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The action of nitric oxide on hepatic haemodynamics during secondary biliary cirrhosis in the rat

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Abstract

The role of nitric oxide (NO) in portal hypertension is poorly understood. The role of NO upon hepatic arterial and portal venous vasoconstrictor responses to noradrenaline and ATP in rats with secondary biliary cirrhosis was evaluated. Cirrhosis was induced by bile duct ligation after which livers were excised and dual-perfused in vitro. Concentration-dependent dose–response curves were then constructed to hepatic arterial and portal venous noradrenaline and ATP. Hepatic arterial responses to noradrenaline and ATP were significantly attenuated in cirrhotic rats. 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) restored attenuated hepatic arterial responses to noradrenaline and ATP in cirrhotic rats. Portal venous responses to noradrenaline in cirrhotic rats were significantly increased compared to controls and were not affected by L-NAME. However, portal venous responses to ATP were significantly attenuated in cirrhotic rats and were also not restored by L-NAME. Hepatic arterial or portal venous responses to noradrenaline did not change after infusion of L-NAME. Hepatic arterial responses to noradrenaline and ATP were significantly attenuated in cirrhotic rats, possibly due to increased production of NO. However, portal venous responses in cirrhotic rats were increased to noradrenaline and attenuated to ATP, and were not related to increased NO production.

Keywords: Nitric oxide (NO); Cirrhosis; L-NAME (N^G-nitro-L-arginine methyl ester); Liver

1. Introduction

It is believed that chronic partial and total extrahepatic bile duct obstruction in man and experimental models leads to secondary biliary cirrhosis, portal hypertension and liver failure. During the development of cirrhosis, ill-defined haemodynamic changes occur in the liver, which may be closely related to, or indeed, responsible for cellular injury. In addition, the mechanisms responsible for these are unclear, and the interactive role of vasoactive agents during the development of secondary biliary cirrhosis induced by chronic bile duct occlusion remains obscure.

Nitric oxide (NO) is believed to be an important modulator of hepatic vascular tone under normal and diseased conditions (Alexander, 1998). A low basal production of NO that modulates vascular resistance is generated by endothelial NO synthase (eNOS) in the normal liver (Mittal et al., 1994; Serra, 2001). The precise role of NO in the regulation of hepatic portal vascular resistance during cirrhosis remains controversial (Clemens, 1998). There is no evidence of basal NO release in the portal vasculature of control or cirrhotic rats and no augmentation of portal venous responses to noradrenaline and sympathetic nerve stimulation following N^G-nitro-L-arginine methyl ester (L-NAME) administration has been demonstrated in the rat liver (Mathie et al., 1996a). Also, NO release from the portal vascular bed does not increase in cirrhotic livers (Mathie et al., 1996b; Mittal et al., 1993). However, L-arginine attenuates portal venous responses to norepinephrine in cirrhotic rats and suggests that endogenous NO production may have a potentially important hypotensive role (Gupta and Groszmann, 1994). Other studies have shown that NOS activity and NO production are reduced in cirrhotic rat livers (Rockey and

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Chung, 1998; Zimmermann et al., 1996) with diminished portal responses to acetylcholine (Wiest and Groszmann, 1999). L-Arginine infusion has been shown to reduce hepatic sinusoidal resistance and therefore increase hepatic blood flow (Kakumitsu et al., 1998). All these data imply that portal hypertension, a major complication of cirrhosis, may be generated from impaired release of NO due to sinusoidal endothelial dysfunction. However, little is known regarding the intrahepatic arterial circulation in the rat which appears to differ from the extrahepatic arterial splanchnic, circulation (Yang et al., 2001) and other experimental models (Alexander and Mathie, 1993). In addition, little is known regarding the differences between intra- and extra-hepatic portal haemodynamics, and this is particularly relevant in the role of Kupffer cells and NO in the regulation of hepatic haemodynamics in health and disease (Reynaert et al., 2002). Moreover, very little is known regarding the interrelationship between the hepatic arterial and portal venous microvascular beds (Yang et al., 1999), the site of confluence of the two systems (Alexander et al., 2001a, 2002) or how NO release from one vasculature may influence hepatic haemodynamics in the adjacent vasculature (Browse et al., 1994). The precise function of NO in the regulation of hepatic haemodynamics, particularly during cirrhosis, is difficult to ascertain in vivo due to the over-riding complications of the presence of other organs and the instability of systemic haemodynamics.

An isolated dual-perfused rat liver preparation (Yang et al., 1999) that achieves haemodynamic stability and reliability reproduces concentration-dependent responses to a variety of vasoactive substances (Yang et al., 2000) over prolonged periods of perfusion has been developed (Yang et al., 2001). The model demonstrated that NO was involved in acetylcholine-induced hepatic arterial vasodilatation and ATP-induced vasoconstriction in the rat (Yang et al., 2001) and rabbit liver (Alexander et al., 1999a; Browse et al., 1994).

The aim of the present study was to determine what haemodynamic changes occur to noradrenaline- and ATP-induced vasoconstrictor dose–response curves in rats with secondary biliary cirrhosis, as a model of portal hypertension, in both the hepatic arterial and portal venous vasculatures. In addition, the putative modulator role of NO upon these dose–response curves in both control and cirrhotic rats was determined by infusion of L-NAME, a non-selective NOS inhibitor.

