Effects of portal hypertension on responsiveness of rat mesenteric artery and aorta

Teresa Cawley, *J. Geraghty, *H. Osborne & †J.R. Docherty

Department of Physiology, Royal College of Surgeons in Ireland, Dublin 2 and *Department of Surgery, Beaumont Hospital, Dublin 9, Ireland.

1 We have examined the effects of pre-hepatic portal hypertension on the responsiveness of rat small mesenteric arteries and aorta. Rats were made portal hypertensive by creating a calibrated portal vein stenosis, or sham-operated.

2 In rat mesenteric arteries, there was no significant difference between portal hypertensive and sham-operated animals in the contractile potency of noradrenaline (NA), but the maximum contractile responses to NA, U46619 and KCl were significantly increased in vessels from portal hypertensive animals. This altered maximum contractile response was not due to alterations in smooth muscle mass.

3 In rat mesenteric arteries, there were no significant differences between portal hypertensive and sham-operated animals in endothelium-dependent relaxations to acetylcholine (ACh). The difference between portal hypertensive and sham-operated rats in the maximum response to U46619 was maintained following a combination of methylene blue (1 μ M) and N^G-monomethyl-L-arginine (100 μ M), suggesting that any differences in endothelial function do not explain differences in the response to vasoconstrictors.

4 In rat aorta, there were no significant differences between portal hypertensive and sham-operated animals in the contractile response to NA or KCl or in the endothelium-dependent relaxations to ACh.

5 In pithed rats, there was no difference between portal hypertensive and sham-operated animals in the pressor potency of NA.

6 It is concluded that portal hypertension produces an increase in the contractile response to the vasoconstrictors NA, U46619 and KCl in rat mesenteric arteries but not in the aorta. This suggests that the diminished responsiveness to vasoconstrictors reported in portal hypertensive rats *in vivo* is not due to a diminished responsiveness at the level of the vascular smooth muscle.

Keywords: Portal hypertension; noradrenaline; endothelium-dependent relaxations; mesenteric artery; acetylcholine; U46619; N^G-monomethyl-L-arginine (L-NMMA)

Introduction

The pre-hepatic portal hypertensive rat is a widely used model of human portal hypertension characterized by the development of portal-systemic shunting (Sikuler *et al.*, 1985; Geraghty *et al.*, 1989; Bosch *et al.*, 1992). It is generally accepted that shunts develop in response to the increased portal pressure, but that a hyperdynamic circulatory state follows, characterized by increased cardiac output and increased splanchnic blood flow. The increased cardiac output and splanchnic blood flow may be a result of diminished responsiveness to vasoconstrictors and/or to the increased entry of gastrointestinal vasodilator hormones into the circulation due to shunting, and vasodilator substances such as glucagon have been implicated (Benoit *et al.*, 1984).

A component of the increased splanchnic blood flow found in portal hypertension may be due to increased function of the vascular endothelium (Valance & Moncada, 1991) or decreased mesenteric vascular responsiveness to vasoconstrictors. Indeed, many authors have reported decreased vascular responsiveness to vasoconstrictors such as noradrenaline (NA) (Kiel *et al.*, 1985; Pizcueta *et al.*, 1990) in the mesenteric vascular bed of portal hypertensive rats *in situ*. Similar findings have been reported for angiotensin but not NA (Murray & Paller, 1985; Villamediana *et al.*, 1988) in the mesenteric vascular bed of cirrhotic rats *in situ*. In contrast, Joh *et al.* (1993) report a diminished vasodilator response to α -adrenoceptor antagonists in the mesenteric bed of portal hypertensive rats, suggesting diminished sympathetic drive:

diminished release of noradrenaline might be expected to cause up-regulation of receptors and increased responsiveness to exogenous noradrenaline. However, the presence of circulating vasodilators in these *in situ* studies probably influenced the actions of vasoconstrictors. Other authors have examined isolated blood vessels. Leehey (1993) found a decreased pressor sensitivity to angiotensin *in vivo*, but no change in the contractile response to angiotensin in isolated aorta from cirrhotic rats, although Castro *et al.* (1993) found decreased responsiveness to angiotensin in aorta. Rat aortic response to NA is reported to be increased or decreased by portal hypertension of 10 or 21 day duration, respectively (Bomzon & Blendis, 1987; Bomzon *et al.*, 1991). Cummings *et al.* (1986) found an increased maximum contraction to 5-hydroxytryptamine (5-HT) and vasopressin, but not to NA, in mesenteric veins from portal hypertensive rats, and no change in the responsiveness to NA in mesenteric arteries. Hence, whereas most studies of pressor responses *in vivo* report a diminished responsiveness to vasoconstrictors in portal hypertension, conflicting results have been obtained *in vitro*.

