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Mesenteric vascular bed responsiveness in bile duct-ligated rats: roles of opioid and nitric oxide systems

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Abstract

Changes in vascular responsiveness are proposed as the basis for some of the cardiovascular complications in cholestasis. Cholestasis is also associated with accumulation of endogenous opioid peptides and evidence of overproduction of nitric oxide (NO). The possible role of NO or opioid system in cholestasis-induced mesenteric vascular bed responsiveness was investigated. Bile duct-ligated and sham-operated rats were treated for 6 days with either normal saline, naltrexone, an opioid antagonist (20 mg/kg/day) or L-NAME (*N*(ω)-nitro-L-arginine methyl ester), a nitric oxide synthase inhibitor (3 mg/kg/day). After 7 days, the superior mesenteric artery was cannulated and the mesenteric vascular bed was perfused according to the McGregor method. Baseline perfusion pressure of the mesenteric vascular bed was decreased in bile duct-ligated compared to sham-operated animals. ED_{50} of phenylephrine-induced vasoconstriction was increased, but vasoconstriction R_{max} was not different in the vascular bed of bile duct-ligated rats and of sham-operated ones. Acetylcholine-induced vasorelaxation was impaired in bile duct-ligated rats (increased ED_{50} and decreased vasorelaxation R_{max}). Sodium nitroprusside-induced vasorelaxation was not different between bile duct-ligated and sham-operated rats, implying that the smooth muscle components of vasorelaxation were intact. Chronic treatment with L-NAME partially restored both the acetylcholine-induced vasorelaxation and phenylephrine-induced vasoconstriction response in bile duct-ligated rats. Naltrexone treatment also partially restored the acetylcholine-induced vasorelaxation and phenylephrine-induced vasoconstriction in bile duct-ligated rats. There is impaired acetylcholine-induced vasorelaxation in cholestatic rats, probably due to a defect in endothelial function. This study also provided evidence for the involvement of increased opioidergic tone and NO overproduction in cholestasis-induced vascular hyporesponsiveness.

Keywords: Cholestasis; Mesenteric vascular bed; Nitric oxide (NO); Opioid; Endothelium dysfunction

1. Introduction

Cholestasis is associated with a tendency to develop hypotension and acute renal failure (Bomzon et al., 1996; Dooley, 1999). The exact etiology of these problems is elusive, but vascular hyporesponsiveness to sympathetic stimulation (Bomzon et al., 1985; Jacob et al., 1993) is thought to play an important part. A number of factors, such as bile salts (Pak and Lee, 1993), endotoxin (Inan et al., 1997) and increased prostaglandin production (Cioffi et al., 1986), are proposed for cholestasis-induced vascular hyporesponsiveness. Recently, we have reported evidence for nitric oxide (NO) overproduction in cholestasis (Nahavandi et al., 1999, 2000; Mani et al., in press). There is also evidence for the role of NO in the cholestasis-induced vascular hyporesponsiveness (Kimpel et al., 1998; Utkan et al., 1996, 2000).

Elevated plasma levels of endogenous opioid peptides, mainly methionine enkephalin, are described in cholestatic patients and rats (Thornton and Lowosky, 1988; Swain et al., 1992). There is now increasing evidence for a role of opioids in the pathophysiology and manifestations of cholestasis (Dehpour et al., 1999, 2000; Jones and Bergasa, 2000). With the aid of their widely distributed receptors, opioids modulate many functions of the cardiovascular system. Their effects on the cardiovascular system are mediated either centrally, such as a role in the pathogenesis of hypotension during blood loss (Schadt, 1989), or peripherally, inhibition of noradrenaline release and hence inhibition of neurogenic vasoconstriction via presynaptic receptors in the portal vein (Szabo et al., 1987), or a direct

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contractile effect in isolated rat aorta at high concentrations (Parra et al., 1995).

We now investigated the effect of bile duct ligation on the vasoconstriction and vasorelaxation responses of the mesenteric vascular bed. We further evaluated the possible roles of opioid and nitric oxide systems in the cholestasis-associated vascular hyporesponsiveness.

2. Materials and methods

2.1. Animals

The animals were handled in accordance with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH US publication 86-23 revised 1985). Seventy-four adult male Sprague-Dawley rats weighing 200-250 g were used. The rats were randomly divided into six groups; each group consisted of six to seven rats. Three groups were sham-operated and another three groups underwent bile duct ligation. Bile duct ligation was performed as described previously (Nahavandi et al., 1999). Laparotomy was performed under anesthesia (ketamine HCl (50 mg/kg) and promazine HCl (10 mg/kg), i.p.). In the sham-operated rats, the bile duct was identified, manipulated, and one untied loose tie was left in place. Because laparotomy distorts the intra-abdominal vasculature, the loose tie was left to mimic this effect of the bile duct ligation procedure. In bile duct-ligated rats, the bile duct was doubly ligated. Then, the abdominal wall was closed in two layers. Finally, all groups were injected i.p. with 5 ml sterile isotonic saline solution.