2. Materials and methods

2.1. Experimental design

Eighteen male Wistar rats weighing 300–350 g were divided into control ($n=8$) and bile duct ligated ($n=10$) groups. Haemodynamic studies were performed at 4–5 weeks following bile duct ligated or sham operation, this being the time period required to develop the optimal characteristics of secondary biliary cirrhosis. Hepatic arterial and portal venous responses to noradrenaline and ATP were initially studied in isolated dual-perfused livers by construction of dose–response curves to bolus drug injections. L-NAME (100 μM) was then infused continuously and hepatic vascular responses to these vasoconstrictors were re-constructed and compared to curves before the addition of L-NAME. Saline was the vehicle used to dissolve all the drugs in the construction of both the control and test curves. Both the present and previous studies (Yang et al., 1999; Alexander et al., 1992) have shown the effects of saline alone to be undetectable.

2.2. Induction of secondary biliary cirrhosis

Secondary biliary cirrhosis was induced by common bile duct ligation as described elsewhere (Kountouras et al., 1984). In brief, the rats were anaesthetised with 3% halothane and, following midline laparotomy, the common bile duct was exposed and double-ligated with a 7–0 silk suture between the bifurcation and just above suprapancreatic segment of the common bile duct. The bile duct was then divided between the two ligatures and the abdominal wall was closed using continuous suturing. Sham operations were performed in controls by exposing but not ligating and sectioning the common bile duct. The animals were then allowed to recover with free access to food and water after surgery. All the procedures were carried out under sterile conditions, and the protocols were approved by the guidelines and legislative procedures outlined by the Home Office of the United Kingdom in the Animal Scientific Procedures Act 1986.

2.3. Histology

Liver tissue from sham and bile duct ligated rats during the time course of 1–4 weeks were fixed with 10% buffered formaldehyde–saline solution and embedded with paraffin. Four-micrometer-thick sections of liver tissue were then stained with haematoxylin and eosin and Gorden and Sweet silver method for reticulin. Histological changes were assessed under light microscope by a liver pathologist without prior knowledge of the treatment.

2.4. Measurement of portal pressure in vivo

After the defined experimental post-operative time period had been achieved, the rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.), the abdomen re-opened through a midline incision and portal venous pressures were measured in vivo. The gastroduodenal artery and vein were carefully exposed and dissected. Following heparinisation (1000 U kg^{-1} i.v.), the gastroduodenal vein was cannulated with a 0.75-mm o.d. (3 FG) cannula (Portex, Hythe, Kent, England) and portal pressure recorded on a Grass 79F

polygraph through a Spectramed (Statham) P23XL physiological pressure transducer (Grass Instrument, Quincy, MA, USA).

2.5. Liver perfusion

The bile duct, hepatic artery and portal vein were cannulated as described elsewhere (Yang et al., 1999). The livers were excised and perfused in vitro with Krebs–Bülbring buffer (pH 7.4, 37 °C), saturated with 95% O_2/5% CO_2 via both the hepatic arterial and portal venous cannulae at constant flow rates of 0.53 ± 0.03 and 1.47 ± 0.09 ml min^{-1} g $liver^{-1}$, respectively, according to our previous protocols (Yang et al., 1999). Dose-dependent response curves were constructed to hepatic arterial and portal venous (0.05 ml) bolus injections of noradrenaline (10^{-6} to 10^{-2} M) and ATP (10^{-6} to 10^{-1} M). Transient increases in perfusion pressure were taken as indicative of vasoconstriction (Alexander et al., 1992; Yang et al., 1999). Dose–response curves were re-examined in the presence of 100 μM L-NAME infused continuously 30 min after completion of the dose–response curves.

2.6. Measurement of NO production

NO has a half-life of only 3 s and therefore highly specialised equipment is required for its measurement on-line. Perfusate effluent concentrations of nitrite/nitrate, the stable end metabolic products of NO, were measured before and after infusion of L-NAME by using a chemiluminescence NO analyzer (Sievers Research, Boulder, CO). A total of 50 μl 800 μM nicotinamide adeninine dinucleotide (NADPH), 40 μl 100 μM flavin adenine dinucleotide (FAD), 300 μl Tris buffer (pH 7.6) and 10 μl nitrate reductase (2 U ml^{-1}) were added to each 100 μl of perfusate sample. The reaction mixture was incubated for 1 h at 37 °C to convert nitrate to nitrite. The conversion rate of nitrate to nitrite is greater than 90%. Samples were then injected into a reaction chamber containing acetic acid and potassium iodide (50 mg ml^{-1}) at a ratio of 4:1. This reduces nitrite to NO, which is purged from the refluxing solution by nitrogen and reacts with ozone before analysis by chemiluminescence. Standards were prepared from 1 M stock solution of sodium nitrite and nitrate and serially diluted with fresh double-distilled reverse osmosis purified water to the a range covering 0–10 μM. Measurements were calibrated against standard curves of sodium nitrite and sodium nitrate using the equations derived from these standard curves.

2.7. Statistics

The results are expressed as mean ± S.E.M. Differences between groups were calculated for statistical significance using analysis of variance (ANOVAR) followed by Student's, two-tailed, unpaired *t*-test with Bonferroni adjustment, unless otherwise stated where paired analysis was used. *P*-values of less than 0.05 were considered to be statistically significant.

3. Results

Portal venous pressures in sham and bile duct ligated rats measured in vivo were 6.8 ± 0.6 and 11.5 ± 1.2 mm Hg, respectively, at 1 week after surgery, and 7.2 ± 0.8 to 16.3 ± 1.5 mm Hg, respectively, at 4–5 weeks, a percentage increase of 126.4%.

