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Nitric oxide-dependent and -independent vascular hyporeactivity in mesenteric arteries of portal hypertensive rats

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1 Increased production of nitric oxide (NO) has been suggested to underlie both the vascular hyporeactivity to vasoconstrictors and the splanchnic vasodilatation seen in portal hypertension. This study assessed the role of NO in the vasoconstrictor hyporeactivity of portal vein-ligated (PVL) rats in isolated and *in situ* perfused mesenteric arterial beds.

2 Isolated perfused mesenteric arteries of PVL rats were significantly less reactive to noradrenaline (NA), methoxamine (METH), arginine vasopressin (AVP) and endothelin-1 (ET-1) than those from sham-operated (Sham) rats.

3 Blockade of NO synthesis with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) in isolated perfused mesenteric arteries from PVL rats restored the reactivity to bolus injections of AVP and ET-1, but had little effect on the hyporeactivity to NA or METH. Cyclo-oxygenase inhibition with indomethacin (5 μM) likewise did not restore reactivity to METH of isolated perfused mesenteric arteries of PVL rats.

4 The hyporeactivity to METH seen in isolated perfused mesenteric arteries from PVL rats was reduced by low concentrations of AVP (20 nM) or ET-1 (1 nM) which *per se* caused only a slight increase in perfusion pressure. When L-NAME (100 μM) was combined with AVP (20 nM) or ET-1 (1 nM), respectively, reactivity to METH of isolated perfused mesenteric arteries of PVL rats was restored to the level seen in Sham rats. These effects of AVP and ET-1 were not mimicked by precontracting the vessels with 5-hydroxytryptamine (5 μM).

5 The differential effects of L-NAME and AVP on the hyporesponsiveness to methoxamine and AVP were corroborated by experiments performed with the *in situ* perfused mesenteric vascular bed preparation.

6 These data indicate that both NO-dependent and NO-independent mechanisms are involved in the vasoconstrictor hyporesponsiveness of mesenteric arteries from portal hypertensive rats. The hyporeactivity to AVP and ET-1 is mediated by NO whereas the reduced responsiveness to adrenoceptor agonists appears to be predominantly NO-independent. AVP and ET-1, in addition, seem to inhibit the NO-independent mechanism of vascular hyporeactivity, since the hyporesponsiveness to METH was reduced in the presence of AVP or ET-1 and abolished by the combination of these peptides with L-NAME.

Keywords: Portal hypertension; mesenteric arterial bed; nitric oxide; noradrenaline; methoxamine; arginine vasopressin; endothelin-1; 5-hydroxytryptamine; indomethacin; N^G-nitro-L-arginine methyl ester (L-NAME)

Introduction

Hyperdynamic splanchnic circulation due to vasodilatation has been demonstrated to be an essential factor in maintaining elevated portal pressure in portal hypertension (Vorobioff *et al*., 1983). Several explanations for the splanchnic vasodilatation have been put forward, including elevated plasma levels of circulating vasodilators as is true for glucagon (Benoit *et al*., 1984; Rodriguez-Perez *et al*., 1993), gastrin (Lam, 1976; Lauritsen *et al*., 1976), calcitonin gene-related peptide (Bendtsen *et al*., 1991) and bile acids (Pak & Lee, 1993), and excess of locally released vasodilator factors such as nitric oxide (NO) (Vallance & Moncada, 1991; Bomzon & Blendis, 1994) or prostanoids (Bruix *et al*., 1985; Wu *et al*., 1993). Conversely, the vascular sensitivity to vasopressors is decreased as found *in vivo* both in animal models (Finberg *et al*., 1981; Kiel *et al*., 1985; Castro *et al*., 1993), cirrhotic patients (Ryan *et al*., 1993) and various *in vitro* preparations (Bomzon & Blendis, 1987; Sieber & Groszmann, 1992a, b; Castro *et al*., 1993). Although NO has been implicated in the vascular hyporeactivity/vasodilatation in portal hypertension (Vallance & Moncada, 1991; Bomzon & Blendis, 1994), some authors found that the splanchnic vasodilatation (Iwata *et al*., 1992; Pizcueta *et al*., 1992; Garcia-Pagan *et al*., 1994; Heinemann *et al*., 1996) and the *in vitro* hyporeactivity to certain vasoconstrictors (Weigert *et al*., 1994; Heinemann & Stauber, 1995) was resistant to blockade of NO formation.