The object of the present study was to investigate whether responses to vasoconstrictors are altered by portal hypertension by examining isolated mesenteric arteries from portal hypertensive and sham-operated rats in their responsiveness to NA, the thromboxane-mimetic, U46619 and KCl in the absence of the influence of vasodilators. Responses to vasoconstrictors in mesenteric arteries might be expected to be reduced or even increased depending on whether these alterations were responsible for, or in response to, the hyper-

[†] Author for correspondence.

dynamic circulation. Responses in mesenteric arteries were compared with responses obtained in the aorta, and in the pithed rat preparation. Secondly, endothelium-dependent responses were investigated to assess whether altered endothelial function occurred. Some of these results have been published in abstract form (Cawley *et al.*, 1994).

Methods

Male Wistar rats (200–400 g) were obtained from Trinity College, Dublin, and tissues were obtained from portal hypertensive or sham-operated animals.

Surgical preparation

Animals were anaesthetized with ether, a midline incision was made in the abdomen, and the portal vein exposed. A 21 gauge needle was placed alongside the portal vein and a suture was tied around both; the needle was then removed resulting in a calibrated stenosis. The abdomen was then closed and animals were examined 14 days after surgery. In sham-operated animals, the portal vein was exposed but no suture was placed around it.

Recording of pressures

Animals were anaesthetized with ether, and maintained with pentobarbitone (Nembutal, 10% solution) injected into the jugular vein following cannulation. The ileocaecal vein and carotid artery were cannulated and connected to blood pressure transducers for pressure measurements.

Radioactive microspheres

Animals were anaesthetized with ether and the ileocaecal vein was cannulated. Radioactive microspheres (^{57}Co , 15 μm diameter, approximately 100,000) were suspended in 10% dextran with 0.01% of the surfactant Tween 80 to prevent clumping, and injected in a volume of 1 ml kg^{-1} . After 10 min, animals were killed and the lung, liver and kidney removed, chopped up and counted in a gamma scintillation counter. The degree of shunting was assessed from the equation

$$\% \text{ shunting} = \text{lung c.p.m.} \times 100 / (\text{lung c.p.m.} + \text{liver c.p.m.})$$

Kidney radioactivity was sampled to assess leaching of microspheres, but radioactivity in kidney samples was not significantly different from background so that no significant leaching occurred.

Pithed rat preparation

Animals were anaesthetized with ether and a tracheal cannula was placed. The rats were then pithed and respired artificially with 100% O_2 (Gillespie *et al.*, 1970; Docherty, 1988). The carotid artery was cannulated for the recording of blood pressure. Following a 30 min equilibration period, a dose-response curve was carried out to NA (10 ng kg^{-1} –100 $\mu\text{g kg}^{-1}$), injected into the jugular vein in 0.5 log unit increments at intervals of 3–5 min.

Aorta

Following stunning and exsanguination, aortic rings of 3–5 mm in length were attached to myograph transducers under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119, NaHCO_3 25, D-glucose 11.1, KCl 4.7, CaCl_2 2.5, KH_2PO_4 1.2, MgSO_4 1.0 and EDTA 0.03. Additionally, cocaine (3 μM), propranolol (3 μM) and indomethacin (10 μM) were present. Tissues were contracted with KCl (40 mM), exposed to ACh (10 μM) to test for endothelium-dependent relaxations (EDR), and washed. Bathing fluid was then changed

every 15 min for the next hour. Tissues were then contracted with noradrenaline (NA) (1 μM), with KCl (40 mM), and when contraction had reached a maximum, ACh (or an equivalent volume of vehicle) was administered cumulatively in 1 log unit increments beginning with 10 nM. After the final concentration of ACh, sodium nitroprusside (SNP) (100 μM) was administered. In some experiments, cumulative concentration-response curves were carried out to NA in 0.5 log unit increments, beginning with 1 nM.