Basal hepatic arterial perfusion pressures in bile duct ligated rats were significantly lower compared to sham rats (70.0 ± 4.7 vs. 100.0 ± 5.1 mm Hg, bile duct ligated vs. sham, $P < 0.0001$). These changes were paralleled with alterations in intrahepatic arterial resistance (145.8 ± 9.9 vs. 208.3 ± 2.3 mm Hg ml^{-1} min g liver, bile duct ligated vs. sham, $P < 0.0001$). However, basal portal venous perfusion pressures in bile duct ligated rats were significantly higher than sham rats (17.6 ± 0.8 vs. 9.9 ± 0.3 mm Hg, bile duct ligated vs. sham, $P < 0.0001$). Similarly, in vitro intrahepatic portal resistance in bile duct ligated rats was increased significantly compared to sham rats (10.5 ± 0.5

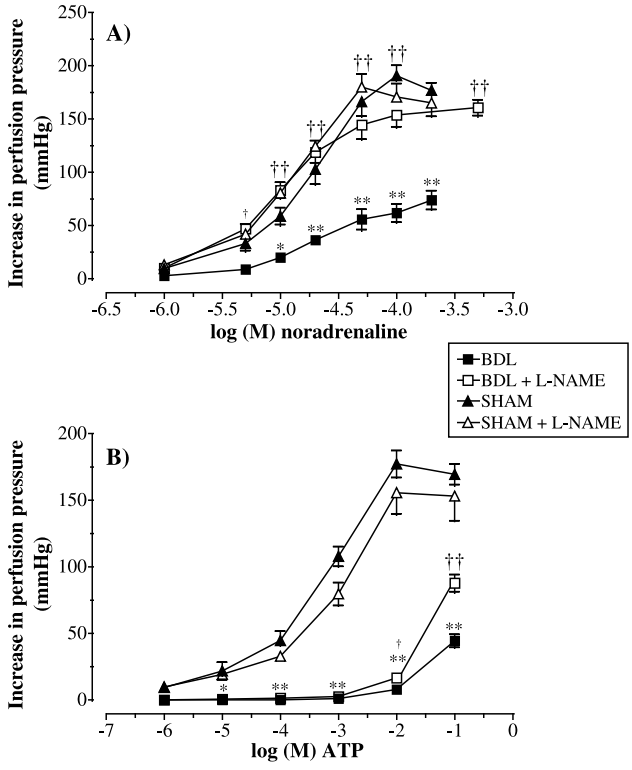

Fig. 1. Hepatic arterial responses to (A) noradrenaline and (B) ATP in bile duct ligated (BDL) ($n = 10$) and sham ($n = 8$) rats before and after infusion of 100 μM L-NAME. Values are mean ± S.E.M. *$P < 0.05$, **$P < 0.01$, BDL vs. sham (Student's unpaired *t*-test); †$P < 0.05$, ††$P < 0.01$, before vs. after L-NAME in BDL (Student's paired *t*-test).

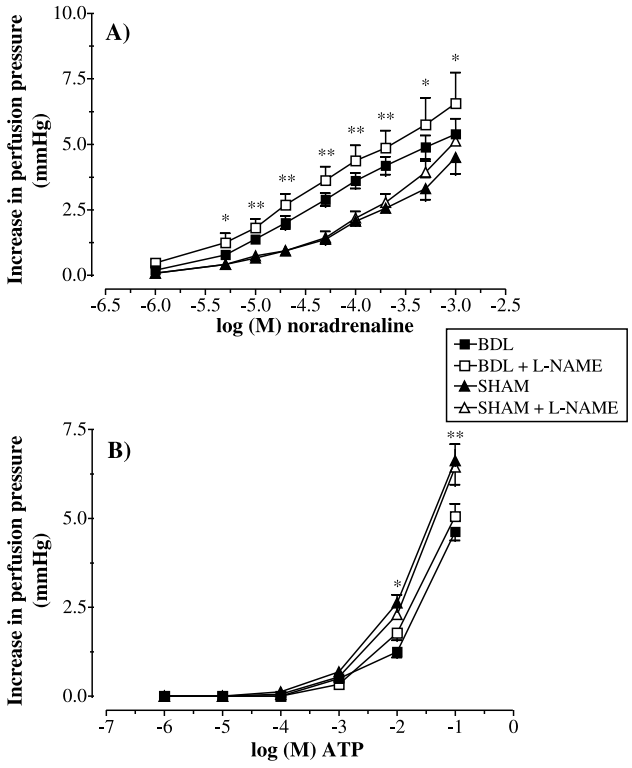

Fig. 2. Portal venous responses to (A) noradrenaline and (B) ATP in BDL ($n=10$) and sham ($n=8$) rats before and after infusion of 100 μM L-NAME. Values are mean ± S.E.M. *$P<0.05$, **$P<0.01$, BDL vs. sham (Student's unpaired t-test). No significant changes in portal venous responses to either noradrenaline or ATP were observed after infusion of L-NAME either in BDL or in sham rats.

vs. 5.9 ± 0.2 mm Hg ml^{-1} min g liver, bile duct ligated vs. sham, $P<0.0001$).

3.1. Hepatic arterial vasoconstrictor responses

Hepatic arterial responses to noradrenaline were significantly reduced in bile duct ligated rats compared to sham rats at all but the lowest concentration of noradrenaline tested $E_{max}=73.9\pm8.8$ and 190.6 ± 9.8 mm Hg, respectively, $P<0.01$, the $-\log$ (Fig. 1A). However, there were no significant differences in ED_{50} between sham and bile duct ligated rats. Responses to ATP were also diminished in bile duct ligated rats compared to sham rats in a competitive, reversible manner shifting the $-\log(M)$ ED_{50} to 2.38 ±0.03 from 4.36 ± 0.24, respectively, $P<0.0001$, and also at all the concentrations shown including the $E_{max}=44.4\pm4.9$ and 177.2 ± 10.2 mm Hg ($P<0.01$) respectively (Fig. 1B).