In order to clarify the role of NO in the haemodynamic derangements seen in portal hypertension we studied isolated perfused mesenteric arteries and *in situ* perfused mesenteric vascular beds from portal hypertensive rats and evaluated their responsiveness to a range of vasoconstrictors in the absence and presence of a NO synthase inhibitor. Since the mechanisms leading to vasoconstriction vary with different vasoconstrictors, it is conceivable that there might also be distinct mechanisms which underlie the hyporeactivity to different vasoconstrictors in portal hypertension.

Methods

Male Sprague-Dawley rats weighing 300–330 g were used for mesenteric arterial perfusion. The average body weight of the study groups was similar.

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Surgical preparation

Portal hypertension was induced by partial portal vein ligation (PVL, Vorobioff *et al*., 1983). Briefly, animals were anaesthetized by i.p. injection of pentobarbitone sodium (40 mg kg^{-1}). The portal vein was exposed and ligated with silk (4-0) over a blunt-tipped 21-gauge needle, which created a calibrated stenosis of the portal vein. Sham-operated (Sham) animals were treated in the same manner but without ligation. Further experiments were carried out 10–12 days after surgery.

Portal venous pressure measurement

To confirm the efficacy of PVL in inducing portal hypertension, a group of PVL and Sham rats was anaesthetized with ketamine (150 mg kg^{-1}, i.m.). The ileocolic vein was cannulated and portal venous pressure was recorded with the zero level set at the mid-point of the animal. PVL rats had markedly elevated portal venous pressures as compared to Sham rats (14.5 ± 0.2 versus 9.1 ± 0.3 mmHg; $P < 0.005$, $n = 6$).

Isolated perfused mesentery

Experiments with isolated perfused mesenteric arterial beds were carried out as originally described by McGregor (1965). After intraperitoneal injection of heparin 1000 iu kg^{-1} the animals were killed by cervical dislocation. The superior mesenteric artery was cannulated through a small incision in the abdominal aorta. The mesenteric arterial bed was dissected free from the intestine, then placed on a heated pad and covered with parafilm. The pad was tilted to facilitate removal of the effluent perfusate. The preparation was perfused at 37°C with oxygenated (95% O_2, 5% CO_2) Krebs solution at a rate of 4 ml min^{-1} by a roller pump. The Krebs solution contained (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11 and EDTA calcium 0.026, at a pH of 7.4. Perfusion pressure was measured via a side arm of the arterial cannula by means of a pressure transducer. Pressor responses were calculated in mmHg as increases above baseline pressure. Zero pressure was determined at the end of each experiment by perfusing the arterial cannula after removal of the vessel preparation.

Two mesenteric arterial beds, of one PVL and one Sham rat, were tested in parallel. The preparations were allowed to equilibrate for 60 min. Pressor responses to increasing doses of noradrenaline (NA), methoxamine (METH), arginine vasopressin (AVP) and endothelin-1 (ET-1) were determined with bolus injections made close to the arterial cannula in a volume of 100 μl over a period of 20 s via a roller pump. The next dose of the vasoconstrictor under study was administered when the perfusion pressure had returned to baseline. Only one dose-response curve was recorded in each preparation. When pressor responses were determined in the presence of N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), indomethacin (5 μM), its vehicle, AVP (20 nM), ET-1 (1 nM) or 5-hydroxytryptamine (5-HT, 5 μM), these substances were infused into the perfusion system throughout the experiment by means of a syringe pump, the infusion starting 30 min before the dose-response curve to the vasoconstrictor under study was constructed.

In situ *perfused mesentery*

In an additional study the mesenteric artery was cannulated and, after ligation of the coeliac trunc and of the inferior mesenteric artery, perfused *in situ*. The chest was opened and the thoracic organs were removed. The effluent perfusate, drained via the inferior vena cava, was continuously removed from the thoracic cavity. The experimental procedure was the same as that described above, except that the intestine was left intact and the vasoconstrictors, METH and AVP, were infused continuously for 5 min at increasing concentrations with adequate washout periods in between.

Substances and statistics

All drugs were purchased from Sigma (Sigma; Vienna, Austria) except AVP (Bachem; Bubendorf, Switzerland) and ET-1 (Peptide Institute; Osaka, Japan). Stock solutions of the drugs were prepared in saline except ET-1 which was dissolved in 0.1 N acetic acid. Further dilutions were made with saline. Indomethacin was dissolved in 3.3% $NaHCO_3$ on the day of the experiment and diluted with saline.

