Portal hypertension increases vasoconstrictor responsiveness of rat aorta

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ABSTRACT

We have examined the effects of pre-hepatic portal hypertension on the responsiveness of aorta from Wistar and Sprague–Dawley rats. Rats were made portal hypertensive by creating a calibrated portal vein stenosis, or sham operated. In rat aorta, there was no significant difference between portal hypertensive and sham-operated animals in the contractile potency of KCl, noradrenaline or phenylephrine. In aortas from Wistar rats, the maximum response to KCl ($0.71\pm0.12$ g) and noradrenaline ($1.00\pm0.17$ g) but not phenylephrine ($0.86\pm0.10$ g) in portal hypertensive animals was significantly increased compared with that in sham-operated animals ($0.45\pm0.04$ g, $0.57\pm0.07$ g, $0.71\pm0.05$ g respectively). In aortas from Sprague–Dawley rats, the maximum response to KCl ($1.21\pm0.21$ g) and phenylephrine ($1.54\pm0.30$ g) but not noradrenaline ($0.93\pm0.09$ g) in portal hypertensive animals was significantly increased compared with that in sham-operated animals ($0.59\pm0.09$ g, $0.76\pm0.11$ g, $1.04\pm0.10$ g respectively). There was no difference between portal hypertensive and sham-operated Wistar rats in the affinity or maximum number of binding sites for $[^3H]$prazosin to $\alpha_1$-adrenoceptors in cardiac ventricular membranes. It is concluded that portal hypertension tends to produce an increase rather than a decrease in the contractile response to vasoconstrictors in aorta from both Wistar and Sprague–Dawley rats. 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.

INTRODUCTION

The pre-hepatic portal hypertensive rat is a widely used model of human portal hypertension characterized by the development of portal-systemic shunting [1–3]. These shunts develop in response to the increased portal pressure, but 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 due to the increased entry of gastrointestinal vasodilator hormones into the systemic circulation or to diminished responsiveness of the systemic vasculature to endogenous vasoconstrictors. A number of vasodilator substances including glucagon and vascular endothelium-derived substances have been implicated [4,5].

It is generally assumed that responsiveness to vasoconstrictors is diminished in portal hypertension [3,6]. Many authors have reported decreased vascular responsiveness to vasoconstrictors such as noradrenaline or angiotensin II in the mesenteric vascular beds of portal vein-ligated or cirrhotic rats in situ [7–10]. However, the presence of circulating vasodilators in these in situ studies may have influenced the actions of vasoconstrictors.

Key words: acetylcholine, aorta, noradrenaline, phenylephrine, portal hypertension, Sprague–Dawley rat, Wistar rat.
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Other authors have examined isolated blood vessels (especially rat aorta), in which the true vascular responsiveness to vasoconstrictors can be assessed in the absence of circulating vasodilators. Rat aortic responsiveness to vasoconstrictors is generally reported to be decreased by portal hypertension (see Discussion), although there is one report of increased responsiveness [11]. Hence, most studies of pressor responses in vivo report a diminished responsiveness to vasoconstrictors in portal hypertension, and in general similar results have been obtained in vitro.

We have previously reported that the maximum contractile responses to noradrenaline, KCl and the thromboxane mimetic U46199 are significantly increased in mesenteric arteries from portal hypertensive Wistar rats compared with sham-operated animals [12,13]. In comparison, many of the above mentioned studies of portal hypertension employed the Sprague–Dawley rat and examined aortic rings. The main purpose of the present study was to determine whether strain differences might explain these discrepant results. We therefore compared the responsiveness of aortic tissue from Wistar and Sprague–Dawley rats to the vasoconstrictors noradrenaline, phenylephrine and KCl, and examined the changes after partial portal vein ligation. A secondary aim of the study was to determine whether changes in responsiveness could be due to changes in the number of \(\alpha_1\)-adrenoceptors.