After infusion of L-NAME, decreased hepatic arterial responses to noradrenaline in bile duct ligated rats were significantly enhanced $-\log(M)$ $ED_{50}=4.92\pm0.09$ vs. 5.29 ± 0.07, before vs. after L-NAME, $P<0.001$, paired t-test, at all concentrations tested including the E_{max} from 73.9 ± 8.8 to 160.6 ± 7.5 mm Hg, $P<0.01$, Student's paired t-test (Fig. 1A). However, there were no significant differences in sham rats before and after L-NAME. Hepatic arterial responses to ATP in bile duct ligated rats were also significantly increased in the presence of L-NAME ($E_{max}=44.4\pm4.9$ vs. 87.8 ± 6.4, before vs. after L-NAME, respectively, $P<0.01$, paired t-test), but were not fully restored to those seen in sham rats as observed in noradrenergic responses. In sham rats, no changes in ATP responses were measured after infusion of L-NAME (Fig. 1B). ED_{50} values were not calculated because maximum responses could not be accurately obtained to ATP in bile duct ligated rats.

3.2. Portal venous vasoconstrictor responses

In contrast to hepatic arterial responses, portal venous responses to noradrenaline in bile duct ligated rats were significantly increased compared to sham rats (Reynaert et al., 2002), $ED_{50}=4.69\pm0.09$ and 3.99 ± 0.08, respectively, $P<0.001$ (Fig. 2A) at all but the lowest concentration of noradrenaline tested, $E_{max}=5.4\pm0.4$ and 4.5 ± 0.4 mm Hg, $P<0.05$. Conversely, portal venous responses to ATP were significantly reduced in bile duct ligated rats compared to sham rats ($E_{max}=4.6\pm0.3$ and 6.6 ± 0.4 mm Hg, respec-

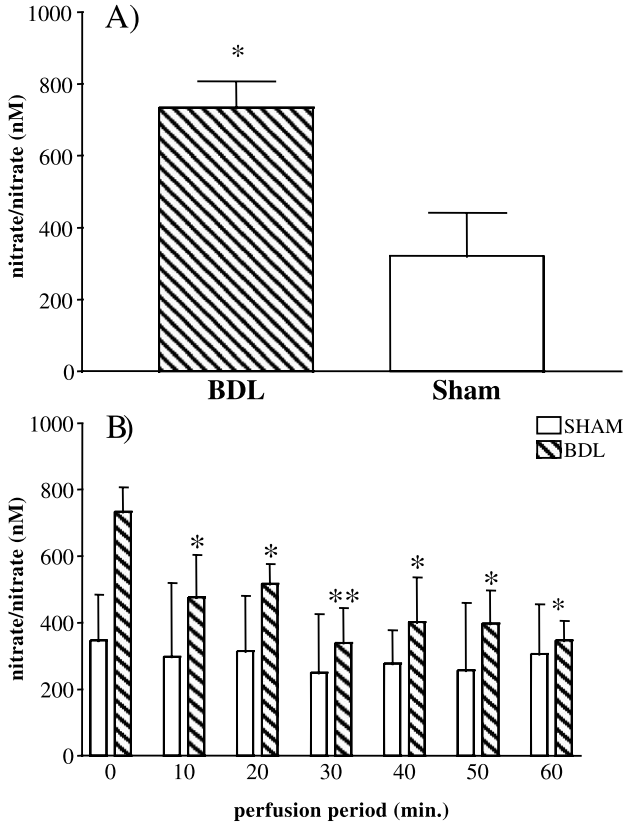

Fig. 3. (A) The concentrations of perfusate effluent nitrite/nitrate in isolated perfused livers from BDL and sham rats after 60 min perfusion, *$P<0.05$. (B) The effect of 100 μM L-NAME, given 30 min prior to the start of effluent collection at $t=0$, on NO production during the period of perfusion. Values are mean ± S.E.M. *$P<0.05$, **$P<0.01$, before ($t=0$) vs. after L-NAME, Student's paired t-test.

tively, $P < 0.001$; Fig. 2B). No significant changes in portal venous responses to either noradrenaline or ATP were observed after infusion of L-NAME either in bile duct ligated or in sham rats.

3.3. NO production in perfusate effluent

Perfusate effluent nitrite/nitrate concentration in bile duct ligated rats were significantly increased compared to shams (733.8 ± 73.4 vs. 396.2 ± 138.1 nM, bile duct ligated vs. sham, $P < 0.05$; Fig. 3A). After infusion of L-NAME, NO release into the perfusate effluent was significantly decreased in bile duct ligated rats during perfusion ($P < 0.01$). However, NO production remained unchanged after L-NAME infusion in sham rats (Fig. 3B).

3.4. Morphological changes in the liver

Sham rat livers showed normal histology using haematoxylin and eosin and reticulin stains (Fig. 4). After 4–5 weeks, bile duct ligated rat livers showed extensive portoseptal fibrous expansion with predominant ductular proliferation. In addition, neoductules protruding from the portal boundaries into the parenchyma, accompanied by thick reticulin fibres, were observed. These divided the parenchyma and led to replacement of plates by ductules and formation of dissecting bridging septa, the equivalent of micronodular biliary cirrhosis (Fig. 5).

