Vascular hyporesponsiveness in aortic rings from cirrhotic rats: role of nitric oxide and endothelium

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1. The role of nitric oxide as mediator of the vascular alterations present in different models of experimental liver cirrhosis is controversial. In the present study, we evaluated the role of nitric oxide and that of the endothelium in the response to phenylephrine and acetylcholine of isolated aortic rings from chronic bile duct-ligated (29 days) rats and their corresponding controls. Experiments were performed in rings with or without endothelium, in rings pretreated with N-o-nitro-L-arginine methyl ester (10^-4 mol/l) to inhibit nitric oxide synthesis and in rings pretreated with aminoguanidine (10^-4 mol/l) to inhibit inducible nitric oxide synthesis.

2. Under basal conditions, the maximum absolute tension developed in response to cumulative addition of phenylephrine was significantly decreased in rings from bile duct-ligated animals (1.62 ± 0.06 g) compared with the control rings (2.15 ± 0.099). This hyporesponsiveness to phenylephrine of rings from bile duct-ligated animals was corrected after treatment with N-o-nitro-L-arginine methyl ester and reduced, but not completely eliminated, in rings without endothelium. In contrast, aminoguanidine did not modify the lower response to phenylephrine rings from bile duct-ligated animals. ED50 values were not different between groups under any experimental conditions.

3. The endothelium-dependent vasodilatation to acetylcholine in phenylephrine-constricted rings was similar in both groups of animals, control and bile duct ligated, under all experimental conditions. N-o-nitro-L-arginine methyl ester pretreatment and removal of the endothelium completely abolished the response to acetylcholine in cirrhotic and control rings.

4. These results demonstrate that in aortic rings from cirrhotic, bile duct-ligated rats, increased production of nitric oxide, mainly of endothelial origin, is responsible for the lower contractile response to phenylephrine. Our data, however, do not support the involvement of the inducible nitric oxide synthase isoform in this alteration. In contrast, endothelial vasodilatory response to acetylcholine is not altered in this model of cirrhosis, which indicates that not all mechanisms of nitric oxide release are abnormal.

INTRODUCTION

Liver cirrhosis can be described as a disease in which there is an imbalance between vasoconstrictor and vasodilator substances [1–3]. Thus, a reduced response to most endogenous vasoconstrictors has been found in several cirrhotic experimental models [4–14]. As plasma levels of noradrenaline, vasopressin and other vasoactive substances are usually elevated in cirrhosis, it is possible that an excess of local or systemic vasodilators is involved in the attenuation of their vasoconstrictor effects. The mechanisms mediating this phenomenon of pressor hyporesponsiveness in cirrhosis are not completely established.

Nitric oxide (NO) is a potent endogenous vasodilator that participates in the control of vascular tone, among other functions. NO is normally synthesized in vascular endothelium by a constitutive NO synthase, and its production can be induced in vascular smooth muscle and phagocytic cells in the presence of endotoxaemia and high levels of cytokines [3]. Recent studies point to an enhanced production of NO as one of the important pathogenetic mechanisms of the systemic and renal alterations present in liver cirrhosis [3, 15]. However, there are conflicting reports regarding the role of NO in the vascular response to vasoconstrictors in different vascular beds. This is especially important in the frequently used rat model of bile duct ligation (BDL) [5, 13–14, 16]. Thus, in the present study, we evaluated the vascular response of aortic rings from rats with chronic biliary cirrhosis to the α-adrenergic vasoconstrictor agent phenylephrine (PE) and the endothelium-dependent vasodilator acetylcholine.

Key words: acetylcholine, aorta, endothelium, liver cirrhosis, nitric oxide, phenylephrine.

Abbreviations: ACh, acetylcholine; AG, aminoguanidine; ANOVA, analysis of variance; BDL, bile duct ligation; L-NAME, N-o-nitro-L-arginine methyl ester; NO, nitric oxide; PE, phenylephrine.

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in order to assess the modulatory role of vascular endothelium. As NO is the major endothelium-derived vasodilating factor, we also analysed its participation by studying the involvement of the main NO synthase isoforms, constitutive and inducible, in the mediation of these vascular responses.

METHODS

All the experiments were performed according to the guidelines for the ethical treatment of the animals of the European Union. Male Sprague–Dawley rats born and raised in the Animal House of the University of Murcia were used in this study.

