Adrenergic Response of Splanchnic Arteries from Cirrhotic Patients: Role of Nitric Oxide, Prostanoids, and Reactive Oxygen Species

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Peripheral and splanchnic vasodilatation in cirrhotic patients has been related to hyporesponsiveness to vasoconstrictors, but studies to examine the vascular adrenergic response provide contradictory results. Hepatic arteries from cirrhotic patients undergoing liver transplantation and mesenteric arteries from liver donors were obtained. Segments 3 mm long from these arteries were mounted in organ baths for testing isometric adrenergic response. The concentration-dependent contraction to noradrenaline (10^{-8} to 10^{-4} *M*) was similar in hepatic and mesenteric arteries, and prazosin (α1-adrenergic antagonist, 10^{-6} *M*), but not yohimbine (α2-adrenergic antagonist, 10^{-6} *M*), produced a rightward parallel displacement of this contraction in both types of arteries. Phenylephrine (α1-adrenergic agonist, 10^{-8} to 10^{-4} *M*) and clonidine (α2-adrenergic agonist, 10^{-8} to 10^{-4} *M*) also produced concentration-dependent contractions that were comparable in hepatic and mesenteric arteries. The inhibitor of cyclooxygenase meclofenamate (10^{-5} *M*), but not the inhibitor of nitric oxide synthesis N^{w}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} *M*), potentiated the response to noradrenaline in hepatic arteries; neither inhibitor affected the response to noradrenaline in mesenteric arteries. Diphenyleneiodonium (DPI; 5×10^{-6} *M*), but neither catalase (1000 U/ml) nor tiron (10^{-4} *M*), decreased the maximal contraction for noradrenaline similarly in hepatic and mesenteric arteries. Therefore, it is suggested that, in splanchnic arteries from cirrhotic patients, the adrenergic response and the relative contribution of α1- and α2-adrenoceptors in this response is preserved, and prostanoids, but not nitric oxide, may blunt that response. Products dependent on NAD(P)H oxidase might contribute to the adrenergic response in splanchnic arteries from control and cirrhotic patients. Exp Biol Med 232:1360–1367, 2007

Key words: hepatic arteries; vasoconstriction; α1-adrenoceptors; α2-adrenoceptors; prostacyclin

Introduction

A remarkable hemodynamic feature in the advanced stage of liver cirrhosis, either human or experimental animals, is the abnormal peripheral and splanchnic vasodilatation, and that vasodilatation has been related to an excessive production of endogenous vasodilator substances or a decreased responsiveness to vasoconstrictors (1, 2). Using experimental models, most of the studies show that cirrhosis induces *in vivo* hyporesponsiveness to several types of vasoconstrictors, and this vascular alteration is also present *ex vivo* (2). The relevance of these studies to the condition in humans, however, is not clear. Because *in vivo* human studies of vascular reactivity can be difficult to interpret (2), isolated blood vessel techniques have been used. With regard to adrenergic reactivity, there are data showing that the response of hepatic arteries from cirrhotic patients to α1-adrenoceptor stimulation is decreased (3, 4). Other studies, however, report that hepatic arteries (5) or mesenteric arteries (6) from cirrhotic patients exhibit unaltered response to α-adrenoceptor and to α1-adrenoceptor stimulation. As far as we know, only one study has been made to examine the response to both selective α1 and α2 adrenergic stimulation, and that study was performed *in vivo*, evaluating blood pressor response (7). Therefore, the relative contribution of α1- and α2-adrenoceptors in the

This work was supported, in part, by Fundación Médica Mútua Madrileña Automovilista and Ministerio de Euducación y Ciencia (BFU2004-04054).

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Received April 27, 2007.
Accepted June 18, 2007.

DOI: 10.3181/0701-RM-112
1535-3702/07/23210-1360$15.00

adrenergic response of splanchnic arteries from cirrhotics is unknown.

The fact that the contraction to KCl was unaltered in arteries that were hyporesponsive to α1-adrenoceptor stimulation (8) has suggested that structural changes are not the cause of functional abnormalities, and that the abnormality probably lies upstream in the signal transduction pathway (9). Smith *et al*. (4) suggest that the decreased response of the hepatic artery to adrenergic stimulation was related to increased production of nitric oxide *via* inducible nitric oxide synthase in the smooth muscle, but these observations have been not supported by others (8). Also, it has been reported that indomethacin, but not N^w-nitro-L-arginine methyl ester (L-NAME), increased the responsiveness of omental arteries from patients with fulminant hepatic failure to noradrenaline, suggesting that in this entity, cyclooxygenase-2 (COX-2) vasodilating by-products are of importance in blunting the effects of augmented vasoconstrictor activity (10). On the other hand, increased formation of reactive oxygen species may occur during cirrhosis (11, 12), and a role for these species in vascular reactivity after cirrhosis has been proposed (13, 14). The role of reactive oxygen species in vasoconstrictor responsiveness during cirrhosis, however, has not, to our knowledge, been examined. Therefore, studies investigating blood vessels from cirrhotic patients may be insufficient, and further studies about vascular adrenergic response are needed. The study of adrenergic reactivity deserves attention because adrenergic mechanisms play a main role in regulating the vascular system, and increased sympathetic activity is present in cirrhotic patients (15).