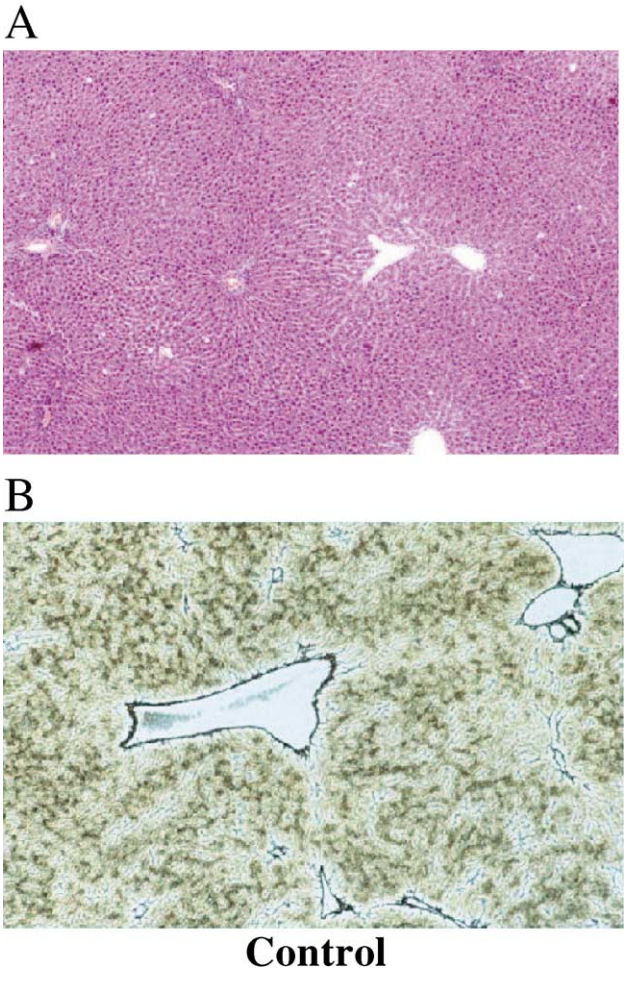

Fig. 4. Representative light photomicrograph of a sham operated rat liver stained with (A) haematoxylin and eosin ×50 and (B) Gorden and Sweet silver method for reticulin ×50.

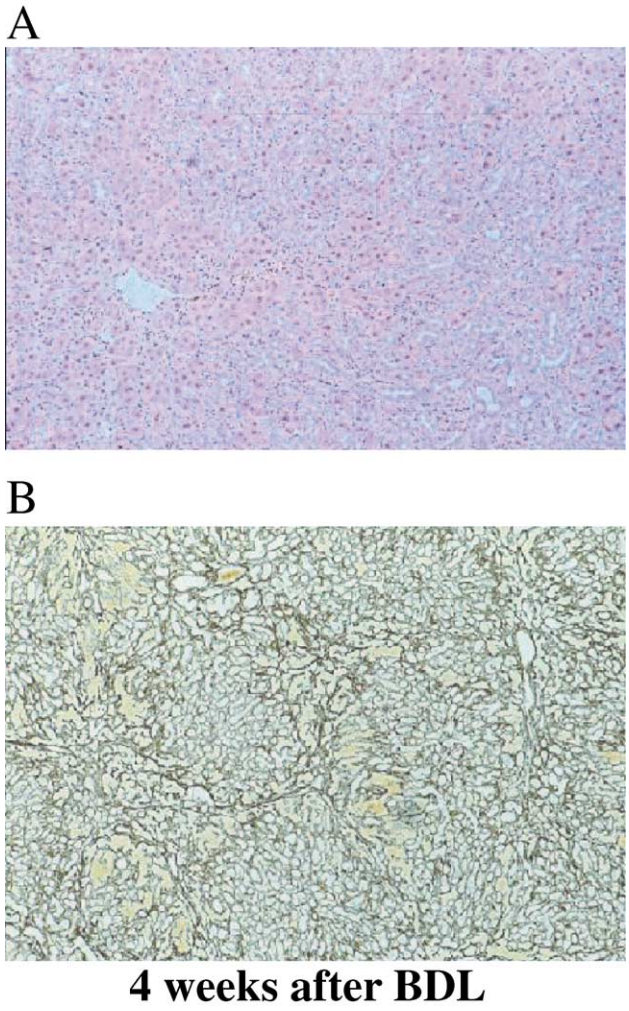

Fig. 5. Representative light photomicrograph of a rat liver 4 weeks after bile duct ligation, stained with (A) haematoxylin and eosin ×100 and (b) Gorden and Sweet silver method for reticulin ×100.

4. Discussion

The data from the present study showed that hepatic arterial vasoconstrictor responses were significantly decreased in bile duct ligated rats and these paralleled the decreased arterial perfusion pressure and resistance to flow in vitro. Attenuated hepatic arterial responses to noradrenaline were fully restored after inhibition of NO synthase by infusion of L-NAME in bile duct ligated rats, and this was likely to be due to increased NO production. This was

further substantiated by increased NO release in the perfusate effluent of bile duct ligated rats, which was significantly reduced by infusion of L-NAME.

NO is produced in small amounts in the liver by the constitutive form of NO synthase (eNOS) (Mittal et al., 1994) and, in diseased conditions such as cirrhosis, a much greater amount of NO may be produced by the inducible form of NO synthase. This is activated by increased circulating endotoxin and release of cytokines such as tumor necrosis factor (Geller et al., 1995). However, it remains unclear whether the activated or induced NO synthase is located within hepatocytes or Kupffer cells (Reynaert et al., 2002). Although in situ hybridisation studies may indicate whether the expression of a particular isoform of NOS is increased or decreased, they cannot confirm biological activity of the enzyme. This can really only be carried out using pharmacological vasoreactivity and/or biochemical techniques, to measure the output of the products of the chemical reaction such as those carried out in the present study. It was impossible to clarify which isoforms of NO synthase were responsible for the hepatic arterial hyporesponsiveness since L-NAME is a non-selective NOS inhibitor (Gadano et al., 1999; Parmentier et al., 1999) and is a weak iNOS inhibitor (Vos et al., 1997). The attenuated hepatic arterial responses to ATP were not restored to original levels following L-NAME infusion. It is not believed this was due to the L-NAME concentration being too low because: (i) noradrenaline responses were fully restored and (ii) NO release in the perfusate effluent decreased after L-NAME infusion.

