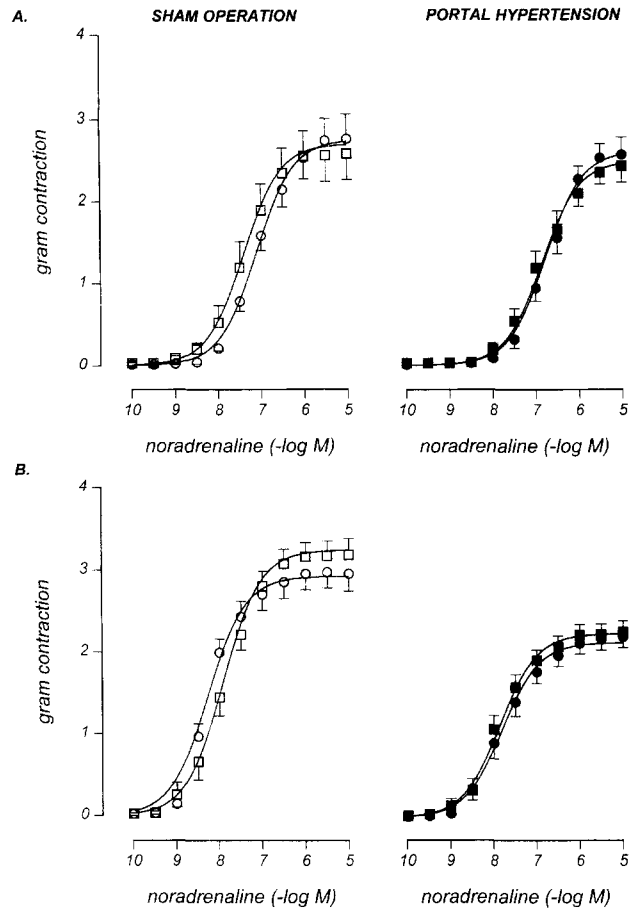

Fig. 5. Concentration–response curves to noradrenaline (10^{-10}–10^{-5} M) in aortic rings with (A) and without (B) endothelium from sham-operated (open symbols) and portal vein-ligated rats (closed symbols), used as time controls. Circles represent the first, squares the second curve. Data are presented as means ± S.E.M. in g contraction from 8–13 experiments.

but not in portal vein-ligated rats; T^m values were not significantly different (Fig. 5; Table 4).

Similarly, the first and second concentration–response curves to noradrenaline were compared in 13 and 11 aortic rings without endothelium from respectively sham-operated and portal vein-ligated rats. There was a significant decrease in pD_2 from the first to the second curve in the sham-operated rats, indicating a desensitization with time, but not in the portal vein-ligated rats; the maximal contractions were significantly higher in the second curve of both sham-operated and portal vein-ligated rats (Fig. 5; Table 4).

Time controls comparing the second and third concentration–response curves to noradrenaline showed no significant differences in pD_2 or T^m (results not shown).

4. Discussion

Recent studies have emphasized the role of increased vascular nitric oxide production in the systemic and splanchnic vasodilatation, and vascular hypo-responsiveness to vasoconstrictors in portal hypertension. Controversy exists whether the constitutive endothelial nitric oxide synthase is upregulated or the inducible nitric oxide synthase is activated in endothelium and/or smooth muscle cells in the vascular wall. In the present study, using aminoguanidine, a selective inhibitor of the inducible nitric oxide synthase in rat aorta, we provide evidence for activation of the inducible nitric oxide synthase in the thoracic aorta from portal vein-ligated rats.

Aminoguanidine in a concentration of 3×10^{-4} M was shown to give maximal inhibition of the inducible nitric oxide synthase in pulmonary artery from endotoxin-treated rats; higher doses did not result in further inhibition (Griffiths et al., 1993).

Comparable to our previous findings (Michielsen et al., 1995a,b), we demonstrated vascular hyporesponsiveness to noradrenaline in portal vein-ligated rats. The pD_2 values for noradrenaline were significantly lower in aortic rings from portal vein-ligated rats as compared to those from sham-operated rats. In aortic rings with endothelium, aminoguanidine reversed this hyporesponsiveness, a finding not observed in endothelium-denuded rings, suggesting increased inducible nitric oxide synthase activity in the endothelium. In endothelium-denuded rings, blockade of both the constitutive and inducible nitric oxide synthase by aminoguanidine and N^G-nitro-L-arginine did not affect the concentration–response curve either, indicating the involvement of a nitric oxide-unrelated substance in the vascular hyporesponsiveness as well. In the rings with endothelium, N^G-nitro-L-arginine induced a further shift of the concentration–response curve to the left, most likely

Table 4
Differences in pD_2 and T^m values of noradrenaline between the first and second concentration–response curves to noradrenaline in rat aortic rings with and without endothelium used as time controls

	ΔpD_2	ΔT^m
With endothelium		
Sham operation ($n = 8$)	+0.30 ± 0.08 ($P < 0.05$)	−0.06 ± 0.10 (ns)
Portal hypertension ($n = 9$)	+0.09 ± 0.06 (ns)	−0.13 ± 0.08 (ns)
Without endothelium		
Sham operation ($n = 13$)	−0.35 ± 0.06 ($P < 0.05$)	+0.32 ± 0.07 ($P < 0.05$)
Portal hypertension ($n = 11$)	+0.06 ± 0.05 (ns)	+0.10 ± 0.04 ($P < 0.05$)

The results are shown as mean differences ± S.E.M. for 8–13 experiments. Maximal contraction (T^m) is expressed in g contraction. ΔpD_2: pD_2 of the second curve minus pD_2 of the first curve. ΔT^m: T^m of the second curve minus T^m of the first curve. ns: not significant.

resulting from blockade of the constitutive endothelial nitric oxide synthase.