The present study was performed to examine the adrenergic response of splanchnic arteries from cirrhotic patients, paying attention to the analysis of the relative contribution of α1- and α2-adrenoceptors, as well as the role of nitric oxide, prostanoids, and reactive oxygen species in this response. This study was performed using isolated hepatic arteries from cirrhotic patients undergoing liver transplantation; hepatic artery is from a vascular territory (splanchnic) that is known to be dilated in cirrhosis (16). The results obtained in these hepatic arteries were compared with those obtained in mesenteric arteries from the corresponding liver donors. Mesenteric arteries were used for comparison because of difficulties encountered in obtaining donor hepatic arteries to act as controls. Nevertheless, it has been reported that human normal hepatic and mesenteric arteries exhibit comparable responses to noradrenaline, phenylephrine, and KCl, and mesenteric arteries are considered to be suitable for use as control vessels in investigations of hepatic arteries (17).

Materials and Methods

Patients. Twenty-one patients with cirrhosis undergoing liver transplantation were included in this study (mean age: 54 years [range: 43–68], 14 males, 7 females). Etiology of cirrhosis was hepatitis virus C or B in eight patients, alcohol-related in seven patients, both alcohol-related and virus C in three patients, and cryptogenic in three patients. Four patients were Child-Pugh Grade A, 10 patients Grade B, and 7 patients grade C. The diagnosis of cirrhosis was made by liver biopsy in all patients. All the patients exhibited systemic hypotension, and had severe portal hypertension as evidenced by the presence of esophageal varices, demonstrated by endoscopy. Two patients had previously bled from esophageal varices, and seven had ascites. Five cirrhotic patients had a history of hypertension and two of diabetes mellitus type II; 11 were smokers (10–40 cigarettes/day). At the time of liver transplantation, three patients had hypercholesterolemia (237–256 mg/dl) and received treatment with propranolol (two patients), noradrenaline (one patient), dobutamine (one patient), and noradrenaline plus dobutamine (one patient). As liver donors, 16 patients were included (mean age: 51 years [range: 26–82], 8 males, 8 females). Of these donors, nine had died as consequence of stroke and seven as consequence of a cranioencephalic traumatism. Six of these donors had history of hypertension and one of diabetes mellitus type II; eight had hypercholesterolemia (202–247 mg/dl), and six were smokers (15–20 cigarettes/day).

Experimental Protocol. In the present study, the right, intermediate, and left branches of the proper hepatic artery in the hilum were taken from the liver removed from cirrhotic patients during transplantation. Also, arteries from branches of the superior mesenteric artery were obtained from the material that had been previously removed from the liver donors. After their removal, the arteries were kept in cold CELSIOR medium (IMTX SANGSTAT, Lyon, France) until they were transported to the laboratory. Then, the CELSIOR medium was replaced by cold physiological saline, where the arteries were dissected free from connective tissue and cut into segments 3 mm in length (the external diameter of these hepatic and mesenteric arterial segments was 3–4 mm). Then, these arterial segments were mounted in 4-ml organ baths for isometric tension recording. The organ baths contained modified Krebs-Henseleit solution with the following composition (m*M*): NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11. The solution was maintained at 37°C and equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Briefly, the method consists of passing two fine stainless steel pins, 150 μm in diameter, through the lumen of the vascular segment. One pin is fixed to the organ bath wall, whereas the other is connected to a strain gauge for isometric tension recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular ring. The recording system included a Universal Transducing Cell UC3 (Statham Instruments, Inc., Oxnard, CA) and a Statham Microscale Accessory UL5 (Statham Instruments). Changes in isometric force were recorded on a Macintosh computer by use of Chart v3.6/s software and a MacLab/8e

data acquisition system (ADInstruments, Colorado Springs, CO). A previously determined optimal passive tension of 2 g was applied to the arterial segments, and they were allowed to equilibrate for 90–120 mins.

First, the ability of each vascular preparation to contract was tested with KCl (100 m*M*), and the solution was renewed by repeated washouts. Then, the arteries were tested with noradrenaline (10^{-8} to 10^{-4} *M*), phenylephrine (α1-adrenergic agonist, 10^{-8} to 10^{-4} *M*), and clonidine (α2-adrenergic agonist, 10^{-8} to 10^{-4} *M*). The response to noradrenaline was examined in the absence and in the presence of prazosin (α1-adrenergic antagonist, 10^{-6} *M*), yohimbine (α2-adrenergic antagonist, 10^{-6} *M*), the inhibitor of nitric oxide synthesis L-NAME (10^{-4} *M*), the inhibitor of cyclooxygenase meclofenamate (10^{-5} *M*), the scavenger of hydrogen peroxide catalase (1000 U/ml), the superoxide dismutase mimetic tiron (10^{-2} *M*), or the inhibitor of NAD(P)H oxidase diphenyleneiodonium (DPI, $5x10^{-6}$ *M*).