Mesenteric arteries

Following stunning and exsanguination, the intestines and mesentery were removed and placed in Krebs-Henseleit solution of the same composition as above. By use of a dissecting microscope, a segment of rat small mesenteric artery, approximately 1.5 mm in length, corresponding to a first or second order branch of the superior mesenteric artery, was carefully dissected free from its vein. The artery was mounted in a small vessel myograph with 40 μm tungsten wires (Mulvany & Halpern, 1977). Data were recorded on a dual channel electronic display recorder (Dual Myograph MK-4) and pen recorder. Vessels were allowed to equilibrate at 37°C and gassed with 5% CO_2 in O_2 . The vessel was set to a tension generated at 0.9 times the diameter of the vessel at 100 mmHg transmural pressure (Mulvany & Warshaw, 1977). Experiments were carried out exactly as described for rat aorta, except that vessels were contracted with a higher concentration of NA (10 μM). The maximum response to the thromboxane-mimetic, U46619, was also obtained, the highest concentration employed was 20 μM , due to cost of this agent, although this concentration gave no further increase in contractile response over that obtained to 1–10 μM . Responses to U46619 were also obtained following inhibition of nitric oxide synthase and guanylate cyclase with a combination of L-NMMA (100 μM) and methylene blue (1 μM) for 1 h. Administration of L-NMMA sometimes produced a contraction, but this appeared to be due to the presence of small amounts of the previously administered vasoconstrictor after washout. Tension returned to baseline before the second administration of U46619 (see also Rees *et al.*, 1990).

Microscopy

Wall thickness of mesenteric arteries was measured by light microscopy and a micrometer eyepiece with the vessel diameter at 0.9 times the diameter at 100 mmHg transmural pressure, and was the average of up to 10 determinations for each vessel.

Drugs

The following were used: acetylcholine chloride (Sigma, Poole, U.K.); cocaine hydrochloride (Sigma); indomethacin (Sigma); methylene blue (Aldrich); L-NMMA (N^G -monomethyl-L-arginine acetate: Sigma); U46619 (9,11-dideoxy-11 α , 9 α -epoxy-methanoprostaglandin $\text{F}_{2\alpha\alpha}$: Sigma); noradrenaline bitartrate (Sigma); propranolol hydrochloride (Sigma); sodium nitroprusside (Sigma). Drugs were dissolved in distilled water, except for indomethacin (100% ethanol).

Statistics

Values are mean $\pm$ s.e.mean. Agonist potency was expressed as a $-\log\text{EC}_{50}$. Differences between groups were compared by Student's *t* test for unpaired data.

Results

Radioactive microspheres

Effectiveness of surgery was assessed by measuring the degree of portal-systemic shunting using the radioactive microsphere

technique. Degree of shunting was $59.6 \pm 7.2\%$ (range 10–98%) ($n = 5$) and $0.08 \pm 0.05\%$ (range 0.02–0.24%) ($n = 5$) in 14 day portal hypertensive and 14 day sham rats, respectively (Student's *t* test: $P < 0.05$).

Portal and systemic arterial pressures

Systolic blood pressure was 117.6 ± 3.8 mmHg ($n = 9$), and 130.8 ± 8.0 mmHg ($n = 6$), and diastolic blood pressure was 80.5 ± 3.0 mmHg and 93.7 ± 6.0 mmHg, in anaesthetized portal hypertensive and sham-operated animals, respectively (no significant differences). Portal pressure was 9.5 ± 0.7 mmHg ($n = 5$) in sham-operated animals (14 days), 25.3 ± 3.4 mmHg ($n = 5$) after acute ligation ($P < 0.01$) and 14.2 ± 0.6 mmHg ($n = 4$) in 14 day portal hypertensive animals ($P < 0.01$ from sham-operated).

Pithed rat preparation

In pithed rats, resting diastolic blood pressure was 41.8 ± 4.0 mmHg ($n = 4$) and 40.0 ± 3.4 mmHg ($n = 4$) in portal hypertensive and sham-operated animals, respectively (no significant difference). NA (10 ng kg^{-1} – $100 \mu\text{g kg}^{-1}$) produced dose-dependent increases in diastolic blood pressure, but there were no significant differences between portal hypertensive and sham-operated animals in the pressor response (Figure 1).

Aorta

In rat aortic rings, NA produced concentration-dependent contractions with $-\log EC_{50}$ values of 7.61 ± 0.18 and 7.70 ± 0.16 in tissues from portal hypertensive and sham-operated animals, respectively (no significant difference). There were no significant differences between tissues from portal hypertensive and sham-operated animals in the maximum contraction to NA, nor in the contraction to KCl (40 mM) (Table 1).