All data are presented as mean ± s.e.mean. Statistical evaluation of the data was performed with the Mann-Whitney U test. Probability values of $P < 0.05$ were considered to be statistically significant.

Results

Isolated perfused mesentery

The values of baseline perfusion pressure, which were recorded immediately before the dose-response curves were constructed, are summarized in Table 1. The basal perfusion pressure in isolated mesenteric arteries of PVL rats was significantly lower than in vessels of Sham rats (Table 1). This difference was not abolished after preincubation with D-NAME, L-NAME (each 100 μM) or indomethacin (5 μM; Table 1).

Mesenteric arteries from PVL rats were significantly less reactive to bolus injections of METH, NA, AVP and ET-1 as compared to preparations from Sham rats. The hyporeactivity to the adrenoceptor agonists NA and METH was more pronounced than that to AVP and ET-1 (Figures 1 and 2). Preincubation of the vessels with L-NAME (100 μM) potentiated the pressor responses to all vasoconstrictors under study both in PVL and Sham preparations. In arteries from PVL rats the hyporeactivity to AVP and ET-1 (Figure 1), but not to METH and NA (Figure 2), was abolished by L-NAME. D-NAME (100 μM) had no effect as compared with the respective responses recorded in the absence of a drug ($n = 5–6$ in each group; data not shown). Indomethacin (5 μM) also failed to abolish the METH hyporeactivity of vessels from PVL rats ($n = 6$; data not shown).

Table 1 Baseline perfusion pressure in isolated mesenteric arteries of portal vein-ligated (PVL) and sham-operated rats

Preincubation	n	*PVL*	*Sham*
–	24	7.5 ± 0.1*	13.1 ± 0.2
D-NAME (100 μM)	21	8.7 ± 0.1*	13.2 ± 0.3
L-NAME (100 μM)	24	8.0 ± 0.3*	14.0 ± 0.5
Indomethacin (5 μM)	6	7.3 ± 0.5*	12.4 ± 0.6
AVP (20 nM)	6	18.7 ± 1.4*	25.8 ± 1.7
AVP (20 nM)	5	17.8 ± 1.7*	26.1 ± 1.9
+ D-NAME (100 μM)			
AVP (20 nM)	6	26.4 ± 1.8*	33.4 ± 1.8
+ L-NAME (100 μM)			
ET-1 (1 nM)	7	20.5 ± 0.9*	28.7 ± 1.9
ET-1 (1 nM)	5	21.0 ± 0.5*	29.8 ± 1.4
+ D-NAME (100 μM)			
ET-1 (1nM)	7	29.4 ± 0.7*	37.2 ± 2.2
+ L-NAME (100 μM)			
5-HT (5 μM)	6	18.2 ± 1.1*	23.2 ± 1.2
5-HT (5 μM)	6	24.0 ± 2.1*	31.3 ± 2.4
+ L-NAME (100 μM)			

Baseline perfusion pressures (mmHg) in mesenteric arteries of portal vein-ligated (PVL) and sham-operated (Sham) rats were recorded after a 60 min period of equilibration. N^G-nitro-D-arginine methyl ester (D-NAME), N^G-nitro-L-arginine methyl ester (L-NAME), indomethacin, arginine vasopressin (AVP), endothelin-1 (ET-1) and 5-hydroxytryptamine (5-HT) were administered by continuous infusion which was started 30 min before the baseline values were recorded. Data are shown as mean ± s.e.mean; *$P < 0.05$ PVL versus Sham.