In each artery, one concentration-response curve for each agonist, in the absence or in the presence of the treatments used, was determined. Prazosin, yohimbine, L-NAME, meclofenamate, tiron, or DPI was applied to the organ bath for 30–40 mins, and catalase was applied to the organ bath for 60 mins, before the response to noradrenaline was tested.

The contractions to the adrenergic agonists are expressed as a percentage of the response induced by KCl (100 m*M*). With these data, graphics showing concentration-response curves were constructed, and the pD_2 (vascular sensitivity) for each concentration-response curve was calculated as the negative logarithm of the concentration producing 50% of the maximal response by geometric interpolation.

The present human study was approved by the local Ethical Committee of Clinical Research (Hospital Universitario "Puerta de Hierro," Madrid, Spain).

The substances used were DL-norepinephrine hydrochloride, R-(–)-phenylephrine hydrochloride, clonidine hydrochloride, prazosin hydrochloride, yohimbine hydrochloride, L-NAME, meclofenamate (2[1,6-dicloro-3-methylphenyl-amino]benzoic acid sodium salt), catalase, tiron, and DPI, all obtained from Sigma (St. Louis, MO). All drugs were dissolved in distilled water and further diluted in physiological saline with 0.01% ascorbic acid immediately before beginning the experiments.

The data are expressed as mean ± SEM. Statistical comparisons of the contraction to KCl in absolute values, as well as the contractions (percentage of the response produced by KCl) and pD_2 values obtained with noradrenaline, phenylephrine, and clonidine in untreated hepatic and mesenteric arteries were made using unpaired Student's *t* test. The effects of the different treatments on the contractions (percentage of the contraction with KCl) to noradrenaline in each type of artery were evaluated by applying one-way, factorial analysis of variance (ANOVA) followed by a Dunnett test. Two-way, factorial ANOVA followed by the Bonferroni test was also applied to analyze whether the effects of the treatments used were different in hepatic and mesenteric arteries. In each case, $P < 0.05$ was considered statistically significant.

Results

The contractile response to KCl (100 m*M*) was comparable ($P > 0.05$) in hepatic arteries (4357 ± 327 mg for 60 segments) and mesenteric arteries (4443 ± 379 mg for 39 segments).

Noradrenaline (10^{-8} to 10^{-4} *M*) produced concentration-dependent arterial contractions, and the maximal response as well as the sensitivity (pD_2) was not significantly distinct in hepatic and mesenteric arteries (Fig. 1 and Table 1). Prazosin (10^{-6} *M*) produced a right parallel shift of the response to noradrenaline in both types of arteries, and this shift was not significantly different ($P > 0.05$) in hepatic arteries (12 times) and mesenteric arteries (28 times). Yohimbine (10^{-6} *M*) did not modify significantly the concentration-response curve for noradrenaline in both hepatic and mesenteric arteries (Fig. 1 and Table 1).

Phenylephrine (10^{-8} to 10^{-4} *M*) and clonidine (10^{-8} to 10^{-4} *M*) also caused concentration-dependent contraction, and the response for each agonist was similar in both types of arteries. The response to phenylephrine was comparable to that of noradrenaline, and it was much higher than that produced by clonidine (Fig. 2 and Table 1).

L-NAME (10^{-4} *M*) did not modify significantly the concentration-dependent contraction for noradrenaline in hepatic and mesenteric arteries (Fig. 3 and Table 1).

Meclofenamate (10^{-5} *M*) potentiated the contraction without changing significantly the sensitivity (pD_2) to noradrenaline in hepatic, but not in mesenteric, arteries (Fig. 3 and Table 1).

Catalase (1000 U/ml) or tiron (10^{-4} *M*) did not modify the concentration-response curve for noradrenaline in hepatic and mesenteric arteries (Fig. 4 and Table 1). DPI (5×10^{-6} *M*) decreased the maximal contraction without changing significantly the pD_2 for noradrenaline in a similar way in hepatic and mesenteric arteries (Fig. 4 and Table 1).

Table 1 summarizes the maximal contractions and pD_2 values obtained with noradrenaline, phenylephrine, and clonidine in hepatic and mesenteric arteries in the different conditions tested.

