Study on the vascular reactivity and α_1 -adrenoceptors of portal hypertensive rats

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1 Vascular hyporesponsiveness in portal hypertension has been demonstrated to various vasoconstrictors including noradrenaline (NA). The present study aimed to determine whether the attenuated vascular responsiveness to NA is due to a change in the affinity or the number of α_1 -adrenoceptors.

2 Partial portal vein ligation (PVL) was performed in Sprague-Dawley rats to produce portal hypertension. Vascular responsiveness to NA was assayed in portal vein, mesenteric artery or tail artery. The affinity and number of α_1 -adrenoceptors were determined by specific binding of [¹²⁵I]-HEAT (2- β -4-hydroxy-3-iodophenylethyl-aminomethyltetralone).

3 In the presence of yohimbine (10^{-7} M, an α_2 -adrenoceptor antagonist), propranolol (10^{-6} M, a β -adrenoceptor antagonist), and two catecholamine uptake inhibitors, desipramine (10^{-7} M) and normetanephrine (10^{-6} M), the maximum responses to NA were decreased in all three blood vessels of PVL rats: 45% decrease in portal vein, 25% in mesenteric artery and 18% in tail artery.

4 The EC₅₀ values of NA and the pA₂ values of prazosin, an α_1 -adrenoceptor antagonist, in all three blood vessels were not significantly different between sham-operated and PVL rats.

5 The K_D and B_{max} values for specific binding of [¹²⁵I]-HEAT or the K_i values for NA in the crude membrane preparations of either mesenteric artery or tail artery were also not significantly different between the two groups.

6 It is concluded that the vascular hyporesponsiveness to NA in the mesenteric artery or tail artery of PVL rats is not due to changes in the affinity or number of α_1 -adrenoceptors.

Keywords: Portal hypertension; portal vein; mesenteric artery; tail artery; α_1 -adrenoceptor; noradrenaline

Introduction

It is known that chronic portal hypertension is associated with increased cardiac output and splanchnic blood flow and decreased total peripheral and splanchnic vascular resistances (Vorobioff *et al.*, 1983). The decreased vascular resistance may, in part, result from the attenuated vascular reactivity to endogenous vasoconstrictors, such as noradrenaline (NA) (Kiel *et al.*, 1985), arginine vasopressin (Murray & Paller, 1986), and angiotensin II (Finberg *et al.*, 1981). The nature of the attenuation of vascular reactivity is not clear at present. Accumulated evidence has suggested that functional antagonism by elevation of vasodilator substances and a defect in the cellular contractile apparatus in the vascular smooth muscle may be involved in the hyporesponsiveness of portal hypertension. Benoit *et al.* (1984) and Pizcueta *et al.* (1990) hypothesized that hyperglucagonism plays a key role in the decreased vascular sensitivity to various vasoconstrictors. Recently, in studying the role of endothelium in regulation of vascular tone, investigators have revealed that nitric oxide (Vallance & Moncada, 1991; Pizcueta *et al.*, 1992; Lee *et al.*, 1993) and endothelin (Yu *et al.*, 1992) may be responsive for the hyporesponsiveness of portal hypertension. However, the hyporesponsiveness was not only to vasoconstrictors but also to vasodilators (Kaumann & Groszmann, 1989; Chao *et al.*, 1992). In addition, there are also studies indicating that the hyporesponsiveness in portal hypertension may be attributed to impairment of intracellular mechanisms (Murray & Paller, 1985; Chao *et al.*, 1992).

Despite the well documented attenuated vascular response to NA it is generally accepted that, in patients with decompensated liver cirrhosis, sympathetic nervous activity is in-

creased (Bichet *et al.*, 1982; Arroyo *et al.*, 1983; Tage-Jensen *et al.*, 1988). Although many reports have described the decreased responsiveness to NA or a related α -adrenoceptor agonist (MacGilchrist *et al.*, 1991; Lee *et al.*, 1992), the vascular receptor number and affinity of NA in portal hypertension have not been studied. It is possible that elevated plasma concentrations of NA may affect the receptor number or the affinity and so affect vascular reactivity (Vilamediana *et al.*, 1988). Using platelets and lymphocytes, MacGilchrist *et al.* (1990) did not find an adrenoceptor down-regulation in cirrhotic patients. However, platelet and lymphocyte receptors may not be representative of vascular receptors. The purpose of this study was to clarify whether the hyporesponsiveness to NA occurs at the receptor level. Therefore, we investigated whether there were alterations in the number and affinity of vascular α -adrenoceptor in portal hypertensive rats.