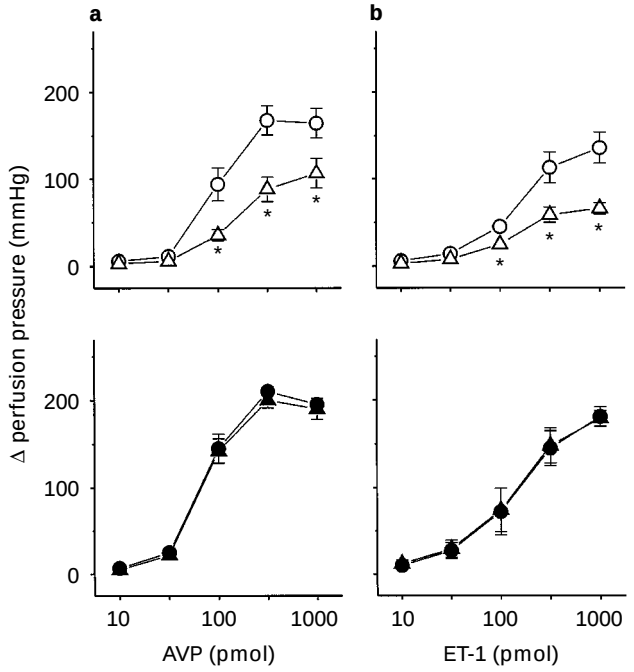

Figure 1 Pressor responses to bolus injections of arginine vasopressin (AVP; a) and endothelin-1 (ET-1; b) in isolated perfused mesenteric arteries from portal vein-ligated (PVL, △, ▲) and sham-operated (Sham, ○, ●) rats. Mesenteric vessels from PVL rats (△) were markedly hyporesponsive as compared to those from Sham rats (○). Preincubation with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) abolished the difference in responsiveness to AVP and ET-1 between the PVL (▲) and Sham group (●). Data are shown as mean and vertical lines indicate s.e.mean; $^*P<0.05$ PVL versus Sham, $n=6$.

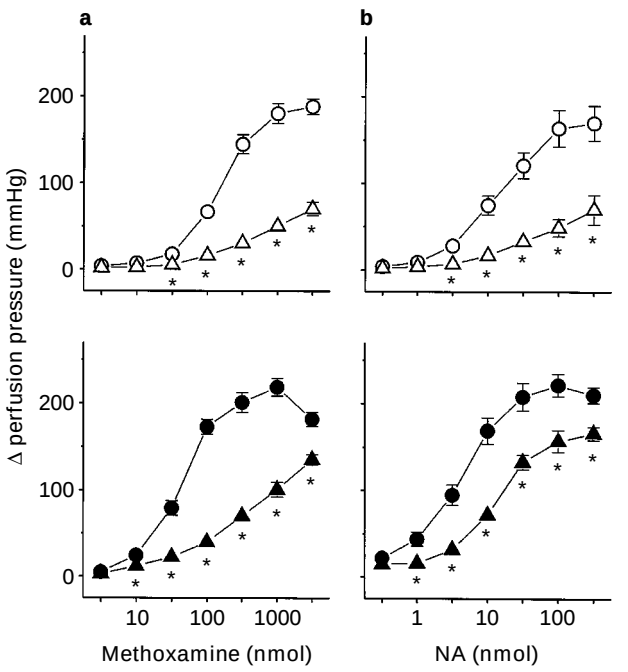

Figure 2 Pressor responses to bolus injections of methoxamine (a) and noradrenaline (NA; b) in isolated perfused mesenteric arteries from portal vein-ligated (PVL, △, ▲) and sham-operated (Sham, ○, ●) rats. Mesenteric vessels from PVL rats (△) were markedly hyporesponsive as compared to those from Sham rats (○). Preincubation with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) potentiated the responses to methoxamine and NA, but did not abolish the difference in responsiveness betwen the PVL (▲) and Sham group (●). Data are shown as mean and vertical lines indicate s.e.mean; $^*P<0.05$ PVL versus Sham, $n=6$.

These findings suggest that the vasoconstrictor hyporeactivity of mesenteric vessels of PVL rats involves two different mechanisms: one mechanism that is sensitive to inhibition of NO synthesis as observed with AVP and ET-1 (Figure 1) and another mechanism that is resistant to L-NAME as found during adrenoceptor stimulation (Figure 2). To elucidate further this apparent discrepancy, dose-response curves for METH were recorded in the presence of low concentrations of AVP, ET-1, or, for comparison, 5-HT. Preincubation of the vessels with AVP (20 nM), ET-1 (1 nM) or 5-HT (5 μM) led to a transient rise of perfusion pressure by about 60 mmHg but this effect gradually vanished so that by the time the METH dose-response curves were constructed the perfusion pressure was only slightly increased (Table 1). The pressor responses to METH were potentiated in the presence of AVP (20 nM) or ET-1 (1 nM) (compare Figure 2a with Figure 3a, b) but, since this effect was more pronounced in the PVL group, the difference between arterial preparations from PVL and Sham rats in terms of responsiveness to METH was diminished (Figure 3a, b). D-NAME (100 μM), when combined with AVP (20 nM) or ET-1 (1 nM), respectively, had no additional effect ($n=5-6$ in each group; data not shown), while the combination of L-NAME (100 μM) with AVP (20 nM) or ET-1 (1 nM), respectively, restored the reactivity to METH in the PVL group to the level seen in the Sham group (Figure 3a, b). Preconstriction of the arteries with 5-HT (5 μM) to a similar extent as achieved by AVP (20 nM) or ET-1 (1 nM) was ineffective in reducing the hyporeactivity to METH, both in the absence and presence of L-NAME (Figure 3c).