Interpretation of the effect of aminoguanidine on endothelium-denuded rings was obscured by a significant decrease of the pD_2 to noradrenaline in sham-operated rats. Due to this shift, the difference between sham-operated and portal vein-ligated rats was eliminated. However, as shown by the data obtained from the time controls, this effect should rather be attributed to a time effect in endothelium-denuded rings of sham-operated rats. No such effect was observed in the other experiments. Therefore we conclude that the vascular hyporesponsiveness to noradrenaline in portal vein-ligated rats results from induction of nitric oxide synthase in the endothelium. This conclusion, however, is compromised to some extent by time-related effects in the rings of sham-operated rats.

A point of discussion is the difference in our conclusion in previous experiments (Michielsen et al., 1995a) and these experiments, concerning the possible involvement of nitric oxide synthase in the aortic smooth muscle layer. In contrast to the data presented here, we suggested induction of nitric oxide synthase in the aortic smooth muscle layer from similar portal vein-ligated rats (Michielsen et al., 1995a). This discrepancy is related to a difference in pD_2 values in control conditions obtained in rings without endothelium from sham-operated as well as portal vein-ligated rats (in sham-operated rats 7.93 ± 0.09 and 8.47 ± 0.09; in portal vein-ligated rats 7.54 ± 0.09 and 8.06 ± 0.08 respectively in the previous and actual series of experiments). As the pD_2 values after blockade of nitric oxide are almost identical in the two series, these differences in pD_2 values in control conditions inevitably lead to different conclusions concerning the location of the increased nitric oxide synthase activity. Although the two series of experiments were performed in similar conditions in the same animal model, possible explanations for this difference in pD_2 might be difference in animal groups or seasonal variations.

Our data are in accordance with other recent preliminary reports in portal vein-ligated rats (Atucha et al., 1994; Lopez-Talavera and Groszmann, 1994). Differences in animal models, tissues studied and the sensitivity of the used assays to assess the activity of constitutive and inducible nitric oxide synthase might explain the conflicting results reported on the involvement of these enzymes in the hyperdynamic circulation of portal hypertension.

As previously reported (Michielsen et al., 1995a,c), the endothelium-dependent acetylcholine-induced relaxations were significantly reduced in portal vein-ligated rats, indicating a reduced activity of the endothelial constitutive nitric oxide synthase. This can be explained by the knowledge that nitric oxide has a negative feedback effect on the nitric oxide synthase activity (Buga et al., 1993; Bult et al., 1990): nitric oxide produced by the inducible nitric oxide synthase could reduce the activity of the constitutive endothelial enzyme. Desensitization of the smooth muscle cells to 3′,5′-cyclic guanosine monophosphate-dependent relaxation due to exposure to nitric oxide (Ahlner et al., 1991) in the portal vein-ligated rats is unlikely since the relaxations to nitric oxide were not statistically different between sham-operated and portal vein-ligated rats, similarly as previously reported (Michielsen et al., 1995a). The relaxations to the endothelium-independent vasodilator nitric oxide also demonstrate the integrity of the relaxatory apparatus in the aortic rings used.

In contrast to our findings in aortic rings preincubated with N^G-nitro-L-arginine (3×10^{-5} M), the acetylcholine-induced relaxations were still present after preincubation with aminoguanidine (3×10^{-4} M), with unaffected pD_2 values in sham-operated rats. However, as after aminoguanidine T^m values for the concentration–response curves to acetylcholine were significantly decreased in both sham-operated and portal vein-ligated rats, in contrast to the increased T^m values in the time controls, some inhibition of the endothelial constitutive nitric oxide synthase by aminoguanidine in the used concentration has to be considered. This inhibition, however, is minimal as compared to the effect of N^G-nitro-L-arginine (3×10^{-5} M), completely abolishing the acetylcholine-induced relaxations. Other investigators reported no effect of aminoguanidine on the acetylcholine-induced relaxations in rat aortic rings (Joly et al., 1994; concentration 10^{-4} M) and rat pulmonary artery (Griffiths et al., 1993; concentration 3×10^{-4} M). The differences with our data might be explained by differences in the used concentrations of aminoguanidine and/or in the vascular preparations.

In portal hypertensive rats, aminoguanidine significantly shifted the relaxation curves to acetylcholine to the left, indicating an enhanced activity of the endothelial constitutive nitric oxide synthase. This finding can be explained by reduced feedback inhibition of the constitutive nitric oxide synthase by nitric oxide synthesized by the inducible enzyme.

From these data, we conclude that the aortic responsiveness to noradrenaline in portal vein-ligated rats results from induction of nitric oxide synthase most likely in the endothelium. In these experiments, we were unable to demonstrate induction of nitric oxide synthase in the smooth muscle wall of aorta from portal vein-ligated rats. Other mechanisms might be involved in the hyporesponsiveness to noradrenaline in endothelium-denuded rings from portal vein-ligated rats. The observed reduction in endothelial constitutive nitric oxide synthase in portal vein-ligated rats most likely results from feedback inhibition by nitric oxide resulting from inducible nitric oxide synthase activity.

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