**METHODS**

Male Wistar and Sprague–Dawley rats (225–300 g) were obtained from Trinity College Dublin and University College Dublin respectively, and tissues were obtained from portal hypertensive or sham-operated animals. Animals were anaesthetized with ether, a midline incision was made in the abdomen, and the portal vein exposed. The bile duct and hepatic artery were separated from the portal vein, a 21-gauge needle was placed alongside the portal vein and a suture was tied around both as close to the liver as possible; the needle was then removed resulting in a calibrated stenosis. The abdomen was then closed and animals were examined 7 days after surgery. In sham-operated animals, the portal vein was exposed but no suture was placed around it. The effectiveness of surgery was assessed by the presence or absence of porto-systemic shunt vessels: all portal vein-ligated rats, and none of the sham rats, had visible porto-systemic shunting (particularly visible were spleno-renal shunts).

**Measurement of portal pressures**

In a small number of Wistar rats, portal pressure was measured. Animals were anaesthetized with ether, and maintained with pentobarbitone (Nembutal\textsuperscript{TM}, 10% solution) injected into the jugular vein after cannulation. The ileocaecal vein was cannulated and connected to a blood pressure transducer for measurement of portal pressure. These animals were not employed in studies of rat aorta.

**Aorta**

After stunning and exsanguination, thoracic 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, gassed with 5% CO\textsubscript{2}/95% O\textsubscript{2}, of the following composition: 119 mM NaCl, 25 mM NaHCO\textsubscript{3}, 11.1 mM d-glucose, 4.7 mM KCl, 2.5 mM CaCl\textsubscript{2}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 1.0 mM MgSO\textsubscript{4} and 0.03 mM EDTA. Additionally, cocaine (3 \(\mu\)M), propranolol (3 \(\mu\)M) and indomethacin (10 \(\mu\)M) were present. In each experiment a total of four rings, two each from a sham and portal hypertensive animal, was examined, and agonist EC\textsubscript{50} or maximum response was taken as the mean of the two values from each animal. In preliminary experiments it was found that the maximum contraction of the aorta to KCl was unaffected by changing the resting tension over the range of 1–5 g, in both sham and portal hypertensive animals. Tissues were contracted with KCl (40 mM), exposed to acetylcholine (10 \(\mu\)M) to test for endothelium-dependent relaxation, and washed. Cumulative concentration–response curves were plotted for noradrenaline or phenylephrine in 1-log-unit increments, beginning with 1 nM, and for KCl (10–120 mM). Only one agonist concentration–response curve was carried out per tissue, and a series of experiments was completed for each agonist individually. A small number of tissues with damaged endothelium which failed to relax to acetylcholine or tissues which failed to give sufficient contraction to vasoconstrictors (maximum contraction < 0.25 g) were discarded.

**Radioligand binding studies**

Preparation of cardiac ventricular membranes from portal hypertensive and sham-operated Wistar rats was carried out by a method described previously [14]. The ventricular muscle was weighed, minced with scissors and then homogenized in 10 volumes of ice-cold wash buffer (50 mM Tris–HCl/5 mM EDTA, pH 7.4 at 4°C). The filtrate was centrifuged at 14000 \(\times\) g for 14 min at 4°C. The supernatant was discarded, the pellet resuspended in fresh buffer and the centrifugation step repeated. The resultant pellets were used immediately or stored at –20°C for later use. Pellets were reconstituted in 10 volumes of incubation buffer (50 mM Tris–HCl/5 mM EDTA, pH 7.4 at 25°C).

In saturation experiments, aliquots of membrane suspension were incubated with various concentrations of \([\text{H}]\)prazosin at 25°C (0.1–10 nM; specific radioactivity 78 Ci/mmol, Amersham). Non-specific binding was
determined in the presence of phentolamine (10 μM). Specific binding of [3H]prazosin was greater than 90% of total binding at the $K_D$ concentration. Assays were terminated by washing with ice-cold incubation buffer, followed by rapid vacuum filtration through Whatman GF/C filters, using a Brandel Cell Harvester. Radioactivity retained on filters was determined by liquid scintillation spectroscopy. The protein content of the rat ventricular homogenate was measured using the Coomassie Brilliant Blue method [15].

**Drugs**

Acetylcholine chloride, cocaine hydrochloride, indomethacin, noradrenaline bitartrate, phenylephrine hydrochloride and propranolol were all obtained from Sigma Chemical Co., Poole, Dorset, U.K. $N^\omega$-Monomethyl-L-arginine and phentolamine mesylate were obtained from Research Biochemicals, Natick, U.S.A. Drugs were dissolved in distilled water, except for indomethacin (100% ethanol).