One group of sham-operated and bile duct-ligated rats served as control and were treated with daily subcutaneous administration of isotonic sterile saline solution (normal saline 1 ml/kg/day, s.c.). A second group of sham-operated and bile duct-ligated rats received L-NAME (*N*(ω)-nitro-L-arginine methyl ester) (3 mg/kg/day, s.c.). The last group of sham-operated and bile duct-ligated received naltrexone (20 mg/kg/day, s.c.) with a half-life of 10 h (Way et al., 1998). These rats received six doses of the drugs mentioned for 6 consecutive days. The first dose was injected the day after surgery, and the last dose was injected 24 h before killing, so little drug or drug action was expected to exist in tissues at the time of the experiment.

2.2. Bilirubin measurement

A sample (3–4 ml) of blood was collected at the time of killing, and bilirubin was determined with a commercially available kit (ZistShimi, Tehran, Iran).

2.3. Preparation of mesenteric vascular bed

After 7 days, the rats were anesthetized with ether, and the mesenteric vascular bed was prepared as originally described by McGregor (1965). The abdominal wall was opened and the superior mesenteric artery was identified and cannulated and gently flushed with modified Krebs–Henseleit solution (containing (mM) NaCl: 118, KCl: 4.7, $CaCl_2$: 2.5, $MgSO_4$: 1.2, dextrose: 11, $NaHCO_3$: 25, NaH_2PO_4: 1.2), bubbled with a mixture of 95% O_2 and 5% CO_2 (final pH: 7.4), and warmed to 37 °C before entering the pump. After 5 min of perfusion with 2 ml/min, the mesentery was separated from intestine by cutting close to the intestinal border of the mesentery. Only the main arterial branches from the superior mesenteric artery running to terminal ileum were perfused. Then, the rate of perfusion was increased to 5 ml/min. The tissue was prevented from drying by hyperperfusion with 0.5 ml/min solution and was warmed by placing on a constant temperature (37 °C) bath. A peristaltic pump (Pump speed control Model 500-1200, Harvard Apparatus, Dover, MA, USA) provided the constant flow. The perfusion pressure was measured using a pressure transducer (Pressure Transducer Model P-1000-A, Narco Biosystem, Houston, TX, USA), which was placed in the circuit between the outlet of the pump and the preparation and was recorded on a Narco Physiograph (Desk Model DMP-4B, Narco Biosystem). After 30-min equilibration, each tissue was used for either vasoconstriction or vasorelaxation response as will be described later.

2.4. Vasoconstriction experiment

For measuring the vasoconstriction response of the mesenteric vascular bed, phenylephrine, an α_1-adrenoceptor agonist, was injected (in doses of 1 nmol to 1 μmol with a 10- to 15-min interval between injections) in the perfusate before it entered the tissue. The injection volume was 0.1 ml, and injection time was 10 s. The vasoconstriction, being recorded as an increase in perfusion pressure, was expressed as mmHg increase in perfusion pressure.

2.5. Vasorelaxation experiment

After 30-min equilibration, the vascular bed was constricted with Krebs–Henseleit solution containing phenylephrine (0.5 μM for sham-operated and 1 μM for other groups) to induce submaximal vasoconstriction (about 90% of vasoconstriction R_{max} of the respective groups). Then, it was left to reach a plateau and to stabilize for 45 min. Then, acetylcholine was injected (in 0.1 ml, in 10 s with a 10- to 15-min interval between injections) in doses (1 pmol to 1 μmol) causing a dose-dependent vasorelaxation, recorded as a decrease in perfusion pressure. The responses were interpreted as percent vasorelaxation of the phenylephrine-induced preconstriction. The integrity of endothelium was confirmed by the presence of maximal vasorelaxation in response to 1 μmol acetylcholine.

In order to evaluate components of acetylcholine-induced vasorelaxation, endothelium and vascular smooth muscle, the response of the vascular bed to sodium nitro-

prusside, an endothelium-independent vasorelaxant, injected in graded doses (0.1 nmol to 10 μmol) was investigated. The responses were expressed as percent of the phenylephrine-induced preconstriction.