In tissues pre-contracted with KCl (40 mM), ACh produced relaxations with $-\log EC_{50}$ values of 6.36 ± 0.15 ($n = 6$) and 6.18 ± 0.23 ($n = 6$), and in tissues pre-contracted with NA ($1 \mu\text{M}$) ACh produced relaxations with $-\log EC_{50}$ values of 6.79 ± 0.18 ($n = 4$) and 6.68 ± 0.17 ($n = 4$), in tissues from portal hypertensive and sham-operated animals, respectively (no significant differences). There were no significant differences between tissues from portal hypertensive and sham-operated animals in the maximum relaxation to ACh, when NA or KCl was used as the contractile agent (Table 2).

Mesenteric arteries

Potency of NA was not significantly altered by portal hypertension (NA $-\log EC_{50}$; portal hypertensive: 6.14 ± 0.07 , $n = 11$; sham: 6.0 ± 0.10 , $n = 9$), but the maximum contraction to NA was significantly greater in mesenteric arteries from portal hypertensive rats than in vessels from sham-operated rats (see Table 1 and Figure 2). Likewise, the contraction to KCl (40 mM) was significantly greater in mesenteric arteries from portal hypertensive rats than in vessels from sham-operated rats (Table 1 and Figure 3). The maximum contraction to U46619 was also significantly greater in arteries from portal hypertensive rats and this difference was maintained following blockade of the actions of endogenous NO with methylene blue ($1 \mu\text{M}$) and L-NMMA ($100 \mu\text{M}$) (Table 1 and Figure 3).

In tissues pre-contracted with KCl (40 mM), ACh produced relaxations with $-\log EC_{50}$ values of 6.77 ± 0.20 ($n = 10$) and 6.86 ± 0.17 ($n = 7$), and in tissues pre-contracted with NA ($1 \mu\text{M}$) ACh produced relaxations with $-\log EC_{50}$ values of 6.92 ± 0.12 ($n = 10$) and 7.15 ± 0.11 ($n = 7$), in tissues from portal hypertensive and sham-operated animals, respectively (no significant differences). There were no significant differ-

Figure 1 Dose-response curves for the increase in diastolic pressure produced by noradrenaline in pithed sham-operated (○) and portal hypertensive rats (●). Values are the mean $\pm$ s.e.mean of 4 experiments.

Figure 2 Concentration-response curves to noradrenaline in mesenteric arteries from sham-operated (○) and portal hypertensive rats (●). Values are the mean $\pm$ s.e.mean of 9–11 experiments. The maximum contraction to noradrenaline was significantly greater in portal hypertensive animals with no significant change in potency.

Table 1 Maximum contractile response to noradrenaline (NA) and U46619 and contractile response to KCl (40 mM) in rat aorta and mesenteric artery from portal hypertensive and sham-operated animals

	Portal hypertensive	Sham-operated
Aorta		
NA	1.35 ± 0.21 g ($n = 6$)	1.17 ± 0.22 g ($n = 6$)
KCl	0.79 ± 0.29 g ($n = 4$)	0.63 ± 0.28 g ($n = 4$)
Mesenteric artery		
NA	1.36 ± 0.12 g** ($n = 11$)	0.82 ± 0.07 g ($n = 9$)
KCl	0.58 ± 0.07 g* ($n = 11$)	0.40 ± 0.02 g ($n = 13$)
U46619	0.92 ± 0.12 g* ($n = 7$)	0.57 ± 0.03 g ($n = 6$)
U46619 (following methylene blue and L-NMMA)	1.26 ± 0.16 g** ($n = 6$)	0.72 ± 0.08 g ($n = 6$)

Responses to U46619 were also obtained following exposure to methylene blue ($1 \mu\text{M}$) and L-NMMA ($100 \mu\text{M}$). Values are mean $\pm$ s.e.mean, with *n* the number of experiments. Asterisks denote responses in tissues from portal hypertensive animals significantly different from responses in tissues from sham-operated animals (Student's *t* test. * $P < 0.05$; ** $P < 0.01$).