Discussion

One of the mechanisms proposed to be involved in the abnormal vasodilatation found in cirrhotic patients is the vascular hyporesponsiveness to vasoconstrictors, but data reported about this particular issue are controversial (2, 9). With regard to the response of human blood vessels to adrenergic stimulation, the data reported are contradictory because this response can be decreased (3, 4) or unaltered (5, 6). Human hepatic artery has been used to examine the responsiveness of blood vessels from cirrhotic patients, and

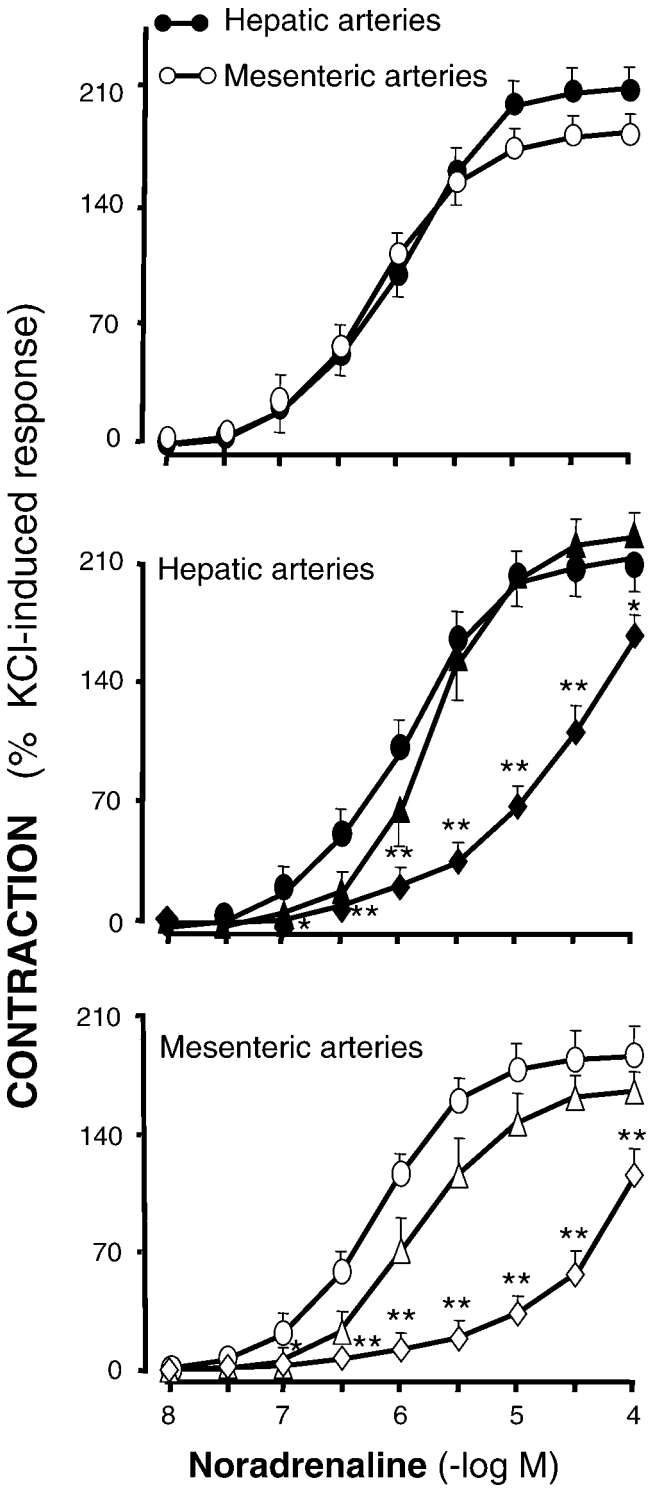

Figure 1. Contraction to noradrenaline in isolated hepatic arteries from cirrhotic patients and isolated mesenteric arteries from liver donors: (Top) Untreated arteries. (Middle) Hepatic arteries, (●) untreated, and treated with (♦) prazosin or (▲) yohimbine. (Bottom) Mesenteric arteries, (○) untreated and treated with (◊) prazosin or (△) yohimbine. Arterial segments used included untreated hepatic arteries (37 segments from 21 patients) and untreated mesenteric arteries (24 segments from 16 donors); arteries treated with prazosin (13 hepatic arteries from 6 patients and 11 mesenteric arteries from 6 donors); arteries treated with yohimbine (14 hepatic arteries from 6 patients and 7 mesenteric arteries from 5 donors). * $P < 0.05$; ** $P < 0.01$.

this artery is considered to be suitable because it is from a vascular territory (splanchnic), which is known to be dilated in cirrhosis (16). We have used mesenteric arteries from liver donors for comparison with hepatic arteries from cirrhotic patients because of difficulties encountered in obtaining donor hepatic arteries to act as controls. Human normal hepatic and mesenteric arteries exhibit a comparable response to noradrenaline, phenylephrine, and KCl (17). Therefore, the comparison between hepatic and mesenteric arteries made in our study may be acceptable. However, we should be cautious with this comparison because, although both types of arteries are splanchnic blood vessels, responsiveness of hepatic and mesenteric arteries might be different during cirrhosis.

Hepatic arteries were obtained immediately after removing the liver during transplantation, and mesenteric arteries were obtained at the moment of transplantation from the material that had been previously removed from the donors, and both types of arteries exhibited a similar, good ability to contract as tested with KCl. The present observations suggest that ability of smooth muscle to contract is comparable in both types of arteries and that this ability may not be affected by cirrhosis. This observation agrees with data previously reported by others (5, 6).