2.6. Drugs

The following drugs were used: phenylephrine hydrochloride, sodium nitroprusside, *N*(ω)-nitro-L-arginine methyl ester (L-NAME), naltrexone HCl and acetylcholine chloride (Sigma, St. Louis, MO, USA). Naltrexone HCl and L-NAME were dissolved in deionized distilled water. Phenylephrine was dissolved in the perfusion medium, Krebs–Henseleit solution. Sodium nitroprusside and acetylcholine were dissolved in the perfusion medium, Krebs–Henseleit containing phenylephrine. All drugs were freshly prepared on the day of the experiment.

2.7. Statistical analysis

The data are expressed as means ± S.E.M. The maximum response (R_{max}) was defined as the maximum response when the response reached its plateau (no further change in response when the dose of mentioned drug is increased or the dose cannot be raised because of unwanted effects of the drug). Half-maximal effective dose (ED_{50}) is the dose of drug that causes the 50% of the maximum response, and is calculated by intrapolation. For statistical analysis, a two-way analysis of variance was used to evaluate the interaction of treatment and bile duct ligation, followed by one-way analysis of variance with Tukey's HSD as post-hoc test to compare the means. $P < 0.05$ was considered significant.

3. Results

3.1. Induction of cholestasis

Two days after bile duct ligation, the animals showed signs of cholestasis (jaundice, dark urine and steatorrhea), which persisted after 2 days. These signs were confirmed biochemically by a significant rise in the level of the serum total bilirubin (Table 1) on the seventh day in bile

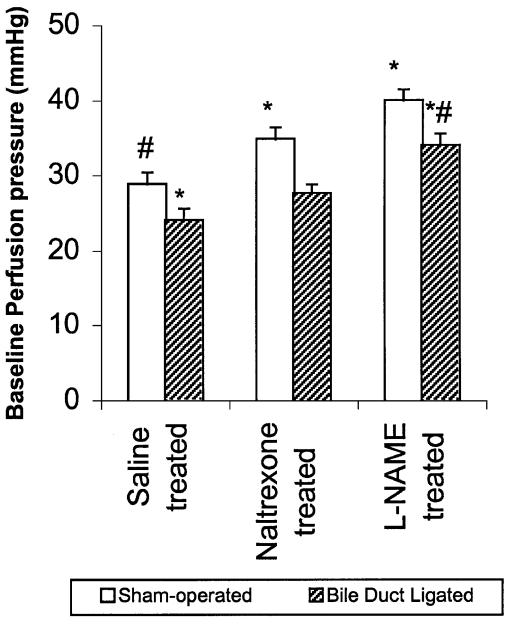

Fig. 1. The baseline perfusion pressure (mm Hg) of mesenteric vascular bed of sham-operated and bile duct-ligated rats, treated with normal saline, naltrexone (20 mg/kg/day), or L-NAME (3 mg/kg/day). Each group consisted of 11–12 rats (*: $P < 0.01$ compared to sham-operated saline-treated group; #: $P < 0.01$ compared to bile duct-ligated saline-treated group).

Table 1
Total bilirubin concentration (in μM) in serum of sham-operated and bile duct-ligated rats (7 days after surgery), treated with saline, naltrexone, or L-NAME.
There was a significant rise in total bilirubin level of bile duct-ligated groups compared with that of the respective sham-operated groups ($P < 0.01$). Treatment with naltrexone or L-NAME did not change the level of serum total bilirubin in either sham-operated or bile duct-ligated rats. Values are expressed as means ± S.E.M ($n = 11$ to 12).

	Normal saline-treated groups (μM)	Naltrexone-treated groups (μM)	L-NAME-treated groups (μM)
Sham-operated	5.1 ± 0.5	6.1 ± 0.7	5.9 ± 0.7
Bile duct-ligated	94.0 ± 12.9[a]	85.5 ± 12.1[a]	85.2 ± 13.6[a]

[a] $P < 0.01$ compared with respective sham-operated group.

Table 2
Half-maximal effective dose (ED_{50}) for the vasorelaxation and vasoconstriction responses of the mesenteric vascular bed of sham-operated and bile duct-ligated rats, treated with normal saline, naltrexone, or L-NAME. Values are expressed as means ± S.E.M ($n = 6$ to 7 in each group).

		Phenylephrine-induced vasoconstriction ED_{50} (nmol)	Acetylcholine-induced vasorelaxation ED_{50} (nmol)
Sham-operated rats	Normal saline treatment	46.37 ± 3.8[a]	0.114 ± 0.0091[a]
	Naltrexone treatment	77.12 ± 8.2	0.949 ± 0.13[b]
	L-NAME treatment	85.12 ± 6.2[b]	26.94 ± 5.17[b]
Bile duct-ligated rats	Normal saline treatment	143.2 ± 3.5[b]	24.17 ± 0.95[b]
	Naltrexone treatment	124.9 ± 10.3	6.05 ± 1.42[a]
	L-NAME treatment	176.6 ± 8.0[c]	2.26 ± 0.28[a]

[a] $P < 0.01$ compared to normal saline-treated (control) bile duct-ligated group.
[b] $P < 0.01$ compared to normal saline-treated (control) sham-operated group.
[c] $P < 0.05$ compared to normal saline-treated (control) bile duct-ligated group.

duct-ligated rats compared with sham-operated ones ($P < 0.01$). None of the rats showed ascites at the time of the experiment.