Bile duct ligation

Rats weighing initially 150–200 g were anaesthetized with ethylid ether and, under aseptic conditions, a double ligature and section of the common bile duct or a sham operation were performed, as previously described [17]. After closure of the abdominal wound, the animals were returned to their home cages, where they had complete access to normal rat chow and tap water. Biliary cirrhosis was diagnosed by clinical inspection (jaundice and coluria) and then confirmed post mortem.

Experimental protocol

Four weeks (±2 days) after BDL or sham operation, the animals were killed by retrocervical dislocation. After opening the thorax, the thoracic aorta was dissected, removed and placed in a Petri dish containing a Krebs–bicarbonate solution (in mmol/l: NaCl, 128; KCl, 4.7; MgCl2·6H2O, 1.2; H2KPO4, 1.2; CaCl2·2H2O, 2.5; EDTA, 0.01; glucose, 11.1; NaHCO3, 24). The aortas were carefully cleaned and cut into 3-mm rings. The rings were mounted horizontally between two stainless-steel hooks in individual organ bath chambers (5 ml) filled with Krebs solution and bubbled with 95% oxygen–5% carbon dioxide and maintained at 37°C and pH 7.4 throughout the experiment. The superior hook was connected to an isometric transducer (Pioden UF-1, Panlab, Barcelona, Spain) to measure changes in tension, which were amplified and recorded on a computer (Cibertec, Madrid, Spain). The rings were equilibrated for 60 min, changing the Krebs every 15 min, at an optimum resting tension of 2 g. A cumulative concentration–response curve for PE (10–9 to 10–5 mol/l) was then obtained. After finishing this curve, rings were repeatedly washed with Krebs and a 60-min period was observed. The rings were then again constricted with a maximum PE dose and a cumulative concentration–response curve for ACh (10–9 to 10–4 mol/l) was obtained.

Statistical analysis

The contractile response to PE is expressed in grams with respect to a basal value of 2 g. The relaxation responses to ACh are expressed as a percentage of the contraction obtained with PE. Results are expressed as the means ± SEM. Statistical analysis was performed by two-way repeated-measures analysis of variance (ANOVA) and post hoc Duncan test where appropriate. The ED50 values were calculated by regression analysis for every ring individually, and the differences between groups were analysed by Student’s t-test. Differences were considered statistically significant at \( P < 0.05 \).

RESULTS

Examination of the BDL rats showed the typical characteristics of secondary biliary cirrhosis, including enlarged liver and spleen, portosystemic shunts, mesenteric oedema and ascites to a variable degree. There were no significant changes in control rats. Body weight was 327.0 ± 18.0 g and 361.5 ± 24.9 g for cirrhotic and control rats respectively.

Constrictor responses (Fig. 1)

The contractile response to PE was significantly lower in cirrhotic than in control aortic rings. The addition of L-NAME significantly increased and shifted to the left the responses to PE in both groups, so that the hyporesponsiveness to PE was eliminated. Similarly, removal of the endothelium induced a significant increase in the response to PE in cirrhotic and control rings. However, the pressor
Endothelial nitric oxide in cirrhotic aortic rings

Fig. 1. Constrictor responses to PE in aortic rings from control and cirrhotic (BDL) rats. E-, rings without endothelium; L-NAME, rings pretreated with the NO synthesis inhibitor L-NAME; AG, rings pretreated with AG.

Table 1. ED50 values and maximum contraction to PE (Max, g) in the experimental groups. Data are expressed as the means±SEM; number in parentheses is number of rings from three to four rats. Statistical significance: *P<0.05 versus control rings; †P<0.05 versus rings in basal conditions.

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>ED50 (10⁻⁷ mol/l)</th>
<th>Max (g)</th>
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<tbody>
<tr>
<td>Basal</td>
<td></td>
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<tr>
<td>Control (11)</td>
<td>0.83±0.14</td>
<td>2.15±0.09</td>
</tr>
<tr>
<td>BDL (12)</td>
<td>1.10±0.15</td>
<td>1.62±0.06</td>
</tr>
<tr>
<td>L-NAME</td>
<td></td>
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<tr>
<td>Control (12)</td>
<td>0.26±0.02†</td>
<td>2.33±0.07†</td>
</tr>
<tr>
<td>BDL (10)</td>
<td>0.53±0.11†</td>
<td>2.29±0.08†</td>
</tr>
<tr>
<td>E-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (9)</td>
<td>0.47±0.06†</td>
<td>2.47±0.13†</td>
</tr>
<tr>
<td>BDL (10)</td>
<td>0.29±0.05†</td>
<td>2.11±0.12‡*</td>
</tr>
<tr>
<td>AG</td>
<td></td>
<td></td>
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<tr>
<td>Control (11)</td>
<td>1.20±0.10†</td>
<td>2.37±0.08†</td>
</tr>
<tr>
<td>BDL (15)</td>
<td>1.27±0.14</td>
<td>1.83±0.06†</td>
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Dilator responses (Fig. 2)