We found that the response to noradrenaline, phenylephrine, and clonidine, as well as the effects of prazosin and yohimbine, on the noradrenaline-induced response is similar in hepatic and mesenteric arteries. In both types of arteries, we observed that prazosin, but not yohimbine, inhibited the response to noradrenaline, that the response to phenylephrine was similar to that produced by noradrenaline, and that the response to clonidine was much lower than that of phenylephrine. This suggests that the relative contribution of α1- and α2-adrenoceptors in the response to noradrenaline is similar in hepatic and mesenteric arteries, and that in both types of arteries, this response is mainly mediated by activation of α1-adrenoceptors with low participation of the α2-adrenoceptors. Thus, the adrenergic reactivity, as well as the relative contribution of α1- and α2-adrenoceptors in this reactivity, in splanchnic blood vessels may be preserved during cirrhosis. With regard to the sensitivity to noradrenaline and phenylephrine, our data are comparable with those obtained in endothelium-denuded hepatic arteries by Hadoke *et al.* (5) and Heller *et al.* (8), although the maximal contraction found in our study was lower than that described by Hadoke *et al.* (5) and higher than that described by Heller *et al.* (8). The presence of both α1- and α2-adrenoceptors in normal human splanchnic arteries has been also reported (18, 19), but further studies are needed to determine the expression of α1- and α2-adrenoceptors in splanchnic arteries from cirrhotic patients. Our observations agree with studies suggesting that the sensitivity and concentration of α-adrenoceptors as well as postreceptorial mechanism involved in the adrenergic contraction in hepatic artery may not be altered after cirrhosis (5). Therefore, a hypothetical decreased adrenergic

Table 1. Maximal Contractions (% of KCl-Induced Response) and pD_2 for Noradrenaline, Phenylephrine and in Hepatic and Mesenteric Arteries, Untreated and Treated with Prazosin, Yohimbine, L-NAME, Meclofenamate, Catalase, Tiron, or DPI[a]

		Hepatic arteries		Mesenteric arteries	
		Contraction	pD_2	Contraction	pD_2
A)	Noradrenaline				
	Untreated	210 ± 15	5.98 ± 0.08	186 ± 9	6.15 ± 0.10
	Prazosin (10^{-6} *M*)	172 ± 12	4.88 ± 0.18**	123 ± 13**	4.69 ± 0.19**
	Yohimbine (10^{-6} *M*)	221 ± 19	5.73 ± 0.10	163 ± 11	5.77 ± 0.22
	L-NAME (10^{-4} *M*)	262 ± 32	6.20 ± 0.21	214 ± 17	5.98 ± 0.22
	Meclofenamate (10^{-5} *M*)	290 ± 29*	6.23 ± 0.21	152 ± 25***	6.21 ± 0.16
	Catalase (1000 U/ml)	174 ± 11	5.98 ± 0.12	173 ± 18	6.41 ± 0.17
	Tiron (10^{-2} *M*)	180 ± 15	5.97 ± 0.08	180 ± 19	6.29 ± 0.11
	DPI (5×10^{-6} *M*)	130 ± 17*	5.55 ± 0.19	106 ± 22**	5.98 ± 0.18
B)	Phenylephrine	234 ± 27	5.52 ± 0.22	172 ± 19	5.25 ± 0.06
C)	Clonidine	11 ± 3	5.91 ± 016	11 ± 1	6.46 ± 0.18

[a] Values are mean ± SEM.
* $P < 0.05$, ** $P < 0.01$, statistical difference between untreated and treated hepatic or mesenteric arteries; *** $P < 0.01$, statistical difference between hepatic and mesenteric arteries in the corresponding conditions.

reactivity in splanchnic vasculature might not underlie the abnormal vasodilatation found in patients with liver cirrhosis. Our observations are difficult to reconcile with studies reporting a decreased response of hepatic artery to adrenergic stimulation (4, 8). Methodological differences between these two studies (4, 8) and ours seem to be unlikely. In previous experiments using hepatic arteries from patients with cirrhosis from causes similar to those in the present study, we observed that the relaxation to acetylcholine, but not to sodium nitroprusside, was much lower in the hepatic arteries than in the mesenteric arteries from liver donors, suggesting that hepatic arteries had an endothelial dysfunction (20). This feature may not have affected the adrenergic response because this response was comparable in both types of arteries. These observations (20) and the present data with L-NAME (see below) suggest that the response of hepatic and mesenteric arteries to adrenergic stimulation may not be modulated by nitric oxide. Also, our data are comparable with those of Hadoke *et al*., who used hepatic arteries without endothelium (5). Therefore, the absence or the presence of the endothelium might not account for differences between our study and those of Smith *et al*. (4) and Heller *et al*. (8). The etiology of cirrhosis probably does not underlie this discrepancy because the studies of Smith *et al*. (4) and Heller *et al*. (8) were made using hepatic arteries from patients predominantly with viral cirrhosis, whereas in the case of Hadoke *et al*. (5), the majority of patients had primary biliary cirrhosis, and in our case the majority of patients had viral or alcohol-related cirrhosis. Discrepancies between studies might reside in the difference in genetic factors or in the previous history of patient, particularly that related to the presence or absence of cardiovascular risk factors. Results from studies using animal models, which sought to clarify the mechanisms underlying the decreased reactivity to adrenergic stimulation in cirrhosis, have been hampered by conflicting results (9).