Methods

Portal hypertensive (PVL) rats

Partial portal vein ligation was performed according to the method described by Chojkier & Groszmann (1981). Briefly, male Sprague-Dawley rats (200–250 g) were anaesthetized with ether. A midline incision was made and the portal vein proximal to the bifurcation was exposed. A 3–0 silk ligature was made around the portal vein and a piece of PE50 tubing (Clay Adams). The PE tubing was then removed and the abdomen closed. On sham-operated rats, no ligature was applied, the abdomen was closed after exposure of the portal vein.

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Pressure measurement

Two weeks after surgery, rat was anaesthetized with ketamine (150 mg kg^{-1}). The ileocolic vein was cannulated with PE50 tubing for measuring portal venous pressure. Portal venous pressures in ligated rats were between 12–17 mmHg, whereas in sham-operated rats were between 5–10 mmHg. Immediately after the portal pressure was confirmed, tail artery, mesenteric artery and portal vein were removed into aerated Krebs-Henseleit solution and cleaned. The composition of Krebs-Henseleit solution was (mM): NaCl 115, CaCl_2 2.1, MgSO_4 1.2, NaH_2PO_4 1.2, NaHCO_3 25, KCl 5.0 and glucose 11.

Tension experiments

The blood vessels were cut into segments and prepared as previously described (Yang *et al.*, 1990; 1991; Chao *et al.*, 1992). The tension generated was recorded on a Grass polygraph (7D) via force displacement transducer (FT03). The tissue chamber was maintained at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2 . Tissues were equilibrated under resting tension for 1 h. Preliminary experiments showed that the optimal tension for the portal vein was 0.7 g, mesenteric artery 1.2 g, and tail artery 1 g. Tissue responsiveness was indicated by the consistent responses of two consecutive tests with KCl (60 mM). After equilibration, cumulative dose-response curves to NA (10^{-8} – $3 \times 10^{-5} \text{ M}$) were first obtained. The tissues were then rinsed and allowed to recover for about 30 min. The dose-response curves to NA were repeated in the presence of 10^{-8} M prazosin (an α_1 -adrenoceptor antagonist). Effects of 10^{-7} M and 10^{-6} M prazosin were tested in the similar manner after a recovery period of 45 min. All of the dose-response curves to NA were performed in the presence of 10^{-7} M yohimbine (an α_2 -adrenoceptor antagonist), 10^{-6} M propranolol (a β -adrenoceptor antagonist), and two catecholamine uptake inhibitors, desipramine (10^{-7} M) and normetanephrine (10^{-6} M) (Han *et al.*, 1990).

Receptor binding assay

The technique for receptor binding assays follows that of Han *et al.* (1987) with some modifications. In brief, the tail artery from two rats and the mesenteric artery from four rats were pooled and homogenized in a glass homogenizer in 1 ml homogenizing buffer (50 mM Tris, 5 mM EDTA, pH 7.4). The homogenate was further diluted with 1 ml of $2 \times$ binding buffer (318 mM NaCl, 2 mM Tris, 10 mM MgCl_2 , pH 7.4) and 4 ml of binding buffer (154 mM NaCl, 20 mM Tris, 5 mM MgCl_2 , pH 7.4) for binding assay. Aliquots of the homogenate preparation (200 μl , 30–60 μg protein per assay) were incubated with [^{125}I]-HEAT (2- β -4-hydroxy-3-iodophenylethyl-aminomethyltetralone, 2200 Ci mmol^{-1} , NEN, Du Pont) at 5–6 concentrations or with 270 pM [^{125}I]-HEAT and seven concentrations of NA (10^{-9} – 10^{-3} M) in a final volume of 250 μl for 30 min at 30°C . Nonspecific binding was assessed by adding prazosin (10^{-5} M). At the end of the incubation, 10 ml of ice-cold wash buffer (150 mM NaCl, 20 mM HEPES, pH 7.4) was added and filtered through a 24 mm glass fibre filter (Whatman GF/C). The filter was then rinsed with additional 10 ml of ice-cold wash buffer and counted in a gamma counter (1277 Gammamaster, Pharmacia, Wallac Oy). All of the determinations were performed in duplicate. The portal vein was not used in this assay since binding was too low (specific bound = 29 c.p.m. with 210 pM [^{125}I]-HEAT added to 30 μg homogenate protein per assay) in a sample pooled from four rats.