In situ *perfused mesentery*

In order to account for possible artefacts due to the isolation of the preparations, a series of experiments was performed in which the mesenteric vascular bed was left *in situ* and attached to the intestine so that the intestinal microcirculation and the mesenteric venous bed were also perfused. In addition, the vasoconstrictors METH and AVP were infused continuously at different concentrations for 5 min, instead of the 20 s exposure in the previous experiments, to check whether the vascular hyporeactivity observed in PVL rats is a function of bolus versus steady-state administration of vasoconstrictors. Baseline perfusion pressure was significantly lower in PVL rats as compared with Sham rats (21 ± 1 versus 26 ± 1 mmHg, respectively: $n=21$, $P<0.01$) and remained unchanged in the presence of L-NAME (100 μM). METH and AVP dose-dependently increased the perfusion pressure, an effect that was sustained during the 5 min periods of methoxamine infusion, whereas tachyphylaxis developed during exposure to higher concentrations of AVP (10–30 nM). The perfusion pressure readily returned to baseline after termination of the vasoconstrictor infusion.

The pressor responses to METH and AVP were blunted in PVL rats relative to Sham rats (Figure 4) although, due to a higher degree of statistical variation than that seen in the isolated arterial preparation, the AVP responses differed significantly only at the agonist concentration of 10 nM. Preincubation with L-NAME (100 μM) abolisehd the hyporeactivity to AVP but left the preparations from PVL rats hyporesponsive to METH (Figure 4). In contrast, the hyporesponsiveness to METH seen in PVL rats was corrected in the presence of AVP (20 nM; Figure 5), a treatment which *per se*, after a transient initial pressor response of 50–70 mmHg, only slightly increased the perfusion pressure by 5–15 mmHg above baseline. The question as to whether the non-significant difference between PVL and Sham rats, which remained in the presence of AVP (20 nM) (Figure 5), was NO-dependent could not be addressed, since combined preincubation with L-NAME (100 μM) and AVP (20 nM) resulted in a sustained