Figure 3 Maximum responses to noradrenaline (NA), KCl (40 mM) and U46619 in mesenteric arteries from sham-operated (open columns) or portal hypertensive (shaded columns) animals. Responses to U46619 in tissues exposed to methylene blue (MB) (1 μ M) and N^G-monomethyl-L-arginine (L-NMMA, 100 μ M) are also shown. Values are mean with s.e. of mean from at least 6 experiments. Asterisks denote significant difference between responses obtained in tissues from portal hypertensive and sham-operated animals (Student's *t* test: **P* < 0.05; ***P* < 0.01).

ences between tissues from portal hypertensive and sham-operated animals in the maximum relaxation to ACh, when NA or KCl was used as the contractile agent (Table 2).

Maximum relaxation to sodium nitroprusside (SNP) (100 μ M) in KCl (40 mM)-contracted tissues was similar in tissues from portal hypertensive ($91.0 \pm 4.3\%$, *n* = 4) and sham-operated animals ($78.0 \pm 5.0\%$, *n* = 4), respectively (no significant difference).

Microscopy

In mesenteric arteries set to a diameter 0.9 times the diameter of the vessel at 100 mmHg transmural pressure, wall thickness was $31.7 \pm 1.5 \mu$ m (*n* = 5) and $30.5 \pm 1.0 \mu$ m (*n* = 6) with lumen diameters of $312 \pm 9.7 \mu$ m and $298 \pm 18.5 \mu$ m in vessels from portal hypertensive and sham-operated animals, respectively (no significant differences). Similarly, wall to lumen ratios did not differ significantly between groups.

Discussion

The major findings from this study are that the maximum contraction to the vasoconstrictors NA, U46619 and KCl is increased in mesenteric arteries but not in aorta from portal hypertensive rats, with no change in endothelium-dependent relaxations. These and other findings will be discussed.

Firstly, it is necessary to consider the effectiveness of portal vein ligation in producing portal hypertension in this study. Portal pressure was significantly increased 14 days after portal vein ligation, as compared with 14 days after sham-operation, and acute ligation more than doubled portal pressure in sham-operated animals. Portal-systemic shunting was effectively nil in sham-operated animals but approximately 60% (range 10–98%) in portal vein-ligated animals, which compares with approximately 60% (range 0–85%) shunting in a previous study using the same methodology (Geraghty *et al.*, 1989). Other authors have injected microspheres into the spleen, rather than into the ileocaecal vein, and reported 95% shunting (range 89–99%) in portal vein ligated animals (Chojkier & Groszmann, 1981). However, this difference may not reflect differing degrees of portal hypertension, but rather

Table 2 Maximum relaxation to acetylcholine (ACh) in rat aorta and mesenteric artery from portal hypertensive and sham-operated animals, contracted with noradrenaline (NA) (aorta: 1 μ M; mesenteric artery: 10 μ M) or KCl (40 mM)

	Portal hypertensive	Sham-operated
<i>Aorta</i>		
NA	$51.5 \pm 12.3\%$ (<i>n</i> = 4)	$50.8 \pm 7.3\%$ (<i>n</i> = 5)
KCl	$42.8 \pm 12.3\%$ (<i>n</i> = 4)	$44.2 \pm 12.6\%$ (<i>n</i> = 4)
<i>Mesenteric artery</i>		
NA	$58.6 \pm 7.5\%$ (<i>n</i> = 10)	$49.9 \pm 5.2\%$ (<i>n</i> = 7)
KCl	$50.7 \pm 6.9\%$ (<i>n</i> = 10)	$44.4 \pm 5.7\%$ (<i>n</i> = 7)

Values are mean $\pm$ s.e. mean, with *n* the number of experiments. There were no significant differences.

an anatomical difference, since we have found similar high degrees of shunting when microspheres were injected into the spleen in our studies (authors, unpublished observations). Overall, we concluded that our portal hypertensive animals had the expected degree of portal systemic shunting and were a useful model of human portal hypertension.

In our studies, we found an increased maximum contraction to NA, U46619 and KCl in mesenteric arteries, but no change in maximum contraction to NA and KCl in aorta, from portal hypertensive animals. An increased maximum response to NA in mesenteric arteries could be due to an increase in number of α_1 -adrenoceptors or a post-receptor change in responsiveness. However, an increase in number of receptors might be expected to increase the potency of NA, rather than the maximum response, in a tissue with spare receptors. Furthermore, the contraction to KCl (40 mM) and U46619 was also significantly increased in mesenteric arteries from portal hypertensive animals, and since KCl acts to produce membrane depolarization rather than at the receptor level, it is more likely that the change in contractile responsiveness occurs beyond the receptor level. Increased contractile response could be due to alterations in second messenger systems, or to alterations in smooth muscle mass. It has been previously reported that an increased maximum contraction to NA and angiotensin II in mesenteric veins from portal hypertensive rabbits was due to an increase in media thickness, and thus to an increase in smooth muscle (Jensen *et al.*, 1987), presumably as an adaptive change to increased pressure. Similar hypertrophy of the portal vein has been reported in portal hypertensive rats, with concomitant increased contraction to KCl (Chao *et al.*, 1992). In the present study, no alteration in wall thickness was found, suggesting that the changes in contractile responsiveness seen in this study are not due to changes in smooth muscle mass.