Calculations

Tension was expressed as mN mm^{-2} (Murphy, 1980). EC_{50} was calculated from the plot of the percentage response

against log dose. pA_2 was calculated according to Arunlakshana & Schild (1959). The density of receptor sites (B_{max}) and the dissociation constant (K_D) were determined by Scatchard analysis (Scatchard, 1949). The inhibition constant K_i was calculated according to Munson & Rodbard (1980). Data are expressed as mean $\pm$ s.e.mean. Significance was determined by the Student's *t* test or one way analysis of variance (ANOVA) followed by Newman-Keuls test at $P < 0.05$.

Results

Tension experiments

As shown in Figure 1, the maximum contractile response of the tail artery to NA was the greatest among the three blood

Figure 1 Maximum contractile response to noradrenaline in blood vessels from portal hypertensive (PVL) and sham-operated (Sham) rats. The data are shown as mean $\pm$ s.e.mean of 6–8 experiments. * $P < 0.05$, significantly different between Sham (open column) and PVL (hatched column) rats by Student's *t* test.

Table 1 The EC_{50} values for noradrenaline on blood vessels of portal hypertensive (PVL) and sham-operated (Sham) rats

Blood vessel	n	EC_{50} (10^{-7} M)	
		Sham	PVL
Portal vein	16	$3.5 \pm 0.4^*$	$3.0 \pm 0.4^{**}$
Mesenteric artery	17	11.5 ± 3.1	12.9 ± 3.2
Tail artery	19	$1.3 \pm 0.2^{**}$	$1.5 \pm 0.2^{***}$

The data are shown as mean $\pm$ s.e.mean. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, significantly different from the corresponding mesenteric artery of sham-operated or PVL rats by Student's *t* test.

Table 2 The pA_2 values for prazosin on blood vessels of portal hypertensive (PVL) and sham-operated (Sham) rats

Blood vessel	n	pA_2	
		Sham	PVL
Portal vein	5	$8.9 \pm 0.1^*$	$9.0 \pm 0.1^{**}$
Mesenteric artery	5	9.6 ± 0.2	9.9 ± 0.2
Tail artery	6	$8.7 \pm 0.1^{**}$	$8.9 \pm 0.1^{**}$

The data are shown as mean $\pm$ s.e.mean. * $P < 0.05$ and ** $P < 0.01$, significantly different from the corresponding mesenteric artery of sham-operated or PVL rats by Student's *t* test.

Figure 2 Cumulative concentration-response curves to noradrenaline in the absence and presence of prazosin (10^{-8} M) of portal vein of portal hypertensive (PVL) and sham-operated (Sham) rats. The data are shown as mean $\pm$ s.e. mean of 5–7 experiments: Sham (○—○), Sham + prazosin (●—●), PVL (○—○), PVL + prazosin (●—●).

vessels tested. When comparing the vascular reactivity to NA between PVL and sham-operated rats, the maximum responses were decreased in all three blood vessels of PVL rats: a 45% decrease in portal vein, 25% in mesenteric artery and 18% in tail artery (Figure 1). However, the EC_{50} values of NA were not changed between the two groups (Table 1). It was noted that the EC_{50} value of NA in the mesenteric artery was much higher than that in the portal vein or tail artery. As shown in Figure 2, the α_1 -adrenoceptor antagonist, prazosin, was able to shift the dose-response curves to NA to the right. In contrast to the affinity of NA, the affinity of prazosin in the mesenteric artery was higher than that in the portal vein or tail artery (Table 2). However, the pA_2 values of prazosin in the three blood vessels were not different between the PVL and sham-operated rats.