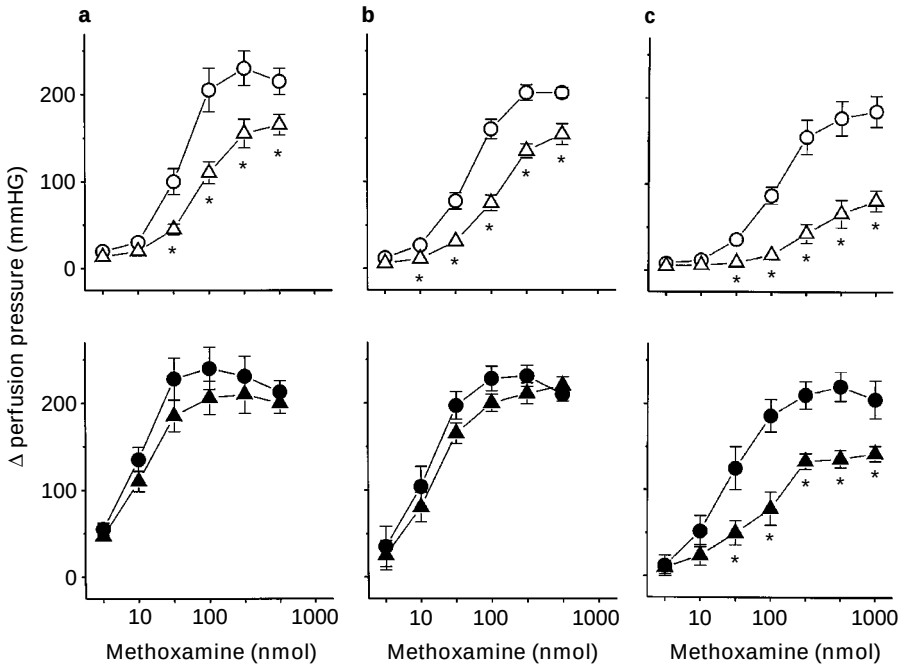

Figure 3 Pressor responses to bolus injections of methoxamine in isolated perfused mesenteric arteries from portal vein-ligated (PVL, △, ▲) and sham-operated (Sham, ○, ●) rats in the presence of arginine vasopressin (AVP, 20 nM; a), endothelin-1 (ET-1, 1 nM; b) and 5-hydroxytryptamine (5-HT, 5 μM; c). Preincubation with AVP or ET-1 alone reduced the hyporesponsiveness of mesenteric vessels from PVL (△) rats as compared to those from Sham (○) rats (a and b). The combination of AVP or ET-1, respectively, with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) restored the responses to methoxamine in the PVL group (▲) to the level observed in the Sham group (●) a and b). 5-HT lacked any effect on the hyporeactivity in the PVL group both in the absence (△, ○, c) and in the presence (▲, ●, c) of L-NAME. Data are shown as mean and vertical lines indicate s.e.mean; $^*P<0.05$ PVL versus Sham, $n=6-7$.

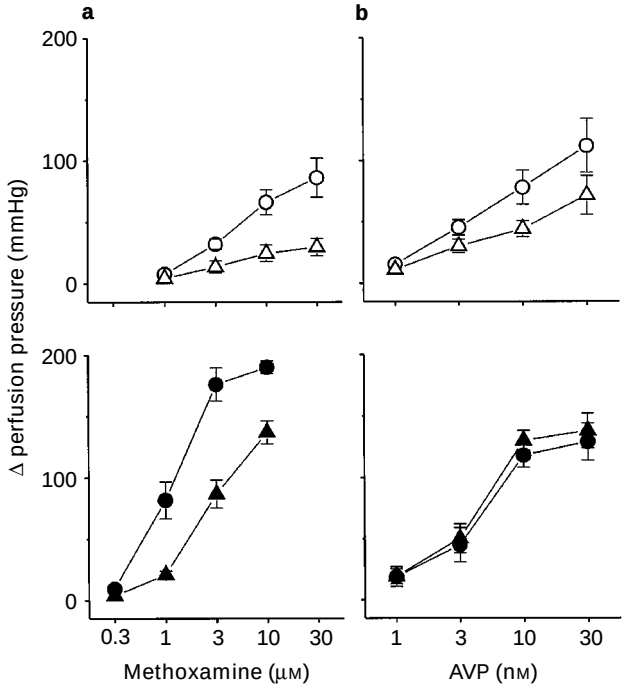

Figure 4 Pressor rsponses to 5 min infusions of methoxamine (a) and arginine vasopressin (AVP, b) in the *in situ* perfused mesenteric preparation of portal vein-ligated (PVL, △, ▲) and sham-operated (Sham, ○, ●) rats in the absence (△, ○) and presence (▲, ●) of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM). Mesenteric vascular beds in PVL rats were hyporesponsive to methoxamine and AVP as compared to those in Sham rats. Preincubation with L-NAME restored the responses to AVP in PVL rats to the level seen in Sham rats, but did not abolish the difference in responsiveness to methoxamine. Data are shown as mean and vertical lines indicate s.e.mean; $^*P<0.05$ PVL versus Sham, $n=7$.

elevation of perfusion pressure by 80–100 mmHg, causing rapid deterioration of the *in situ* preparations due to extensive oedema.

Discussion

In the present study isolated perfused mesenteric arteries from PVL rats were found to be hyporesponsive to a variety of vasoconstrictors, as has been previously shown by Sieber & Groszmann (1992a, b). This contrasts with the findings of Cawley *et al.* (1995) and Cahill *et al.* (1996) that isolated segments of the mesenteric artery from portal hypertensive rats are actually hyperresponsive to NA and ET-1, which suggests that hyporeactivity to vasoconstrictors in portal hypertension is a function of the mesenteric arterioles. This is in line with the concept that the haemodynamic resistance of vascular beds is mainly regulated by arterioles and, as a consequence, increased reactivity of larger arterial segments which are of the conduit type does not fundamentally affect the overall resistance. The hyporeactivity observed here does not seem to be a consequence of the preparation procedure which involved isolation of the mesenteric arteries from the intestinal microcirculation and the portal venous system, because it was also observed in mesenteric vascular beds that were left attached to the intestine and were perfused *in situ*.