An alternative explanation for the globally reduced ATP-induced vasoconstriction in the present study may simply have been due to purine receptor denaturation. Our laboratory has previously shown that vascular purine receptors are more vulnerable to denaturation than adrenergic (Gryf-Lowczowski et al., 1997) or cholinergic receptors (Alexander et al., 1999b, 2001b) following storage at 4 °C in University of Wisconsin solution. Secondary biliary cirrhosis may stimulate or permit entry of unprocessed blood or toxins such as oxygen-free radicals into the systemic circulation. Previous studies from our laboratory have suggested that oxygen-free radicals can be generated following ischaemia or storage of blood vessels at 4 °C and that purine receptors are most vulnerable to their toxic and disruptive effects (Alexander et al., 1999b, 2001b).

Portal venous perfusion pressure, intrahepatic resistance to flow and vascular responses to noradrenaline were significantly enhanced in bile duct ligated rats compared to those in sham rats. However, ATP-induced portal venous vasoconstriction was reduced in bile duct ligated rats although only at the two highest concentrations, which corresponded to ATP concentrations between 10^{-6} and 10^{-4} M (Yang et al., 1999). Decreased portal venous compliance in response to increased inflow in bile duct ligated rats has been proposed as a cause of increased vasoconstrictor activity (Wiest and Groszmann, 1999). This may be due to: (i) increased endogenous release of endothelin-1 that may modify vasoconstriction specifically elicited by noradrenaline (Vos et al., 1997) and not ATP; and (ii) endothelial dysfunction, leading to impaired release of endothelial vasodilators (Gupta et al., 1997). The enhanced noradrenergic portal venous vasoconstriction observed above and in the present study cannot be attributed to decreased release of a single vasodilator since simultaneous unidirectional changes would have been expected in both ATP and noradrenaline and this did not happen.

It could be argued that the data from the present study may be idiosyncratic of the model where the liver was dual-perfused in vitro. It is believed that this is unlikely for several reasons. Firstly, the model has previously been shown to be close-to-physiological (Yang et al., 2000) and to produce reliable and reproducible data for perfusion periods in excess of those quoted here (Yang et al., 1998). Secondly, the results from the present study suggest that increased endogenous NO release is not the reason for the reduction in ATP-induced portal venous vasoconstriction in bile duct ligated rats since portal venous responses to either noradrenaline or ATP were not significantly changed in either group after infusion of L-NAME. This is consistent with other studies, where (i) portal venous noradrenergic responses in carbon tetrachloride-induced cirrhotic rats were enhanced compared to controls (Mathie et al., 1996a) and (ii) decreased portal venous responsiveness to ATP was unaffected by NOS inhibition (Mittal et al., 1993). However, attenuated noradrenergic vasoconstriction in aortic rings of bile duct ligated and partial portal vein ligated Sprague–Dawley rats was fully reversed by constitutive NOS inhibition (Gadano et al., 1999). In contrast, increased noradrenergic contractile responses were reported in aortic rings from partial portal vein ligated Wistar rats but not Sprague–Dawley rats (Connolly et al., 1999). The same group earlier reported increased noradrenergic responses in mesenteric arteries but not aortic rings from partial portal vein ligated Wistar rats (Cawley et al., 1995b) with no evidence for NOS modulation upon acetylcholine-induced vasodilatation (Cawley et al., 1995a). Species differences may explain differences between different laboratories rather than the model of portal hypertension used. For example, all the laboratories quoted including the present study that used Wistar rats reported increased responses to noradrenaline in the splanchnic vasculature of the models of portal hypertension used (Cawley et al., 1995b; Mathie et al., 1996a; Aboud et al., 1997). In contrast, models of portal hypertension that used Sprague–Dawley rats reported either attenuated (Cahill et al., 1996; Karatapanis et al., 1994; Gadano et al., 1999; Chagneau et al., 2000) or unchanged (Connolly et al., 1999) noradrenergic responses in aortic rings or in the splanchnic vasculature of portal hypertensive rats. Only one group reported attenuated noradrenergic responses in aortic rings from Wistar rats whether the duration of partial portal vein ligated-induced portal hypertension was 6 months (Michielsen et al., 1997), or 3 weeks

(Michielsen et al., 1995) compared to between 14 and 28 days in the other groups. One group using inbred strains reported inconclusive evidence (Bomzon et al., 1991) or elevated noradrenergic responses in an unspecified inbred strain (Bomzon and Blendis, 1987); however, both of these studies did exceed 10 days duration of partial portal vein ligation. Therefore, the species of rat used, the location of vessel under investigation (Yang et al., 2001), whether vasodilator or vasoconstrictor responses are under scrutiny and the experimental model of portal hypertension being used must be taken into consideration when comparing different studies. Thus, it appears, in general, that models of portal hypertension using Sprague–Dawley rats have attenuated noradrenergic responses, which may be reversed with NOS inhibitors, and those that use Wistar rats are increased, at least in the splanchnic vasculature.