Receptor binding assay

As shown in Figure 3a (the representative of five similar experiments), the binding of [125 I]-HEAT to the tail artery homogenate of sham-operated rats appeared to be saturable. Scatchard analysis of such binding data indicated that [125 I]-HEAT bound to a single population with an apparent K_D of

Figure 3 Binding of [125 I]-HEAT to tail artery homogenates of sham-operated rats. The data are shown as the average of duplicate determinations from one experiment. This figure is representative of five similar experiments with duplicate determinations. (a) Saturation binding; total bound (○), nonspecific bound (●), specific bound (▽); (b) Scatchard plot.

Figure 4 Binding of [125 I]-HEAT to mesenteric artery homogenate of portal hypertensive (PVL) rats. The data are shown as the average of duplicate determinations from one experiment. This figure is representative of three similar experiments with duplicate determinations. (a) Saturation binding; total bound (○), nonspecific bound (●), specific bound (▽); (b) Scatchard plot.

Table 3 The K_D and B_{max} values of [125 I]-HEAT on blood vessels of portal hypertensive (PVL) and sham-operated (Sham) rats

	n	Mesenteric artery		n	Tail artery	
		K_D (pM)	B_{max} (fmol mg ⁻¹ protein)		K_D (pM)	B_{max} (fmol mg ⁻¹ protein)
Sham	3	80 ± 4	20 ± 4	5	133 ± 35	82 ± 9**
PVL	3	78 ± 19	19 ± 4	5	139 ± 27	87 ± 17*

The data are shown as mean ± s.e.mean. * $P < 0.05$ and ** $P < 0.01$, significantly different between mesenteric artery and tail artery of Sham-operated or PVL rats.

Table 4 The K_i values for noradrenaline on blood vessels of portal hypertensive (PVL) and sham-operated (Sham) rats

	Mesenteric artery		Tail artery	
	n	K_i (μM)	n	K_i (μM)
Sham	3	17.9 ± 9.7	5	9.7 ± 1.0
PVL	3	7.0 ± 3.5	5	30.4 ± 9.3

The data are shown as mean ± s.e.mean.

Figure 5 Competition of noradrenaline with [125 I]-HEAT for specific binding to mesenteric artery or tail artery homogenates of sham-operated rats. The data are shown as the average of duplicate determinations from one experiment as (○) for mesenteric artery and (●) for tail artery. This figure is representative of 3–5 similar experiments with duplicate determinations. [125 I]-HEAT = 270 pM.

122 pM and density of binding sites (B_{max}) of 107 fmol mg⁻¹ protein (Figure 3b). Similarly, a single population of [125 I]-HEAT binding sites was observed in the mesenteric artery. Figure 4 (representative of three similar experiments) illustrates that the K_D and B_{max} values were 96 pM and 22 fmol mg⁻¹ protein, respectively, in the mesenteric artery homogenate of PVL rats. It appeared that the receptor number in the tail artery was about 4 fold greater than that in the mesenteric artery (Table 3). When sham-operated and PVL rats were compared, the K_D and B_{max} values for [125 I]-HEAT in either the mesenteric artery or tail artery were not significantly different. Figure 5 shows the competition of NA for [125 I]-HEAT binding to mesenteric and tail artery homogenates of sham-operated rats. The data were well fitted to a simple, one-site model but not multi-site models. The K_i for NA in either mesenteric artery or tail artery was not significantly different between sham-operated and PVL rats (Table 4).

Discussion

The present study is the first to demonstrate that the α_1 -adrenoceptor number and the affinity of NA are not altered in the vascular smooth muscle of PVL rats. Tension experiments *in vitro* demonstrated that the maximum response, but not the ED_{50} value of NA, was decreased in portal vein, mesenteric artery, tail artery in PVL rats. The results suggest a decrease in efficacy but not the sensitivity to NA. Receptor binding experiments showed that B_{max} and K_D for [125 I]-HEAT and K_i for NA were not significantly changed in either the mesenteric artery or tail artery of PVL rats, suggesting the changes in vascular reactivity were not due to changes in receptor density or affinity. Therefore, the present results suggest that the vascular hyporesponsiveness to NA in PVL rats may be due to a defect at the post-receptor level.