With regard to the role of nitric oxide, we found that L-NAME did not affect the response to noradrenaline in either hepatic or mesenteric arteries, suggesting that nitric oxide does not modulate the adrenergic response in arteries from either control or cirrhotic patients. With respect to this issue, evidence for (21, 22) and against (10, 23, 24) the role of nitric oxide in modulating the adrenergic response of human normal splanchnic arteries has been described. Several studies suggest that production of nitric oxide is increased in the splanchnic and peripheral vasculature of cirrhotic patients (2, 9, 25), and this increased production of nitric oxide has been related to vasodilatation in splanchnic and peripheral vasculature present in cirrhotic patients on the basis that nitric oxide may cause this vasodilatation and/or may decrease vascular response to vasoconstrictors (2, 9). The role of nitric oxide in reducing the adrenergic vasoconstrictor response in this vasculature is, however, controversial because there are studies in favor (4, 21, 23, 25, 26) and against (3, 6, 8, 10, 27) an increased production of nitric oxide underlying the adrenergic hyporesponsiveness of human hepatic arteries during cirrhosis. Experiments performed in splanchnic arteries from cirrhotic patients, after removing (5, 8) or not removing (6, 10, 27) the endothelium, show that inhibition of nitric oxide synthesis did not change the adrenergic response. In agreement with these observations, the present results suggest that nitric oxide may not be involved in the adrenergic vasoconstrictor response of hepatic arteries during cirrhosis. That finding, however, does not exclude the possibility that the production of nitric oxide is increased in this entity because that increase might occur at the same time without the nitric oxide modulating the adrenergic response, as suggested by observations in normal arteries (10, 23, 24, present study).

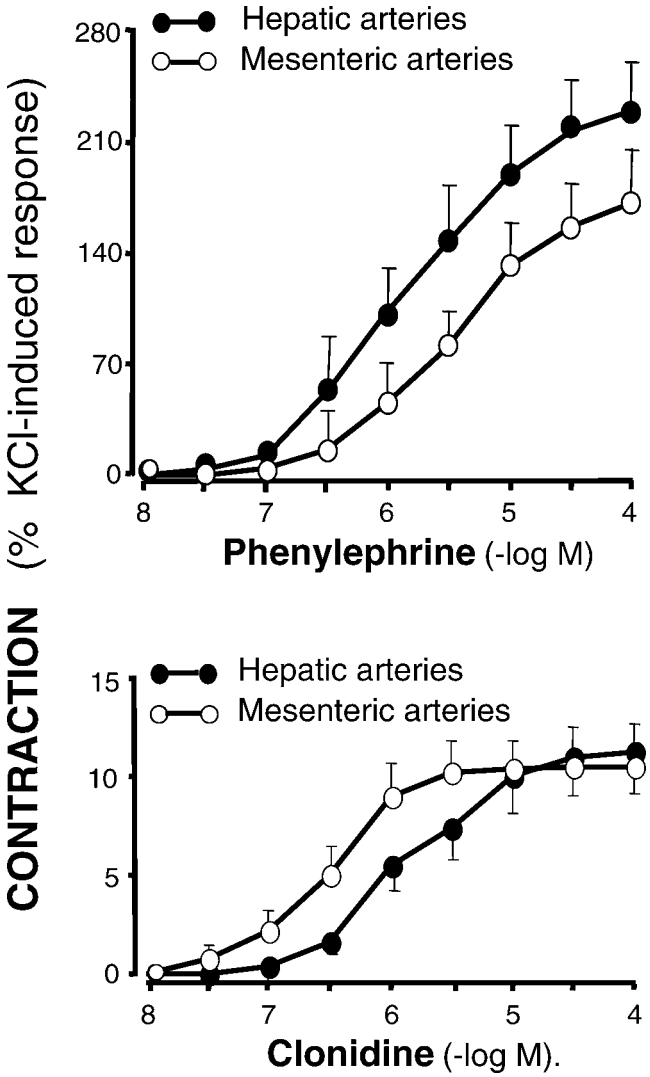

Figure 2. Contraction to (top) phenylephrine and (bottom) clonidine in isolated hepatic arteries from cirrhotic patients and isolated mesenteric arteries from liver donors. Arterial segments used for phenylephrine included 13 hepatic arteries from 6 patients and 9 mesenteric arteries from 4 donors; for clonidine, 10 hepatic arteries from 4 patients and 6 mesenteric arteries from 3 donors.