In the basal situation, ACh induced a similar relaxation in both groups of rings and there were no differences between control and cirrhotic rings. The relaxation in response to ACh was eliminated in the presence of L-NAME and after removal of the endothelium in both control and BDL rings. However, AG administration did not modify the relaxation responses to ACh in any group. The ED50 for ACh responses was significantly lower in intact rings from BDL rats than in rings from control rats and was not modified in the AG-pretreated rings (Table 2). Endothelium removal did not alter the response of the smooth muscle in any group, as sodium nitroprusside relaxed in a similar way both cirrhotic and control denuded rings and there were no differences between them (control: 10⁻⁶, 100.0±2.7%; 10⁻⁵, 104.4±2.9%; 10⁻⁴, 105.2±2.8%; BDL: 10⁻⁶, 94.9±1.3%; 10⁻⁵, 98.7±1.6%; 10⁻⁴, 101.2±1.3%).

DISCUSSION

Vascular vasodilatation induced by an excess of local and systemic vasodilators is one of the mechanisms involved in the lower vascular response to endogenous and exogenous vasoconstrictors characteristic of liver cirrhosis. Among all the possible vasodilator substances suggested to play a role in the hyporesponsiveness to vasoconstrictors in cirrhosis, NO has received considerable interest during recent years, and it has been suggested that
an increase in NO production might be largely responsible for this vascular alteration. Thus, different authors have shown that NO synthesis inhibition increases, and in some cases completely corrects, the poor vasoconstrictor responses of cirrhotic and portal hypertensive rats [6–8, 11, 14]. However, in some cases, a role for NO could not be demonstrated [9, 13, 16]. Specifically, the role of endothelium and NO in the modulation of the vasoconstrictor and vasodilator responses in aortas from chronic BDL rats has not been studied. In the present study, we demonstrate that aortic rings from cirrhotic BDL rats show the characteristic hyporesponsiveness to a vasoconstrictor, as already found in the same vessel type of the same [13–14] or a different rat model [11]. However, whether this alteration represents the confirmation of an enhanced vasodilatory influence operative in these cirrhotic rats is not clear to all authors. Thus, data obtained by Cària et al. [11] support the idea of an enhanced contribution of NO because of their finding of an increased vasodilatory response to ACh, whereas recently Lee et al. [13] have concluded that, as endothelium-dependent vasodilation with bethanechol was not altered, an increased NO activity could not explain the lower contractile response to PE. Our conclusion, however, while based on similar results to those of Lee et al. [13], is different. The present results clearly indicate that the reduced vascular responsiveness to PE exhibited by aortic rings from cirrhotic rats is due to the enhanced vasodilatory influence of NO, mainly of endothelial origin.

The endothelium releases several vasodilatory compounds, and our results indicate that synthesis inhibition of one of them, NO, results in correction of the reduced vasoconstrictor response of the cirrhotic aortic rings. This clearly indicates that an enhanced NO activity is mainly responsible for this vascular hyporesponsiveness. However, one might imagine that this excess of NO in response to the vasoconstrictor adrenergic agonist would also be present when challenging the vessel with ACh, an endothelium-dependent vasodilator. However, this

Table 2. EDSO values and percentage relaxation to ACh in the experimental groups. Data are expressed as the means ± SEM. ND, not determined. Statistical significance: *P < 0.05 versus control rings; †P < 0.05 versus rings in basal conditions.