In summary, it is concluded that the data from present study show that hepatic arterial responses to noradrenaline and ATP are significantly attenuated in bile duct ligated rats, partially due to increased production of NO. This was further substantiated by the fact that the perfusate effluent NO production was significantly increased in bile duct ligated livers and this was inhibited by L-NAME. However, portal venous responses in bile duct ligated rats were enhanced to noradrenaline and attenuated to ATP and did not appear to be directly related to increased NO production. Further studies are being completed at the present time using more specific iNOS inhibitors such as *S*-methyl-isothiourea and 1400 W, which is 5000 times more selective for iNOS than eNOS (Garvey et al., 1997), in order to clarify the nature of the hyporeactivity in bile duct ligated rats. Caution should be applied to the interpretation of data from models of portal hypertension between different species of rat.

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References

Aboud, R.W., Connolly, C., McCormick, P.A., Docherty, J.R., 1997. Vascular actions of octreotide in the portal hypertensive rat. Br. J. Pharmacol. 122, 698–702.

Alexander, B., 1998. The role of nitric oxide in hepatic metabolism. Nutrition 14, 376–390.

Alexander, B., Mathie, R.T., 1993. Diminished hyperaemic response of the hepatic artery to portal venous occlusion (the buffer response) in Asian hybrid minipigs: a comparison of the response to that observed in dogs. J. Comp. Physiol., B 163, 5–10.

Alexander, B., Mathie, R.T., Ralevic, V., Burnstock, G., 1992. An isolated dual-perfused rabbit liver preparation for the study of hepatic blood flow regulation. J. Pharmacol. Toxicol. Methods 27, 17–22.

Alexander, B., Browse, D.J., Benjamin, I.S., 1999a. Hypoxia attenuates hepatic arterial vasodilatation and enhances portal venous vasoconstriction to ATP in the perfused rabbit liver. Eur. J. Pharmacol. 385, 181–189.

Alexander, B., Gryf-Lowczowski, J.V., Sherlock, D., Salisbury, J., Benjamin, I.S., 1999b. Paradoxical cholinergic and purinergic vascular reactivity of rabbit thoracic aorta cold-stored in University of Wisconsin solution. J. Pharm. Pharmacol. 51, 623–630.

Alexander, B., Cottam, H., Naftalin, R., 2001a. Hepatic arterial perfusion regulates portal venous flow between hepatic sinusoids and intrahepatic shunts in the normal rat liver in vitro. Pflugers Arch. 443, 257–264.

Alexander, B., Gryf-Lowczowski, J.V., Marway, J.S., Salisbury, J., Sherlock, D., Preedy, V., Benjamin, I.S., 2001b. Functional and compositional studies of arteries stored in University of Wisconsin solution compared with Krebs–Bülbring buffer. Exp. Mol. Pathol. 71, 265–273.

Alexander, B., Rogers, C., Naftalin, R., 2002. Hepatic arterial perfusion decreases intrahepatic shunting and maintains glucose uptake in the rat liver. Pflugers Arch. 444, 291–298.

Bomzon, A., Blendis, L.M., 1987. Vascular reactivity in experimental portal hypertension. Am. J. Physiol. 252, G158–G162.

Bomzon, A., Jacob, G., Lee, S.S., Meddings, J., 1991. In vitro vascular responsiveness to norepinephrine in experimental portal hypertension. Clin. Invest. Med. 14, 63–71.

Browse, D.J., Mathie, R.T., Benjamin, I.S., Alexander, B., 1994. The transhepatic action of ATP on the hepatic arterial and portal venous vascular beds of the rabbit: the role of nitric oxide. Br. J. Pharmacol. 113, 987–993.

Cahill, P.A., Redmond, E.M., Hodges, R., Zhang, S., Sitzmann, J.V., 1996. Increased endothelial nitric oxide synthase activity in the hyperemic vessels of portal hypertensive rats. J. Hepatol. 25, 370–378.

Cawley, T., Breslin, E., Geraghty, J., Osborne, H., Docherty, J.R., 1995a. Investigations of the function of the vascular endothelium in portal hypertensive rats. Pharmacology 51, 381–390.

Cawley, T., Geraghty, J., Osborne, H., Docherty, J.R., 1995b. Effects of portal hypertension on responsiveness of rat mesenteric artery and aorta. Br. J. Pharmacol. 114, 791–796.

Chagneau, C., Tazi, K.A., Heller, J., Sogni, P., Poirel, O., Moreau, R., Lebrec, D., 2000. The role of nitric oxide in the reduction of protein kinase C-induced contractile response in aortae from rats with portal hypertension. J. Hepatol. 33, 26–32.

Clemens, M.G., 1998. Does altered regulation of ecNOS in sinusoidal endothelial cells determine increased intrahepatic resistance leading to portal hypertension? Hepatology 27, 1745–1747.

Connolly, C., Cawley, T., McCormick, P.A., Docherty, J.R., 1999. Portal hypertension increases vasoconstrictor responsiveness of rat aorta. Clin. Sci. (Colch.) 96, 41–47.

Gadano, A.C., Sogni, P., Heller, J., Moreau, R., Bories, P.N., Lebrec, D., 1999. Vascular nitric oxide production during the development of two experimental models of portal hypertension. J. Hepatol. 30, 896–903.

Garvey, E.P., Oplinger, J.A., Furfine, E.S., Kiff, R.J., Laszlo, F., Whittle, B.J., Knowles, R.G., 1997. 1400 W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase in vitro and in vivo. J. Biol. Chem. 272, 4959–4963.

Geller, D.A., de Vera, M.E., Russell, D.A., Shapiro, R.A., Nussler, A.K., Simmons, R.L., Billiar, T.R., 1995. A central role for IL-1 beta in the in vitro and in vivo regulation of hepatic inducible nitric oxide synthase. IL-1 beta induces hepatic nitric oxide synthesis. J. Immunol. 155, 4890–4898.