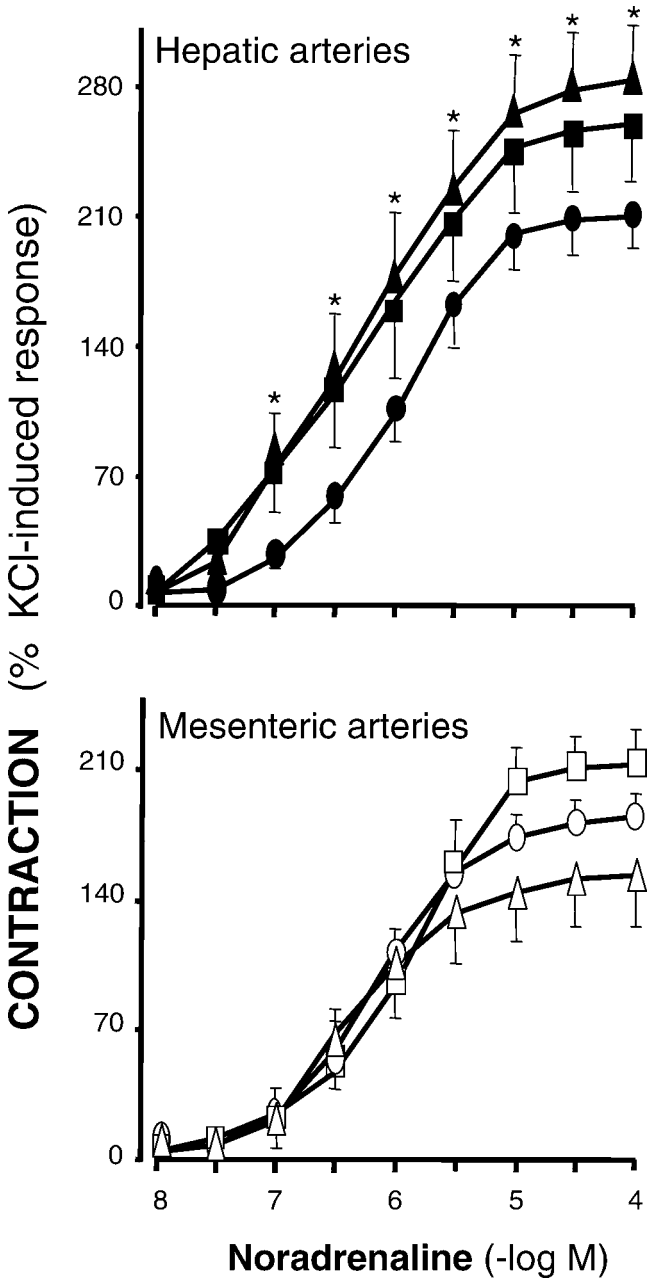

Figure 3. Contraction to noradrenaline in (top) isolated hepatic arteries from cirrhotic patients and (bottom) isolated mesenteric arteries from liver donors, untreated (●, ○) and treated with (■, □) L-NAME or (▲, Δ) meclofenamate. Arterial segments used included hepatic and mesenteric arteries untreated as in Figure 1; treated with L-NAME, 13 hepatic arteries from 7 patients and 7 mesenteric arteries from 4 donors; treated with meclofenamate, 13 hepatic arteries from 7 patients and 8 mesenteric arteries from 5 donors. * $P < 0.05$, statistical difference between hepatic arteries in the absence and in the presence of meclofenamate.

Therefore, nitric oxide might not affect the adrenergic response in splanchnic blood vessels during cirrhosis. Why there is discrepancy between studies showing that the inhibition of nitric oxide synthesis either modifies or does not modify the adrenergic response in splanchnic blood vessels from cirrhotic patients is not clear.

Studies on omental arteries from controls and from patients with cirrhogenous hepatitis describe the COX inhibitor indomethacin as not significantly modifying the contractile response to noradrenaline, but in arteries from patients with fulminant hepatic failure, indomethacin increased that contractile response (10). These authors suggest that mechanisms underlying that hyperreactivity are unknown, but based on similar observations in omental arteries from septic shock patients (28), they suggest that the COX-2 vasodilating by-products are of importance in blunting the effects of augmented vasoconstrictor activity only in fulminant hepatic failure patients (10). We observed that meclofenamate potentiated the response to noradrenaline in hepatic arteries, whereas it tended to decrease, although not significantly, that response in mesenteric