**Statistics**

Values are expressed as means ± S.E.M. Agonist potency was expressed as $-\log EC_{50}$. Differences between groups were compared by Student’s $t$-test for unpaired data and analysis of variance. Ligand binding data were analysed and agonist $EC_{50}$ values were obtained using the GraphPad Prism program for PC (GraphPad Software Inc., CA, U.S.A.).

**RESULTS**

**Portal pressures**

Portal pressure was 7.8 ± 1.9 mmHg ($n = 3$) in sham-operated Wistar rats (7 days) and 14.4 ± 0.6 mmHg ($n = 3$) in 7-day portal hypertensive Wistar rats ($P < 0.05$ compared with sham-operated animals).

<table>
<thead>
<tr>
<th></th>
<th>Portal hypertensive</th>
<th>Sham-operated</th>
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<tbody>
<tr>
<td>Wistar aorta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>$7.56 ± 0.11$ ($n = 6$)</td>
<td>$7.52 ± 0.15$ ($n = 8$)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>$7.69 ± 0.08$ ($n = 14$)</td>
<td>$7.70 ± 0.11$ ($n = 9$)</td>
</tr>
<tr>
<td>KCl (EC$_{50}$: mM)</td>
<td>$17.7 ± 4.0$ ($n = 6$)</td>
<td>$21.1 ± 3.4$ ($n = 6$)</td>
</tr>
<tr>
<td>Sprague-Dawley aorta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>$7.78 ± 0.09$ ($n = 7$)</td>
<td>$7.89 ± 0.15$ ($n = 7$)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>$7.66 ± 0.11$ ($n = 8$)</td>
<td>$7.47 ± 0.10$ ($n = 8$)</td>
</tr>
<tr>
<td>KCl (EC$_{50}$: mM)</td>
<td>$17.4 ± 4.4$ ($n = 7$)</td>
<td>$20.8 ± 2.9$ ($n = 7$)</td>
</tr>
</tbody>
</table>

**Aorta**

In aortic rings from Wistar and Sprague-Dawley rats, noradrenaline, phenylephrine and KCl produced concentration-dependent contractions but there was no significant difference in potency between sham and portal hypertensive animals (Table 1).

In Wistar rat aorta, the maximum contractions to noradrenaline and KCl were significantly increased in portal hypertensive animals compared with sham-operated animals [noradrenaline: $1.00 ± 0.17$ g, $n = 5$, versus $0.57 ± 0.07$ g, $n = 8$, $P < 0.01$ (Figure 1); KCl: $0.71 ± 0.12$ g, $n = 5$, versus $0.45 ± 0.04$ g, $n = 6$, $P < 0.05$ (Figure 2)]. However, in Wistar rat aorta, the maximum contraction to phenylephrine was not significantly in-
increased in portal hypertensive animals \([0.86 \pm 0.10 \text{ g}, n = 13, \text{versus } 0.71 \pm 0.05 \text{ g}, n = 9 \text{ (Figure 3)}]\).

In Sprague–Dawley rat aorta, the maximum contraction to noradrenaline was not significantly increased in portal hypertensive animals compared with sham-operated animals \([0.93 \pm 0.09 \text{ g}, n = 7, \text{versus } 1.04 \pm 0.10 \text{ g}, n = 7 \text{ (Figure 4)}\). However, the maximum contractions to phenylephrine and KCl were significantly increased in portal hypertensive animals [phenylephrine: \(1.54 \pm 0.30 \text{ g}, n = 7, \text{versus } 0.76 \pm 0.11 \text{ g}, n = 7, P < 0.05 \text{ (Figure 5)}\); KCl: \(1.21 \pm 0.21 \text{ g}, n = 7, \text{versus } 0.59 \pm 0.09 \text{ g}, n = 7, P < 0.05 \text{ (Figure 6)}\)]. These differences were maintained after nitric oxide synthase inhibition with 

100 \(\mu\text{M}\) \(N\text{G}-\text{monomethyl-L-arginine} \text{ (phenylephrine: } 1.62 \pm 0.33 \text{ g}, n = 7, \text{versus } 0.84 \pm 0.19 \text{ g}, n = 7, P < 0.05; \text{ KCl: } 1.32 \pm 0.24 \text{ g}, n = 7, \text{versus } 0.66 \pm 0.08 \text{ g}, n = 7, P < 0.05\).