The major conclusion to be drawn from the current data is that two different mechanisms account for the vascular hyporeactivity in portal hypertension. This hypothesis is based on the observation that in vessels from PVL rats blockade of NO synthase with L-NAME abolished the hyporesponsiveness to AVP and ET-1, but was ineffective in restoring the responsiveness to NA and METH. This finding confirms that NO contributes to the vascular hyporeactivity to AVP and ET-1 in portal hypertension (Sieber & Groszmann, 1992b; Hartleb *et al.*, 1994). The inability of L-NAME to abolish the hyporesponsiveness to the adrenoceptor agonists NA and METH in

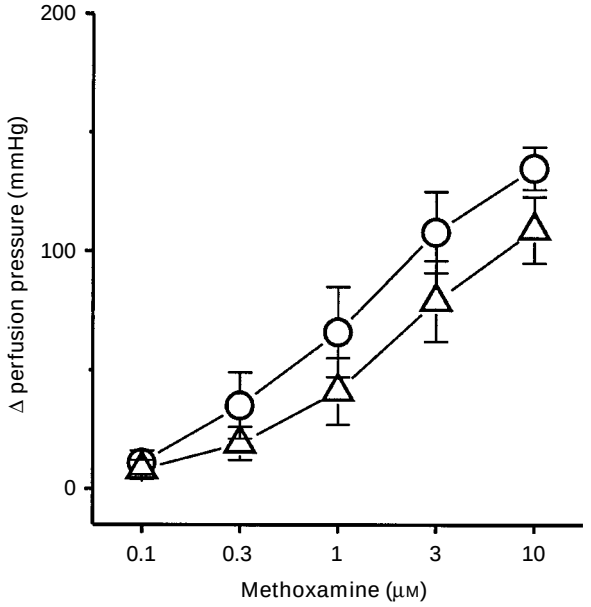

Figure 5 Pressor responses to 5 min infusions of methoxamine in the *in situ* perfused mesenteric preparation of portal vein-ligated (PVL, △) and sham-operated (Sham, ○) rats in the presence of arginine vasopressin (20 nM). The hyporesponsiveness to methoxamine of mesenteric vascular beds from PVL rats which is shown in Figure 4 was corrected by preincubation with arginine vasopressin. Data are shown as mean and vertical lines indicate s.e.mean, $n=7$.

PVL rats indicates that factors other than NO have to be held responsible for the major part of the hyporesponsiveness to adrenoceptor stimulation. This contrasts with previous studies of Sieber & Groszmann (1992a, b) who found that the hyporesponsiveness to NA and METH of isolated mesenteric arteries from PVL rats was reversed by inhibition of NO synthesis. This apparent contraindication might be due to methodological differences. Sieber & Groszmann (1992a, b) ligated the portal vein over a needle that had a larger diameter than the needle which we used. Therefore, in our model the degree of portal vein stenosis and thus of porto-systemic shunting was presumably higher (Stauber *et al*., 1991). This, in turn, may have triggered an additional NO-independent mechanism leading to vascular hyporesponsiveness resistant to blockade of NO formation. This hypothesis is supported by the fact that in the present study mesenteric arteries from PVL rats were less responsive to METH and NA than in the studies of Sieber & Groszmann (1992a, b), while the responsiveness in Sham rats was similar. The possibility that the concentration of 100 μM L-NAME, which was used in the current study, was insufficient to suppress NO formation fully in mesenteric arteries has been ruled out in previous studies (Adeagbo & Triggle, 1993; Parsons *et al*., 1994; Heinemann & Stauber, 1995; 1996). Downregulation of α-adrenoceptors as another possible cause of the reduced responsiveness to NA and METH is unlikely as the affinity and density of α-adrenoceptors have been found to be unchanged in vessels of PVL rats (Liao *et al*., 1994). Cyclo-oxygenase products also do not seem to be involved as inferred from the lack of effect of indomethacin. Furthermore, the possibility that the NO-independent hyporesponsiveness to α-adrenoceptor agonists in PVL rats reflects a peculiarity of isolated mesenteric arteries subjected to non-equilibrium conditions of receptor activation has been ruled out, because the hyporeactivity to METH, both in the absence and presence of L-NAME, was the same when the drug was administered by a 20 s bolus injection (isolated arteries) or when it was infused for 5 min to reach a steady-state concentration (*in situ* mesenteric vascular bed).