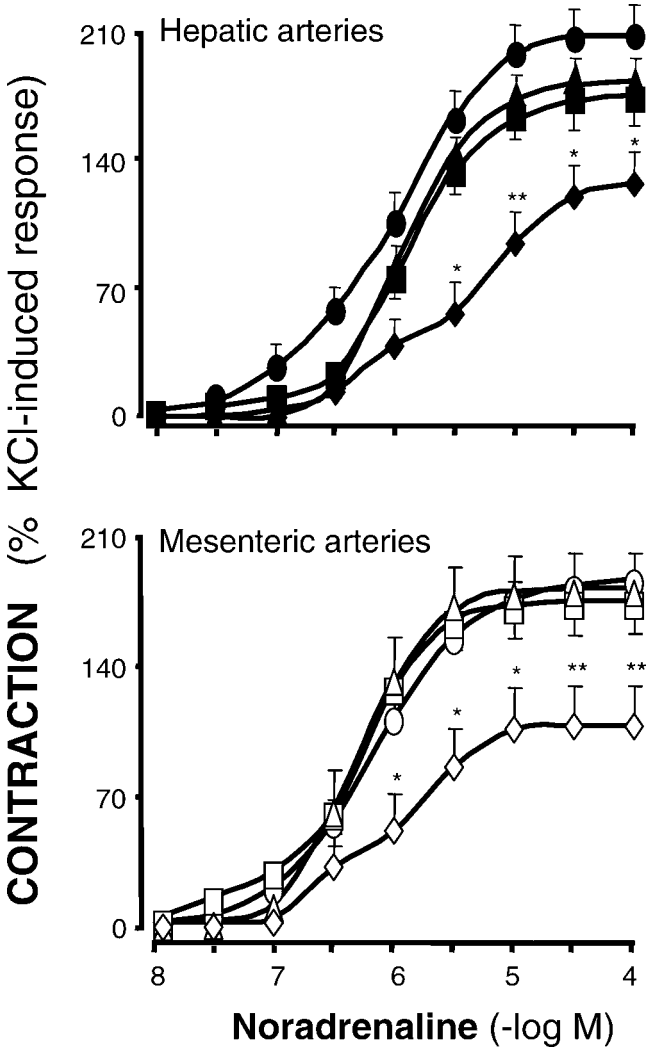

Figure 4. Contraction to noradrenaline in (top) isolated hepatic arteries from cirrhotic patients and (bottom) isolated mesenteric arteries from liver donors, (●, ○) untreated and (■, □) treated with catalase, (▲, Δ) tiron, or (♦, ◊) DPI. Arterial segments used included hepatic and mesenteric arteries untreated as in Figure 1; treated with catalase, 13 hepatic arteries from 7 patients and 10 mesenteric arteries from 5 donors; treated with tiron, 11 hepatic arteries from 6 patients and 9 mesenteric arteries from 5 donors; treated with DPI, 10 hepatic arteries from 5 patients and 8 mesenteric arteries from 4 donors. * $P < 0.05$ and ** $P < 0.01$, statistical difference between arteries in the absence and in the presence of DPI.

arteries. The hepatic arteries tested with meclofenamate were obtained from seven patients that had alcohol-related cirrhosis (three), cryptogenic cirrhosis (one), and cirrhosis related with virus C (two) or virus C plus alcohol (one). This suggests that, at least in some types of cirrhosis, the COX-2 vasodilating by-products might also be of importance in blunting the augmented vasoconstrictor effects after adrenergic activation in splanchnic arteries from cirrhotic patients. As meclofenamate did not modify significantly the response to noradrenaline in mesenteric arteries, we suggest that prostanoids may not be involved in the adrenergic effects in normal splanchnic arteries. From our data, we can hypothesize that production of COX-2 vasodilating by-products is increased in the walls of splanchnic arteries during cirrhosis, and these byproducts contribute to the abnormal vasodilatation found in cirrhotic patients.

Increased formation of reactive oxygen species (11, 12) may occur during cirrhosis, and a role for these species in the development of the hyperdynamic circulatory state has been proposed (13, 14). The role of these species on vasoconstrictor responsiveness during cirrhosis has not yet been examined to our knowledge. In our experiments, catalase (scavenger of hydrogen peroxide) and tiron (mimetic of superoxide dismutase) failed to modify the contractile effects of noradrenaline in both hepatic and mesenteric arteries. Only DPI (an inhibitor of NAD(P)H oxidase) changed (reduced) the response to noradrenaline in a similar way in hepatic and mesenteric arteries. As catalase and tiron produced no effect, we can propose that hydrogen peroxide might not be involved, and because DPI reduced the response to noradrenaline, we can also propose that products dependent on NAD(P)H oxidase might be involved in the adrenergic vasoconstriction of splanchnic arteries from both control and cirrhotic patients. It has been reported that radical superoxide rather than hydrogen peroxide may contribute to renal adrenergic vasoconstriction (29). The significance of our data with DPI in pathophysiology of vascular alterations during cirrhosis is at present uncertain. In a previous study from our laboratory, we found that catalase, tiron, and DPI were able to reverse the decreased acetylcholine-induced relaxation in hepatic arteries from cirrhotic patients, suggesting that reactive oxygen species may cause endothelial dysfunction with decrement of the modulatory role of nitric oxide and prostacyclin in the cholinergic vasodilatation of splanchnic vasculature during cirrhosis (20).

In conclusion, the present data suggest that, in splanchnic arteries from cirrhotic patients, the adrenergic response and the relative contribution of α1- and α2-adrenoceptors in this response are preserved and that prostacyclin, but not nitric oxide, may blunt that response. Products dependent on NAD(P)H might contribute to the adrenergic response in splanchnic arteries from control and cirrhotic patients.

We thank Esther Martínez and Hortensia Fernández-Lomana for technical assistance.

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