**Ligand binding studies**

\([^{3}H]\text{Prazosin bound to a single population of sites in cardiac ventricular membranes from portal hypertensive and sham-operated Wistar rats. There were no differences between portal hypertensive and sham animals in the affinity (}K_{d}\text{) or maximum number of binding sites (}B_{\text{max}}\text{) for }[^{3}H]\text{prazosin in saturation studies (Table 2)}\).
DISCUSSION

The major findings from this study are that the maximum contraction to the vasoconstrictors noradrenaline, phenylephrine and KCl was unchanged or increased in aortic rings from portal hypertensive Wistar and Sprague–Dawley rats. To understand the reason for carrying out this study, it is necessary to review the reported effects of portal vein ligation and experimental cirrhosis on vasoconstrictor responsiveness of rat aorta (Table 3). It can be seen that the majority of studies found a decreased responsiveness to vasoconstrictors in aorta from partial portal vein-ligated or cirrhotic rats, compared with the relevant controls. These results, and studies of other isolated blood vessels, have led to the general view that portal hypertension results in a diminished responsiveness to vasoconstrictors. In our studies of portal hypertensive Wistar rats, we found an increased responsiveness in mesenteric artery [12] and aorta (present results), but, since the majority of published studies employed Sprague–Dawley rats (see Table 3), we expanded our study to examine aorta from Sprague–Dawley rats.

At 7 days, portal hypertensive Wistar rats had significantly elevated portal pressure compared with sham-operated controls (present results), and the level was similar to that previously reported by us for 14-day portal hypertensive Wistar rats [12]. In a study of the time course of change in portal pressure in portal hypertensive Sprague–Dawley rats, highest pressures were reached 2 days post ligation, falling by 8 days to about 15 mmHg, with a similar pressure at 14 days [1]. Hence, portal pressure stabilizes at about 7 days post ligation in both portal hypertensive Wistar and Sprague–Dawley rats to a level significantly higher than that of sham-operated animals.

In our studies we found increased or unchanged maximum contractions to noradrenaline, phenylephrine and KCl in aorta from portal hypertensive animals of both Wistar and Sprague–Dawley strains. Specifically, contractions to KCl were significantly increased in vessels from portal hypertensive animals of both strains, but responses to the α1-adrenoceptor agonists were less consistently increased: responses to noradrenaline and phenylephrine were significantly increased in vessels from portal hypertensive Wistar and Sprague–Dawley

Table 2  Affinity ($K_d$) and maximum number of binding sites ($B_{max}$) for $[^{3}H]$prazosin in saturation studies of ventricular membranes from portal hypertensive and sham-operated Wistar rats

<table>
<thead>
<tr>
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<th>$K_d$ (nM)</th>
<th>$B_{max}$ (fmol/mg protein)</th>
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<tr>
<td>Portal hypertensive rats ($n = 7$)</td>
<td>$1.1 \pm 0.10$</td>
<td>$63.6 \pm 5.9$</td>
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<tr>
<td>Sham-operated rats ($n = 6$)</td>
<td>$1.1 \pm 0.10$</td>
<td>$62.7 \pm 4.8$</td>
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</table>

Table 3  Effects of portal vein ligation and experimental cirrhosis on responsiveness of rat isolated aorta to vasoconstrictors, based on published information

<table>
<thead>
<tr>
<th>Strain</th>
<th>Days</th>
<th>Agonist</th>
<th>Effect of treatment on maximum</th>
<th>Ref.</th>
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<td>[26]</td>
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<td>[31]</td>
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<td>[11]</td>
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<td>No change</td>
<td>[11]</td>
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<tr>
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<td>[12]</td>
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<td>Noradrenaline</td>
<td>Increase</td>
<td>[32]</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>7</td>
<td>Phenylephrine, KCl</td>
<td>Increase</td>
<td>Present results</td>
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<tr>
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<tr>
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<td>70</td>
<td>Noradrenaline</td>
<td>Increase</td>
<td>[32]</td>
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</table>
rats respectively. In a previous study by our laboratory [12], responses to noradrenaline and KCl (40 mM) were increased, but not significantly, in aorta from portal hypertensive Wistar rats. In none of our studies have we found a significant decrease in contractile response in vessels (aorta or mesenteric artery) from portal hypertensive compared with sham-operated animals. We did not relate contractions to vessel wall thickness or mass. However, in our previous study of rat small mesenteric artery, significantly increased maximum contractions were obtained in vessels from portal hypertensive rats with no change in vessel wall thickness [12].