3.2. Baseline perfusion pressure

Perfusion of the mesenteric vascular bed resulted in a baseline perfusion pressure in the range of 19–44 mmHg. Bile duct ligation and treatment has no significant statistical interaction on baseline perfusion pressure ($F = 0.684$; $df = 2$; $P = 0.509$). The baseline perfusion pressure of bile duct-ligated groups was significantly lower than that of the respective sham-operated groups ($P < 0.01$ for all of the three groups). In sham-operated rats, treatment with either naltrexone or L-NAME significantly increased the baseline perfusion pressure ($P < 0.01$ compared with sham-operated controls). The baseline perfusion pressure was also significantly higher in bile duct-ligated rats, treated with either naltrexone or L-NAME, compared to their respective sham-operated groups ($P < 0.01$). The baseline perfusion pressure of sham-operated controls was not different from that of bile duct-ligated rats treated with naltrexone ($P = 0.89$). The baseline perfusion pressure in bile duct-ligated rats treated with L-NAME was significantly higher than that of sham-operated controls ($P < 0.01$) (Fig. 1).

3.3. Phenylephrine-induced vasoconstriction

Phenylephrine, an α_1–adrenoceptor agonist (1 nmol to 3 μmol), induced dose-dependent vasoconstriction, manifested as an increase in the perfusion pressure (Table 2). Maximum vasoconstriction was achieved at 1 μmol. No further significant increase in perfusion was observed with higher doses (Fig. 2).

Seven days after surgery, the ED_{50} of the bile duct-ligated control group was significantly higher than the ED_{50} of sham-operated controls ($P < 0.01$). The R_{max} did not differ between the sham-operated and bile duct-ligated controls ($P = 0.49$) (Fig. 2A).

The R_{max} was higher in sham-operated rats treated with naltrexone (20 mg/kg/day for 6 days) than in sham-operated controls ($P < 0.01$), while their ED_{50} was not significantly different from that of the sham-operated controls ($P = 0.06$). There was a significant increase in R_{max} of the naltrexone-treated bile duct-ligated group compared to that