Villamediana *et al.* (1988) reported that there was no difference in response to NA in the femoral artery of cirrhotic rats compared with control *in vitro*, whereas Kiel *et al.* (1985) reported an increase in the ED_{50} value of NA in perfused intestinal vasculature of portal hypertensive rats. The differences may relate to the nature of the experimental preparations. *In vivo* experiments will be affected more profoundly by the circulating substances. The present study showed that the attenuated maximum response to NA was most prominent in the portal vein and mesenteric artery, indicating the splanchnic circulation was more affected by portal hypertension.

In tension experiments, the present study also showed that the affinity for NA (as indicated by ED_{50} value) was lower, but the affinity for prazosin (as indicated by the pA_2 value) was higher in the mesenteric artery than in either the tail artery or portal vein. The tension studies were complicated by differences in maximal responses to the same agonist between different blood vessels. However, De Mey & Vanhoutte (1981) reported that the ED_{50} values of NA differed significantly between canine isolated blood vessels: it was the lowest in the femoral artery, followed by the saphenous and femoral vein, and was highest in the splenic artery. They also speculated that the presence of mainly α_1 -adrenoceptors in arteries, and of both α_1 - and α_2 -adrenoceptors in veins, demonstrates the heterogeneous pharmacological behaviour of vascular smooth muscle. Since yohimbine was added to block the activation of α_2 -adrenoceptors by NA in our tension experiments, it is unlikely that α_2 -adrenoceptors are involved in the lower affinity for prazosin in the portal vein or tail artery. Medgett & Langer (1984) suggested that the wide range in prazosin affinity is a result of its ability to differentiate between two subtypes of α_1 -adrenoceptors. They proposed a directly innervated α_{1A} -adrenoceptor subtype with a high affinity for prazosin (the affinity constant $K_B < 0.4$ nM) and that the remaining α_1 -adrenoceptors are located farther away from the nerve terminals and have a lower sensitivity to prazosin ($K_B > 1.6$ nM). The higher affinity for prazosin in the densely innervated mesenteric artery in the present study seems consistent with this proposal. Molecular cloning studies have identified at least three distinct subtypes (α_{1A} , α_{1B} and α_{1C}) of α_1 -adrenoceptors; however, the K_i values for prazosin for these three α_1 -adrenoceptor subtypes are similar (Schwinn & Lomasney,

1992). Therefore, the nature of the different potency of prazosin against the α_1 -adrenoceptor response in isolated blood vessels remains to be determined.

MacGilchrist *et al.* (1990) reported that the binding characteristics of α -adrenoceptors in platelet and lymphocyte were not different between cirrhotic patients and healthy subjects. By comparing the pressor reactivity to sympathetic and non-sympathetic agonists, they further concluded that the impaired responses were not caused by sympathetic desensitization. In addition, Murray & Paller (1985) found that the decreased pressor response to angiotension II in cirrhotic rats was the result of a post-receptor defect. The present study also provides evidence to suggest that the vascular hyporesponsiveness to NA may be due to a post-receptor mechanism. Our previous paper (Chao *et al.*, 1992) demonstrated that vascular hyporesponsiveness also occurred in response to potassium chloride, a depolarizing agent that induces vasoconstriction by receptor-independent mechanisms. In studying the actions of synthetic parathyroid hormone, this paper (Chao *et al.*, 1992) showed that the utilization of calcium may be involved

in the vascular hyporesponsiveness of PVL rats. At the present time, it is not known whether there are changes in the contractile apparatus in vascular smooth muscle cells of PVL rats.

Recently, evidence has accumulated that the vascular endothelium plays an important role in regulation of vascular tone (Furchgott, 1983). Vallance & Moncada (1991) proposed that the overproduction of nitric oxide may contribute to the hyperdynamic circulation in cirrhosis. By using nitric oxide synthase inhibitor Claria *et al.* (1992), Lee *et al.* (1992) and Pizcueta *et al.* (1992) have confirmed this hypothesis. Furthermore, the preliminary data from our laboratory also demonstrated an elevation in both cyclic AMP and cyclic GMP contents in blood vessels of PVL rats. It is also possible that overproduction of nitric oxide functionally antagonizes the constrictor response to vasoconstrictors in portal hypertension. However, this explanation is not suitable for the elevation of cyclic AMP, since the *in vitro* experiment is devoid of circulating vasodilators. The role of cyclic nucleotides is currently under investigation.

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