The finding that NO-independent hyporeactivity in PVL rats was seen only when vasoconstriction was induced by METH or NA, but was not seen with AVP or ET-1, raises the question as to whether the NO-independent portion of the vascular hyporeactivity is specifically related to the activation of adrenoceptors or whether it is generally present but is inhibited by AVP and ET-1. The findings in PVL rats that low concentrations of AVP and ET-1 reduced the METH hyporesponsiveness of isolated perfused mesenteric arteries and that a low concentration of AVP corrected the METH hyporesponsiveness of the *in situ* mesenteric vascular bed, indicate that AVP and ET-1 inhibit the NO-independent part of vasoconstrictor hyporesponsiveness in portal hypertension. The difference in the responsiveness to METH between PVL and Sham rats, which in the isolated perfused arteries remained in the presence of AVP or ET-1, was related to NO, since the difference was completely eliminated by a combination of L-NAME, but not D-NAME, with AVP or ET-1. The enhancement by AVP or ET-1 of the responsiveness to METH in the PVL group appeared to be independent of their small effect to increase baseline perfusion pressure, as inferred from the lack of effect of preconstricting the preparations with 5-HT. It is worth noting here that terlipressin, which is a vasopressin analogue lacking intrinsic activity on vasoconstrictor vasopressin receptors and, therefore, does not affect basal persusion pressure, is likewise able to correct the *in vitro* hyporeactivity to METH in PVL rats (Heinemann & Stauber, 1996). As can be seen in Figures 1–3, the maximal vasoconstrictor responses that can be achieved in isolated perfused mesenteric arteries are between 200 and 250 mmHg. Since both AVP and ET-1, on the one hand, and L-NAME on the other, potentiated the METH-induced vasoconstriction it may be argued that the combined effects of L-NAME and AVP or ET-1 on METH hyporeactivity might be related to a 'ceiling phenomenon'. However, this is ruled out by the observation that after these pretreatments the vasoconstrictor responses to METH were identical in PVL and Sham rats over the whole range of the dose-response curve (i.e. even with lower doses of METH which did not cause maximal vasoconstriction).

Although decreased sensitivity to vasoconstrictors is regarded as a major cause of the splanchnic vasodilation in portal hypertension (Bomzon & Blendis, 1994), it may be difficult to extrapolate the present observations to the *in vivo* situation. Nonetheless, the NO-independent hyporesponsiveness to adrenoceptor activation is probably a crucial issue in portal hypertension, since vasoconstrictor tone *in vivo* is largely maintained by endogenous catecholamines while AVP and ET-1 play a lesser role. This concept is supported by *in vivo* studies (Iwata *et al*., 1992; Pizcueta *et al*., 1992; Heinemann *et al*., 1996) which have shown that the splanchnic hyperaemia in PVL rats persists after inhibition of NO formation. On the other hand, AVP and terlipressin have recently been found to abolish the difference in mesenteric blood flow between PVL and Sham rats (Heinemann *et al*., 1996), which indicates that the beneficial effect of vasopressin on vascular responsiveness is maintained *in vivo*. The mechanism by which AVP and ET-1 abolished the NO-independent hyporesponsiveness of vessels from PVL rats remains a matter of speculation. It is a well known, although poorly understood, phenomenon that AVP and ET-1 potentiate the vasopressor effects of adrenoceptor agonists *in vitro* (Karmazyn *et al*., 1978; Henrion & Laher, 1993). As the present study showed this effect to be far more pronounced in PVL rats than in Sham rats, it would appear that AVP and ET-1 interfere with some factor that is a negative modulator of adrenoceptor-mediated vasoconstriction and is enhanced in portal hypertension. AVP and ET-1 are known to have multiple effects on intracellular signal transduction, including inactivation of ATP-sensitive potassium channels (Wakatsuki *et al*., 1992), activation of protein kinase C (Thibonnier, 1992; Murray *et al*., 1992; Henrion & Laher, 1993) and inhibition of the adenylate cyclase/protein kinase A pathway (Eikvar *et al*., 1993; Minami *et*

al., 1993; Kohan *et al*., 1993). Since some of these effector mechanisms have been shown to be involved in the vascular derangements in portal hypertension (Moreau *et al*., 1994; Wu *et al*., 1994; Huang *et al*., 1995; Trombino *et al*., 1996), it is conceivable that AVP and ET-1 exert their positive effect on vascular reactivity at the smooth muscle level.

In conclusion, our data support the concept that NO is involved in the *in vitro* hyporesponsiveness to vasoconstrictors of mesenteric arterial beds of portal hypertensive rats. However, our data also show that additional factors distinct from NO are operative in portal hypertension and are in particular responsible for the decreased responsiveness to adrenoceptor agonists. It appears that this NO-independent mechanism is inhibited by AVP and ET-1.

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