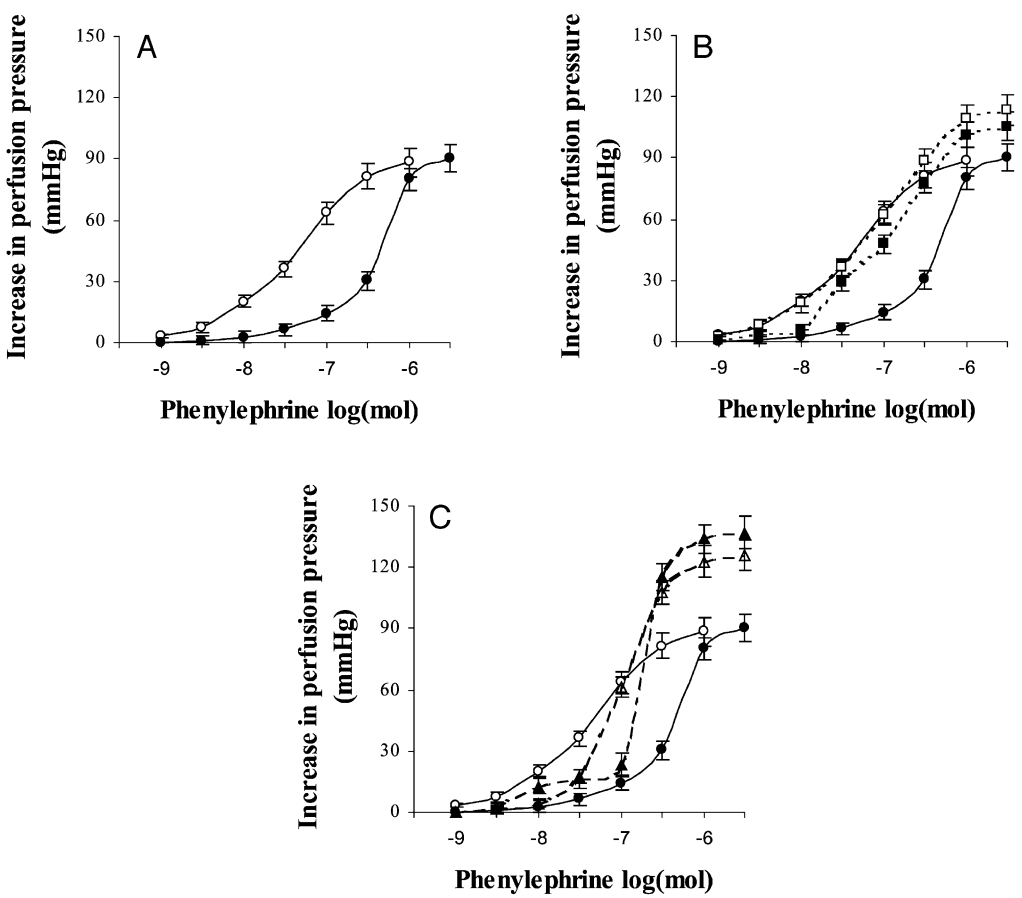

Fig. 2. The vasoconstriction response curve to phenylephrine in mesenteric vascular bed of (A) sham-operated and bile duct-ligated rats treated with saline; (B) sham-operated and bile duct-ligated rats treated with either saline or naltrexone; (C) sham-operated and bile duct-ligated rats treated with either saline or L-NAME. Each group consisted of six to seven rats (○ sham-operated rats treated with saline; ● bile duct-ligated rats treated with saline; □ sham-operated rats treated with naltrexone (20 mg/kg/day); ■ bile duct-ligated rats treated with naltrexone; △ sham-operated rats treated with L-NAME (3 mg/kg/day); ▲ bile duct-ligated rats treated with L-NAME).

of bile duct-ligated controls ($P < 0.05$), but there was no difference between the ED_{50} of these groups ($P = 0.51$) (Fig. 2B). Thus, it can be concluded that naltrexone treatment has little effect on the phenylephrine-induced vasoconstriction in the mesenteric vascular bed.

Among sham-operated rats, the L-NAME-treated group, compared with the saline-treated group, showed a significant increase in both ED_{50} ($P < 0.01$) and R_{max} ($P < 0.05$). Similarly, among bile duct-ligated groups, L-NAME-treated group showed a significant increase in both ED_{50} ($P < 0.05$) and R_{max} ($P < 0.01$), compared to those of the saline-treated group (Fig. 2C). This effect of L-NAME treatment to attenuate phenylephrine-induced vasoconstriction in bile duct-ligated rats was mainly observed at higher doses of phenylephrine (greater than 0.3 μmol).

3.4. Acetylcholine-induced vasorelaxation

Phenylephrine (0.5 μM for sham-operated controls and 1 μM for other groups) caused submaximal vasoconstriction in the mesenteric vascular bed. Submaximal vasoconstriction is defined as a vasoconstriction of about 90% of the vasoconstriction R_{max} of the respective groups. After the perfusion pressure reached a plateau, bolus infusion of acetylcholine (1 pmol to 1 μmol) produced dose-dependent vasorelaxation, which was manifested by a sharp drop and slow recovery of perfusion pressure. Higher doses of acetylcholine (greater than 1 μmol) caused less vasorelaxation through their contractile effect via the acetylcholine receptors of the vascular smooth muscle (Table 2 and Fig. 3).

There was a significant decrease in R_{max} ($P < 0.01$), and a significant increase in ED_{50} ($P < 0.01$) in the mesenteric vascular bed of the bile duct-ligated control group compared with sham-operated controls (Fig. 3A).

In sham-operated rats, the naltrexone-treated group had a significant increase in ED_{50} ($P < 0.01$) and a significant decrease in R_{max} ($P < 0.01$) compared to sham-operated controls. Naltrexone-treated bile duct-ligated rats had a significantly lower ED_{50} ($P < 0.01$), but a significantly higher R_{max} than did bile duct-ligated controls (Fig. 3B).