Mesenteric vessels were compared at the same level of transmural pressure (see Mulvany & Halpern, 1977), although it might be argued that mesenteric vessels from sham-operated animals may have experienced a lower mean perfusion pressure *in vivo* than those from the portal hypertensive animals due to decreased mesenteric vascular resistance in the latter group (see Pizcueta *et al.*, 1992). However, we have previously found that there is no significant difference between contractile responses to NA in mesenteric arteries from normal rats when the resting tension is changed from 90% of the diameter at 100 mmHg to 90% of the diameter at 67 mmHg (Breslin, 1993), so that this is probably not a major problem.

The increased contractile responsiveness of the isolated mesenteric artery found in this study contrasts with reports of decreased responsiveness of the *in situ* mesenteric vascular bed of pre-hepatic portal hypertensive rats of cirrhotic rats to

vasoconstrictors such as NA (Kiel *et al.*, 1985; Pizcueta *et al.*, 1990; Villamediana *et al.*, 1988), endothelin-1 (Yu *et al.*, 1992) and angiotensin (Villamediana *et al.*, 1989). Pressor responses to angiotensin also decreased in bile duct-ligated dogs (Finberg *et al.*, 1981). The most likely reason for this apparent discrepancy is that endogenous vasodilators produced in the mesenteric bed enter the systemic circulation through shunt vessels, resulting in increased circulating levels of these vasodilators in portal hypertension (Benoit *et al.*, 1984). These vasodilators act as physiological antagonists of the mesenteric vasoconstrictor response *in vivo*, and may cause an adaptive change to occur in the responsiveness of the mesenteric artery to vasoconstrictors. Such a physiological antagonism by vasodilators would be absent *in vitro*, but the altered contractile responsiveness would be revealed. However, in the present study, responses to the vasoconstrictors NA, U46619 and KCl were increased in mesenteric artery but not aorta, although both tissues were presumably exposed to vasodilators *in vivo*. This difference may be due to differing susceptibilities to the actions of vasodilators such as glucagon. In other studies of portal hypertension, the contractile response to NA was reported to be increased in the aorta and decreased in the portal vein of portal hypertensive rats (Bomzon *et al.*, 1991), unchanged in femoral artery (Villamediana *et al.*, 1988) from cirrhotic rats, but increased in mesenteric veins from portal vein ligated rabbits (Jensen *et al.*, 1987). Other studies of isolated blood vessels report no change in the contractile response to angiotensin II in aorta (Leehey, 1993) or femoral artery (Villamediana *et al.*, 1989) from cirrhotic rats, and an increased contraction to 5-HT in mesenteric veins from portal hypertensive rats (Cummings *et al.*, 1986).

In the present study, we also examined pressor responses to NA in the pithed rat, a preparation in which vasoconstrictor responses can be examined in the absence of cardiovascular reflexes (Gillespie *et al.*, 1970), and found that these responses were unaffected by portal hypertension. This contrasts with a decreased pressor responsiveness to the α -adrenoceptor agonist, phenylephrine, in conscious portal vein

ligated rats, a difference in responsiveness that was abolished by ganglion block. This effect of ganglion blockade suggests that differences in sympathetic drive and baroreflex function contribute to altered pressor responsiveness to NA (Battarbee *et al.*, 1989) and angiotensin II (Villamediana *et al.*, 1989) in conscious or anaesthetized portal vein-ligated and cirrhotic rats, and this complication is avoided by examining the pithed rat preparation. There is evidence for diminished sympathetic tone to mesenteric vessels from portal hypertensive rats, as demonstrated by decreased vasodilatation to α -adrenoceptor antagonists (Joh *et al.*, 1993).