<table>
<thead>
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<th>EDSO (10⁻⁶ mol/l)</th>
<th>(%)</th>
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<tr>
<td>Acetylcholine</td>
<td></td>
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<tr>
<td>Basal Control</td>
<td>0.24 ± 0.05</td>
<td>91.1 ± 3.1</td>
</tr>
<tr>
<td>BDL</td>
<td>0.13 ± 0.02*</td>
<td>94.4 ± 2.9</td>
</tr>
<tr>
<td>L-NAME</td>
<td></td>
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<tr>
<td>Control</td>
<td>ND</td>
<td>0.9 ± 0.6†</td>
</tr>
<tr>
<td>BDL</td>
<td>ND</td>
<td>3.5 ± 1.1†</td>
</tr>
<tr>
<td>E⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>ND</td>
<td>6.8 ± 0.8†</td>
</tr>
<tr>
<td>BDL</td>
<td>ND</td>
<td>4.5 ± 1.1†</td>
</tr>
<tr>
<td>AG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.16 ± 0.03</td>
<td>89.7 ± 2.7</td>
</tr>
<tr>
<td>BDL</td>
<td>0.13 ± 0.01</td>
<td>91.8 ± 3.7</td>
</tr>
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</table>
was the case, and the vasodilator response to ACh was unaltered in the cirrhotic rings compared with that of the controls, although the ED50 was lower in the cirrhotic rats, indicative of a somewhat greater sensitivity. Overall, this result would suggest that not all NO release mechanisms are altered in this cirrhotic model. As already mentioned, similar results have recently been published [13] in the same cirrhotic model. However, we and other authors have found that ACh produces greater relaxations in vessels from carbon tetrachloride-treated cirrhotic rats [11, 12], supporting different roles for NO and different pathophysiological mechanisms depending on the experimental model studied.

As already mentioned, some studies [3, 7, 8] suggest the existence of a systemic activation of the constitutive endothelial NO synthase as the main isoform responsible for the enhanced activity of NO in cirrhosis. However, we found that removal of the endothelium did not completely correct the hyporesponsiveness to PE, which may be indicative of the existence of a muscular vasodilatory mechanism that also contributes to this vascular alteration. As l-NAME corrected the hyporesponsiveness to PE of the cirrhotic rings, we hypothesized that a muscular NO synthase could also be involved in the vascular hyporesponsiveness of cirrhotic rings.

It has been reported that the inducible isoform of NO synthase can be found in smooth muscle cells, as well as in endothelium [18, 19]. Moreover, cirrhosis is a disease in which there are elevated levels of endotoxaemia and cytokines, both known stimuli for the inducible NOS. At the dose used in the present study, AG has been identified as a selective inhibitor of this isoform [20–23], and our results show that AG did not significantly affect the response to ACh, indicating that the constitutive endothelial NO synthase isoform was not altered at this dose. However, the pressor response to PE was not modified by pretreatment with AG, and the rings from the cirrhotic animals still exhibited a reduced pressor response. Other factors, including cyclo-oxygenase-derived products or smooth muscle alteration, may be involved in the residual hyporesponsiveness shown by the endothelium-denuded cirrhotic rings. Overall, the present results suggest that the hyporesponsiveness of the cirrhotic aortic rings is not due to activation of the inducible NO enzyme and point to the constitutive isoform, mostly of endothelial origin, as the main factor responsible for this vascular alteration.

In the present study, pretreatment of the aortic rings with an NO inhibitor or endothelium removal decreased the ED50 to phenylephrine in both groups, which indicates the important role played by NO in particular, and the endothelium in general, as a mechanism that counteracts the action of vasoconstrictors. This has been also previously shown in different types of vessels [24, 25].

It is now clear that NO can be released by several mechanisms [26]. One way is that used by endothelium-dependent vasodilators, such as ACh, which increase NO production as a result of the elevation in endothelial cell calcium levels brought about by agonist-mediated G-protein activation. As already mentioned, our results and those of Lee et al. [13] indicate that this pathway is not altered in the rat cirrhotic BDL model. Another mechanism is secondary to the binding of the vasoconstrictor, such as PE, to specific receptors in smooth muscle, α-adrenergic receptors in this case. In this situation, at least two mechanisms, the vasoconstriction-inducing stretching of the vessel wall or a shear-stress related mechanism, could activate the release of endothelial NO. It is this mechanism that our data identifies as defective in cirrhosis.

In summary, aortic rings from cirrhotic BDL rats exhibit a reduced pressor response to PE that can be corrected by inhibition of NO synthesis. The NO synthase isoform involved is mainly of endothelial origin and is constitutively expressed. Finally, our results also indicate that the endothelium-dependent vasodilatation is normal in this model, and this suggests that not all the NO release mechanisms are altered.

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