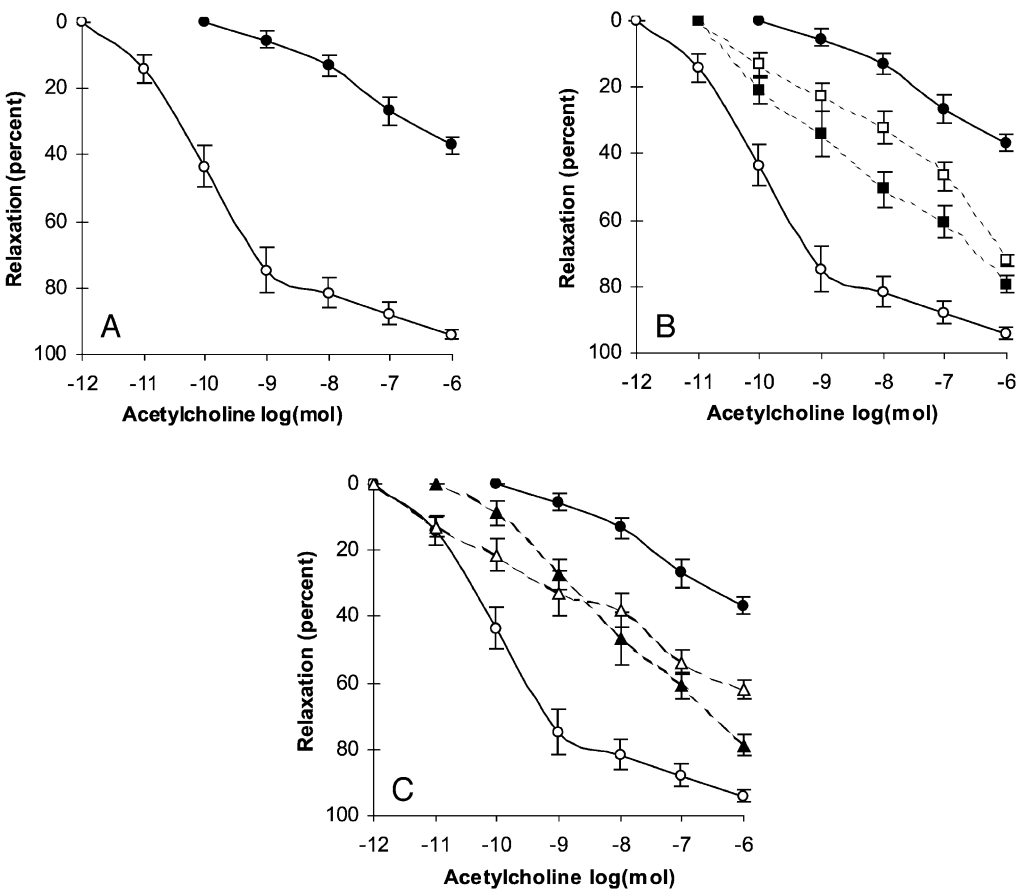

Fig. 3. Endothelium-dependent vasorelaxation response curve to acetylcholine in mesenteric vascular bed, preconstricted with phenylephrine, of (A) sham-operated and bile duct-ligated rats treated with saline; (B) sham-operated and bile duct-ligated rats treated with either saline or naltrexone; (C) sham-operated and bile duct-ligated rats treated with either saline or L-NAME. Each group consisted of six to seven rats (○ sham-operated rats treated with saline; ● bile duct-ligated rats treated with saline; □ sham-operated rats treated with naltrexone (20 mg/kg/day); ■ bile duct-ligated rats treated with naltrexone; △ sham-operated rats treated with L-NAME (3 mg/kg/day); ▲ bile duct-ligated rats treated with L-NAME).

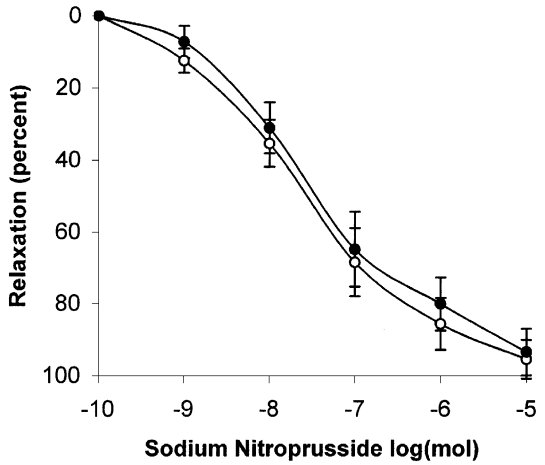

Fig. 4. Endothelium-independent vasorelaxation in response to sodium nitroprusside in mesenteric vascular bed, preconstricted with phenylephrine, of sham-operated and bile duct-ligated rats treated with saline. Each group consisted of six rats (○ sham-operated rats treated with saline; ● bile duct-ligated rats treated with saline).

The ED_{50} of the L-NAME-treated sham-operated group was significantly higher than the ED_{50} of sham-operated controls ($P < 0.01$); however, the R_{max} of the L-NAME-treated sham-operated group was significantly lower than that of sham-operated control ones ($P < 0.01$). The ED_{50} of the L-NAME-treated bile duct-ligated group was significantly lower ($P < 0.01$), but its R_{max} was significantly higher ($P < 0.01$) than that of bile duct-ligated controls (Fig. 3C).