Gryf-Lowczowski, J.V., Sherlock, D., Salisbury, J., Benjamin, I.S., Alexander, B., 1997. Cold-storage of rabbit thoracic aorta in University of Wisconsin solution reduces endothelium-independent vasodilation. J. Pharm. Pharmacol. 49, 1096–1101.

Gupta, T.K., Groszmann, R.J., 1994. Pressure ameliorates hepatic hyperreactivity in experimental cirrhosis. Hepatology 20, 200A.

Gupta, T.K., Chen, L., Groszmann, R.J., 1997. Pathophysiology of portal hypertension. Bailliere's Clin. Gastroenterol. 11, 203–219.

Kakumitsu, S., Shijo, H., Yokoyama, M., Kim, T., Akiyoshi, N., Ota, K., Kubara, K., Okumura, M., Inoue, K., 1998. Effects of L-arginine on the

systemic, mesenteric, and hepatic circulation in patients with cirrhosis. Hepatology 27, 377–382.

Karatapanis, S., McCormick, P.A., Kakad, S., Chin, J.K., Islam, M., Jeremy, J., Harry, D., McIntyre, N., Burroughs, A.K., Jacobs, M., 1994. Alteration in vascular reactivity in isolated aortic rings from portal vein-constricted rats. Hepatology 20, 1516–1521.

Kountouras, J., Billing, B.H., Scheuer, P.J., 1984. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br. J. Exp. Pathol. 65, 305–311.

Mathie, R.T., Ralevic, V., Burnstock, G., 1996a. Portal vascular responsiveness to sympathetic stimulation and nitric oxide in cirrhotic rats. J. Hepatol. 25, 90–97.

Mathie, R.T., Ralevic, V., Moore, K.P., Burnstock, G., 1996b. Mesenteric vasodilator responses in cirrhotic rats: a role for nitric oxide? Hepatology 23, 130–136.

Michielsen, P.P., Boeckxstaens, G.E., Sys, S.U., Herman, A.G., Pelckmans, P.A., 1995. The role of increased nitric oxide in the vascular hyporeactivity to noradrenaline in long-term portal vein ligated rats. J. Hepatol. 23, 341–347.

Michielsen, P.P., Boeckxstaens, G.E., Sys, S.U., Herman, A.G., Pelckmans, P.A., 1997. Aminoguanidine reverses aortic hyporeactivity to noradrenaline in portal vein-ligated rats. Eur. J. Pharmacol. 329, 137–146.

Mittal, M.K., Lee, F.Y., Groszmann, R.J., 1993. Role of nitric oxide in the intrahepatic portal circulation of cirrhotic rats. Gastroenterology 104, A956.

Mittal, M.K., Gupta, T.K., Lee, F.Y., Sieber, C.C., Groszmann, R.J., 1994. Nitric oxide modulates hepatic vascular tone in normal rat liver. Am. J. Physiol. 267, G416–G422.

Parmentier, S., Bohme, G.A., Lerouet, D., Damour, D., Stutzmann, J.M., Margaill, I., Plotkine, M., 1999. Selective inhibition of inducible nitric oxide synthase prevents ischaemic brain injury. Br. J. Pharmacol. 127, 546–552.

Reynaert, H., Thompson, M.G., Thomas, T., Geerts, A., 2002. Hepatic stellate cells: role in microcirculation and pathophysiology of portal hypertension. Gut 50, 571–581.

Rockey, D.C., Chung, J.J., 1998. Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. Gastroenterology 114, 344–351.

Serra, W.C., 2001. The nitric oxide synthase family of proteins. J. Vasc. Res. 31, 131–143.

Vos, T.A., Gouw, A.S., Klok, P.A., Havinga, R., van Goor, H., Huitema, S., Roelofsen, H., Kuipers, F., Jansen, P.L., Moshage, H., 1997. Differential effects of nitric oxide synthase inhibitors on endotoxin-induced liver damage in rats. Gastroenterology 113, 1323–1333.

Wiest, R., Groszmann, R.J., 1999. Nitric oxide and portal hypertension: its role in the regulation of intrahepatic and splanchnic vascular resistance. Seminars in Liver Disease 19, 411–426.

Yang, W., Benjamin, I.S., Sherwood, R., Alexander, B., 1998. Correlation of endothelium-dependent and -independent vasodilatation with liver function tests during prolonged perfusion of the rat liver. J. Pharmacol. Toxicol. Methods 40, 227–234.

Yang, W., Benjamin, I.S., Alexander, B., 1999. Localisation of hepatic vascular resistance sites in the isolated dual-perfused rat liver. Eur. J. Pharmacol. 364, 13–21.

Yang, W., Benjamin, I.S., Sherwood, R., Salisbury, J., Alexander, B., 2000. Effect of perfusate albumin on organ viability and vascular responses in the in vitro dual-perfused rat liver. J. Pharmacol. Toxicol. Methods 43, 225–231.

Yang, W., Benjamin, I.S., Alexander, B., 2001. Nitric oxide modulates acetylcholine-induced vasodilatation in the hepatic arterial vasculature of the dual-perfused rat liver. Acta Physiol. Scand. 171, 413–418.

Zimmermann, H., Kurzen, P., Klossner, W., Renner, E.L., Marti, U., 1996. Decreased constitutive hepatic nitric oxide synthase expression in secondary biliary fibrosis and its changes after Roux-en-Y choledochojejunostomy in the rat. J. Hepatol. 25, 567–573.