In rat mesenteric arteries, there was no evidence for altered endothelium-dependent relaxation to ACh in tissues from portal hypertensive animals. Furthermore, the increased maximum contraction to U46619 in mesenteric arteries from portal hypertensive animals was not due to altered endothelial function since the difference in maximum response was maintained when actions of NO were blocked by a combination of methylene blue (inhibition of guanylate cyclase, and additional actions: Mayer *et al.*, 1993) and L-NMMA (inhibition of nitric oxide synthase; Martin *et al.*, 1992). Hence, although the vascular endothelium may contribute to the hyperdynamic circulation in portal hypertension and cirrhosis (Valance & Moncada, 1991; Pizcueta *et al.*, 1992; Sieber & Groszmann, 1992; Castro *et al.*, 1993), there is no evidence for increased function *in vitro*, at least in terms of relaxations to ACh.

In conclusion, portal hypertension produces an increase in the contractile response to the vasoconstrictors NA, U46619 and KCl in rat mesenteric arteries but not in the aorta or in the pithed rat preparation. No alterations in endothelium-dependent responses to ACh were found. The diminished responsiveness to vasoconstrictors reported in portal hypertensive rats *in vivo* is not due to a diminished responsiveness at the level of the vascular smooth muscle.

This work was supported by the Charitable Infirmary Trust and the Health Research Board (Ireland).

References

- BATTARBEE, H.D., FARRAR, G.E. & SPEARS, R.P. (1989). Pressor responses in conscious rats with chronic portal venous hypertension. *Am. J. Physiol.*, **257**, G773–G781.
- BENOIT, J.N., BARROWMAN, J.A., HARPER, S.L., KVIETYS, P.R. & GRANGER, D.N. (1984). Role of humoral factors in the intestinal hyperemia associated with chronic portal hypertension. *Am. J. Physiol.*, **247**, G486–G493.
- BOMZON, A. & BLENDIS, L.M. (1987). Vascular reactivity in experimental portal hypertension. *Am. J. Physiol.*, **252**, G158–G162.
- BOMZON, A., JACOB, G., LEE, S.S. & MEDDINGS, J. (1991). *In vitro* vascular responsiveness to norepinephrine in experimental portal hypertension. *Clin. Invest. Med.*, **14**, 63–71.
- BOSCH, J., PIZCUETA, P., FEU, F., FERNANDEZ, M. & GARCIA-PAGAN, J.C. (1992). Pathophysiology of portal hypertension. *Gastroenterol. Clin. N. Am.*, **21**, 1–14.
- BRESLIN, E. (1993). Factors affecting vascular function in human and animal tissues. *Ph.D. Thesis, National University of Ireland*.
- CASTRO, A., JIMENEZ, W., CLARIA, J., ROS, J., MARTINEZ, J.M., BOSCH, M., ARROYO, V., PIULATS, J., RIVERA, F. & RHODES, J. (1993). Impaired vascular responsiveness to angiotensin II in experimental cirrhosis; role of nitric oxide. *Hepatology*, **18**, 367–372.
- CAWLEY, T., GERAGHTY, J., OSBORNE, H. & DOCHERTY, J.R. (1994). Effects of portal hypertension on responsiveness of rat mesenteric arteries and aorta. *J. Physiol.*, **475**, 45P.
- CHAO, T.-W., YU, P.-C., KUO, J.-S., PANG, P.K.T. & YANG, M.C.M. (1992). Responsiveness to synthetic parathyroid hormone in the portal vein of hypertensive rats. *J. Hepatol.*, **16**, 326–331.
- CHOJKIER, M. & GROSZMANN, R.J. (1981). Measurement of portal-systemic shunting in the rat by using g-labeled microspheres. *Am. J. Physiol.*, **240**, G371–G375.
- CUMMINGS, S.A., GROSZMANN, R.J. & KAUMANN, A.J. (1986). Hypersensitivity of mesenteric veins to 5-hydroxytryptamine- and ketanserin- induced reduction of portal pressure in portal hypertensive rats. *Br. J. Pharmacol.*, **89**, 501–513.
- DOCHERTY, J.R. (1988). The effects of ageing on vascular α -adrenoceptors in pithed rat and rat aorta. *Eur. J. Pharmacol.*, **146**, 1–5.
- FINBERG, J.P.M., SYROP, H.A. & BETTER, O.S. (1981). Blunted pressor response to angiotensin and sympathomimetic amines in the bile duct ligated dog. *Clin. Sci.*, **61**, 535–539.
- GERAGHTY, J.G., ANGERSON, W.J. & CARTER, D.C. (1989). Portal vein pressure and portasystemic shunting in experimental portal hypertension. *Am. J. Physiol.*, **257**, G52–G57.
- GILLESPIE, J.S., MACLAREN, A. & POLLOCK, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmacol.*, **40**, 257–267.
- JENSEN, L.S., JUHL, C.O. & MULVANY, M.J. (1987). Mechanical, morphological and pharmacological properties of oesophageal varices and small mesenteric veins in portal hypertensive rabbits. *Acta Physiol. Scand.*, **130**, 649–656.
- JOH, T., GRANGER, D.N. & BENOIT, J.N. (1993). Endogenous vasoconstrictor tone in intestine of normal and portal hypertensive rats. *Am. J. Physiol.*, **264**, H171–H177.
- KIEL, J.W., PITTS, V., BENOIT, J.N., GRANGER, D.N. & SHEPHERD, A.P. (1985). Reduced vascular sensitivity to norepinephrine in portal-hypertensive rats. *Am. J. Physiol.*, **248**, G192–G195.
- LEEHEY, D.J. (1993). Dissociation between pressor sensitivity *in vivo* and contractile reactivity *in vitro* to angiotensin II in rats with experimental cirrhosis. *Clin. Sci.*, **84**, 525–529.