3.5. Sodium nitroprusside-induced vasorelaxation

Sodium nitroprusside, a soluble guanylyl cyclase activator, caused dose-dependent vasorelaxation (0.1 nmol to 10 μmol) in the phenylephrine-preconstricted mesenteric vascular bed. The response was not significantly different between groups for ED_{50} and vasorelaxation R_{max} (Fig. 4).

4. Discussion

In the present study, we demonstrated that phenylephrine-induced vasoconstriction and acetylcholine-induced endothelium-dependent vasorelaxation are impaired in the mesenteric vascular bed of 7-day bile duct-ligated rats. The vasorelaxation response to sodium nitroprusside, an NO donor, is not different between groups, implying that the sensitivity of the smooth muscle soluble guanylyl cyclase/vasorelaxation pathway does not differ.

Previous studies had used mainly isolated conductance arterial rings (Bomzon et al., 1996; Utkan et al., 1996; Utkan et al., 2000) to investigate the vascular problems of cholestatic rats. The present study used the mesenteric vascular bed, which allows investigation of the responsiveness of the intact vascular bed. Vascular resistance in the splanchnic circulation is more dependent on small resistance arteries (Benoit and Granger, 1988); thus, it seemed necessary to investigate the effect of bile duct ligation on a vascular system with resistance properties. Finally, the main role of the mesenteric vascular bed in the portal circulation and its contribution to the development and maintenance of portal hypertension is another reason for the importance of this model.

In the present study, the impaired acetylcholine-induced vasorelaxation, accompanied by an intact vasorelaxation response to sodium nitroprusside, may have been due either to a defect in NO release from endothelium or to increased NO inactivation. Previous studies had shown conflicting results for vasorelaxation in cholestasis. Bomzon et al. (1996) reported that the vasorelaxant response of isolated endothelium-intact arterial rings from 3-day bile duct-ligated rats to acetylcholine was identical to that of endothelium-intact arterial rings from control rats. In another study, Utkan et al. (1996, 2000) found that acetylcholine-induced vasorelaxation of isolated femoral and renal arterial rings of 7-day bile duct-ligated dogs was significantly increased. However, the present results showed impaired acetylcholine vasorelaxation in the mesenteric vascular bed. The difference may have been due to the different responses of large conductance vessels, such as aortic ring, and small resistance vascular elements, such as mesenteric vascular bed, to acetylcholine (Feletou and Vanhoutte, 1999) and cytokines (Balligand et al., 1995). Many other studies were done on cirrhotic rats with bile duct ligation of longer (more than 4 weeks) duration (Bomzon and Blendis, 1994). Because no portal hypertension is reported to exist in the first week in bile duct-ligated rats (Bomzon et al., 1985) and there is some evidence that the vascular changes in bile duct-ligated rats become irreversible after the third week (Nagahata et al., 1997), it seems that results of these studies on cirrhosis and portal hypertension cannot be directly compared with the present results.

Several factors may contribute to endothelial dysfunction in the mesenteric vascular bed of bile duct-ligated rats. Increased endothelial intracellular calcium is a possible mechanism. Possibly, an increased level of endogenous opioid peptides in bile duct-ligated rats, acting through μ_3- and δ_2-opioid receptors on the endothelium (Stefano et al., 1998), may chronically increase intracellular calcium (Way et al., 1998). Acetylcholine can thus no longer increase the intracellular calcium level to stimulate constitutive NO synthase (NOS) to release NO. Bile salts are also able to increase intracellular calcium in an endothelial cell line (Nakajima et al., 2000), but their in vivo effect is not well defined. The oxidative stress, which is reported to exist in the cholestatic state (Orellana et al., 2000), is another possibility. Finally, the dysfunction mentioned may be due to the negative feedback of NO on NOS activity (Buga et al., 1993). Cholestasis is known to be associated with endotoxaemia (Clements et al., 1998) and endotoxaemia

induces inducible NOS (iNOS) (Radomski et al., 1990). Increased NO production by iNOS can exert a negative feedback on endothelial constitutive NOS (cNOS) activity and decrease its activity. Further studies, measuring intracellular Ca^{2+}, assessing oxidative stress, or using selective NOS inhibitors, are to be done to verify these theories.