An increased maximum response to noradrenaline or phenylephrine in aorta could be due to an increase in the number of $\alpha_1$-adrenoceptors or a post-receptor change in responsiveness. However, an increase in the number of receptors might be expected to increase the potency of noradrenaline, rather than the maximum response, in a tissue with spare receptors. Since any change in $\alpha_1$-adrenoceptor number due to changes in sympathetic activity might be expected to affect a range of cardiovascular tissues, we examined $\alpha_1$-adrenoceptor number in ventricular membranes and found no significant difference between sham-operated and portal hypertensive Wistar rats in the affinity of prazosin or the maximum number of binding sites. This agrees with the results of Liao et al. [16] who reported no difference in $\alpha_1$-adrenoceptor binding in the mesenteric artery or tail artery of portal hypertensive Sprague–Dawley rats compared with sham-operated animals. Furthermore, we demonstrated that the maximum contraction to KCl was significantly increased in aorta from portal hypertensive animals of both strains. As KCl acts to produce membrane depolarization rather than at the receptor level, it is more likely that the changes in contractile responsiveness occur at the post-receptor level.

The increased contractile responsiveness of the aorta found in this study, and of the mesenteric artery in our previous study [12,13], contrasts with reports of decreased responsiveness of the in situ mesenteric vascular bed of pre-hepatic portal hypertensive rats or cirrhotic rats to vasoconstrictors such as noradrenaline, endothelin-1 and angiotensin II [7–9,17,18]. Pressor responses to angiotensin II also decreased in bile duct-ligated dogs [19]. 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 [4]. These vasodilators act as physiological antagonists of vasoconstrictor responses in vivo, and may cause an adaptive change to occur in the responsiveness of blood vessels to vasoconstrictors. Such a physiological antagonism by vasodilators would be absent in vivo, but any altered contractile responsiveness would be revealed. Some other studies of portal hypertension report increased contractile responsiveness in vitro: the contractile response to noradrenaline was increased in mesenteric veins from portal vein-ligated rabbits [20], and the contractile response to 5-hydroxytryptamine was increased in mesenteric veins from portal hypertensive rats [21]. In human hepatic arteries, vessels from cirrhotic patients tended to give a larger maximum contraction to noradrenaline, phenylephrine and KCl, although this did not reach significance [22].

The vascular endothelium may contribute to the hyperdynamic circulation in portal hypertension and cirrhosis [5,23–25], hence some authors have examined whether removal of the endothelium or addition of inhibitors of nitric oxide synthase would restore vasoconstrictor responses to normal. In rat aorta, there are reports that nitric oxide synthase inhibitors failed to restore responses to normal in portal hypertensive animals [26] or that nitric oxide synthase inhibitors restored responses to normal in portal hypertensive [27] and cirrhotic animals [28]. Removal of the endothelium failed to restore responses in portal hypertensive [29] and cirrhotic animals [28], but was found in another study to restore responses in aorta from cirrhotic animals [30].

Finally, how do we explain the wide range of results reported in the literature for the effects of vasoconstrictors on aorta from portal hypertensive rats? There are two main possibilities. First, in reality there may be no overall difference between portal hypertensive and sham animals (hence some variability in the present results), so that the reported results represent different ends of the spectrum, and there may be unpublished findings showing no change. Studies comparing basal parameters in two groups of animals are always likely to be more problematic than studies making a comparison before and after an intervention. Second, there may be subtle changes occurring with portal vein ligation which are dependent on the strain, agonist, and duration and degree of stenosis, leading to differing results between laboratories. However, the present study would tend to rule out strain or agonist dependence. The effects of duration and degree of stenosis are worthy of further study.

In conclusion, in our study portal hypertension tended to produce an increase in the maximum contractile response to the vasoconstrictors noradrenaline, phenylephrine and KCl in aorta from Wistar or Sprague–Dawley rats. These results question the widely held belief that the diminished responsiveness to vasoconstrictors reported in portal hypertensive rats in vivo is due to a diminished responsiveness at the level of the vascular smooth muscle.

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