- MARTIN, G.R., BOLOFO, M.L. & GILES, H. (1992). Inhibition of endothelium-dependent vasorelaxation by arginine analogues, a pharmacological analysis of agonist and tissue dependence. *Br. J. Pharmacol.*, **105**, 643–652.
- MAYER, B., BRUNNER, F. & SCHMIDT, K. (1993). Inhibition of nitric oxide synthesis by methylene blue. *Biochem. Pharmacol.*, **45**, 367–374.
- MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41**, 19–25.
- MULVANY, M.J. & WARSGAW, D.M. (1977). The active tension-length of vascular smooth muscle related to its cellular components. *J. Gen. Physiol.*, **74**, 85–104.
- MURRAY, B.M. & PALLER, M.S. (1985). Decreased pressor reactivity to angiotensin II in cirrhotic rats. Evidence for a post-receptor defect in angiotensin action. *Circ. Res.*, **57**, 424–431.
- PIZCUETA, M.P., CASAMITJANA, R., BOSCH, J. & RODES, J. (1980). Decreased systemic vascular sensitivity to norepinephrine in portal hypertensive rats: role of hyperglucagonism. *Am. J. Physiol.*, **258**, G191–G195.
- PIZCUETA, M.P., PIQUE, J.M., BOSCH, J., WHITTLE, B.R.J. & MONCADA, S. (1992). Effects of inhibiting nitric oxide biosynthesis on the systemic splanchnic circulation of rats with portal hypertension. *Br. J. Pharmacol.*, **105**, 184–190.
- REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.
- SIEBER, C.C. & GROSZMANN, R.J. (1992). Nitric oxide mediates hyporeactivity to vasopressors in mesenteric vessels of portal hypertensive rats. *Gastroenterology*, **103**, 235–239.
- SIKULER, E., KRAVETZ, D. & GROSZMANN, R.J. (1985). Evolution of portal hypertension and mechanisms involved in its maintenance in a rat model. *Am. J. Physiol.*, **248**, G618–G625.
- VALANCE, P. & MONCADA, S. (1991). Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet*, **337**, 776–778.
- VILLAMEDIANA, L.M., DIEGUEZ, G., SANTOS, J.C., GARCIA-VILLALON, A.L., CAMELO, C. & LOPEZ-NOVOA, J.M. (1988). Vascular reactivity to norepinephrine in rats with cirrhosis of the liver. *Can. J. Physiol. Pharmacol.*, **66**, 567–572.
- VILLAMEDIANA, L.M., GARCIA-VILLALON, A.L., CAMELO, C. & LOPEZ-NOVOA, J.M. (1989). Effect of dietary sodium intake on the pressor reactivity to angiotensin II in rats with experimental cirrhosis of the liver. *Can. J. Physiol. Pharmacol.*, **67**, 1506–1511.
- YU, P.-C., KUO, J.-S., LIN, H.-C. & YANG, M.C.M. (1992). Effects of endothelin in portal hypertensive rats. *Clin. Sci.*, **83**, 165–170.

(Received September 5, 1994

Revised October 13, 1994

Accepted October 27, 1994)