In the present study, the phenylephrine-induced vasoconstriction response was impaired. Previous studies had yielded similar results for different vascular elements of cholestatic rats (Bomzon et al., 1985; Cioffi et al., 1986). The exact mechanism for this impairment is not yet clearly defined. The right shift in vasoconstriction dose–response in bile duct-ligated rats compared to sham-operated controls suggests that a receptor defect may be present. However, Dabagh et al. (1999) reported that the affinity and number of α_1-adrenoceptors are unchanged in acute cholestasis. The possibility of post-receptor defects, present in many receptors of cirrhotic rats (Jaue et al., 1997), needs to be studied. It is reported that NOS inhibition or endothelium denudation (Utkan et al., 2000; Kimpel et al., 1998) will reverse the hyporesponsiveness of vascular elements of cholestatic animals, providing evidence for the role of endothelium-derived NO in this matter. There are some recent reports on the role of the decreased protein kinase C activity, mediated via NO overproduction, in the hyporesponsiveness of portal vein-stenosed rat aorta to vasoconstrictors (Chagneau et al., 2000), and the role of potassium channels (Atucha et al., 2000) in the hyporesponsiveness of cirrhotic rats to vasoconstrictors. The same possibilities may exist in cholestasis, which is to be investigated in further studies.

Another finding of this study was that baseline perfusion pressure is decreased in bile duct-ligated rats, compared to that of sham-operated controls. Previous studies on cirrhotic (Sieber et al., 1993) and portal hypertensive rats (Sieber and Groszmann, 1992) have shown similar results. In the present study, L-NAME treatment (for 6 days) increased the baseline perfusion pressure. However, a previous study (Sieber and Groszmann, 1992) demonstrated no change in baseline perfusion pressure with in vitro NO synthesis blockade. Since there is a similar increase in the baseline perfusion pressure of sham-operated and of bile duct-ligated rats with L-NAME treatment, it cannot be concluded that this decrease in baseline perfusion pressure of bile duct-ligated rats was mediated via NO. In other words, chronic NOS inhibition increases the baseline perfusion pressure, while in vitro NOS blockade has no such effect. This effect may be due to protection from structural changes, induced by NO overproduction, similar to structural changes in rats treated long-term with a vasodilator, minoxidil (Tsoporis et al., 1991). Studies using a longer period of treatment and bile duct ligation are to be done to elucidate this effect.

The present study also showed that, in L-NAME-treated bile duct-ligated rats, both phenylephrine-induced vasoconstriction and acetylcholine-induced vasorelaxation were restored toward normal to some extent. L-NAME is a non-selective NOS inhibitor, and, as mentioned, may prevent the structural changes caused by overproduction of NO, a known vasodilator. It may also prevent the inhibitory effect of overproduced NO by iNOS on endothelial constitutive NOS. These results provide further evidence for the role of NO overproduction in complications of cholestasis, and suggest a new approach toward managing cholestasis-induced vascular aberrations.

Treatment with naltrexone, a non-selective opioid receptor blocker, restored mainly the vasorelaxation response toward normal, with little effect on the vasoconstriction response. This may have been due to prevention of the effect of an increased opioidergic tone on intracellular calcium. Another possibility may be related to the effect of opioids on arterial nerve stimulation. Arterial nerves of the mesenteric vascular bed are known to have δ-opioid receptors (Illes et al., 1986), activation of which results in a decrease in neuroeffector release and consequent neurogenic hyporesponsiveness. Although we have not studied nerve electric stimulation, the effect of local nerves on the vascular responses and the chronic evolution of neurogenic hyporesponsiveness during the cholestatic period may produce some vascular changes. The beneficial effect of naltrexone provides evidence for the role of an increased opioidergic tone in cholestasis-associated hyporesponsiveness in the mesenteric vascular bed.

Although unrelated to cholestasis, the effects of L-NAME and naltrexone treatment on the vascular responsiveness should be mentioned. In sham-operated rats, L-NAME treatment increases the baseline perfusion pressure, increases phenylephrine-induced vasoconstriction, and decreases the acetylcholine-induced vasorelaxation. The effect of 6-day NOS inhibition on acetylcholine-induced vasorelaxation is opposite to the effect of a similar regimen on the acetylcholine-induced vasorelaxation of the bile duct-ligated rats. This implies a beneficial role of basal NO release in acetylcholine-induced vasorelaxation in the noncholestatic state. Naltrexone treatment also increased the baseline perfusion pressure, decreased the acetylcholine-induced vasorelaxation, with minimal effect on the phenylephrine-induced vasoconstriction. These results may be explained by the beneficial role of the opioid system in the mesenteric vascular bed responsiveness in the noncholestatic state. These effects deserve further studies.

As a conclusion, there is impaired acetylcholine-induced endothelium-dependent vasorelaxation in the mesenteric vascular bed of bile duct-ligated rats, possibly due to a defect in the endothelium. NOS inhibition improved vascular responsiveness of bile duct-ligated rats, suggesting a role for increased NO overproduction in cholestasis. Naltrexone treatment also restores vascular responsiveness, especially the acetylcholine-induced vasorelaxation, implying a possible role for an increased opioidergic tone in cholestasis-induced vascular hyporesponsiveness.

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