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Vasoconstrictor responsiveness of the rat mesenteric arterial bed in cirrhosis

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1 The effects of cirrhosis on mesenteric vascular reactivity were assessed in constantly perfused mesenteric arterial beds isolated from cirrhotic rats (carbon tetrachloride with phenobarbitone, $n=6$), and from phenobarbitone-treated and untreated age-matched controls ($n=4,5$).

2 At a constant flow rate of 5 ml min^{-1} there was no difference in basal perfusion pressure between the groups. Electrical field stimulation (EFS; 4-32 Hz, 90V, 1 ms, 30 s) of perivascular nerves caused frequency-dependent increases in perfusion pressure which were not different between the groups. Dose-dependent vasoconstrictor responses to exogenous noradrenaline (NA), methoxamine (an α_1-adrenoceptor agonist), adenosine 5'-triphosphate (ATP) and vasopressin were also similar between the groups.

3 The nitric oxide (NO) synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 30 μM) augmented constrictor responses to NA, EFS, methoxamine and vasopressin in all groups, and as shown for EFS and NA, this was reversed by L-arginine (300 μM). However, the maximum constrictor responses of cirrhotic preparations in the presence of L-NAME were significantly lower than those of both groups of control animals at the highest frequency of EFS (32 Hz) and highest doses of NA (0.15 and 0.5 μmol) and, compared to phenobarbitone-treated controls, methoxamine (5 μmol). Responses to ATP were significantly augmented by L-NAME only in the cirrhotic group.

4 A step-wise increase in perfusate flow to 10, 15 and 20 ml min^{-1} produced a broadly similar increase in perfusion pressure within each group. At increased flow rates, cirrhotic preparations were hyporesponsive to NA (15 nmol) compared to the phenobarbitone-treated animals but not the untreated controls. Glibenclamide (5 μM) or L-NAME (30 μM) had no significant effect on the relationship between flow and perfusion pressure or on responses to NA at the different flow rates.

5 We conclude that sympathetic neurotransmission is unchanged in cirrhosis. Endogenous NO is important in modulation of constriction in both normal and cirrhotic states. Changes in NO may occur in cirrhosis, although the role of this in hyporesponsiveness of cirrhotic preparations to NA at higher flow rates and to the greater potentiation of ATP-mediated constriction in the presence of L-NAME, together with the impact of factors such as changes in calcium and potassium channels, is not entirely clear.

Keywords: Cirrhosis; endothelium-derived relaxing factor; glibenclamide; hyporesponsiveness; nitric oxide, rat mesenteric arterial bed

Introduction

Decompensated cirrhosis is characterized by an increase in cardiac output and systemic vasodilatation. Current evidence suggests that vasodilatation of the mesenteric vascular bed contributes significantly to the decrease in systemic vascular resistance in cirrhosis. Several mechanisms have been suggested to contribute to splanchnic hyperaemia including an increase in the vasodilators adenosine, glucagon, prostacyclin and atrial natriuretic peptide (Champigneulle *et al.*, 1991; Lee *et al.*, 1992; Stark & Szurszewski, 1992) or diminished responsiveness to vasoconstrictors such as catecholamines, angiotensin II or arginine vasopressin (Kiel *et al.*, 1985; Wu *et al.*, 1991; Mesh *et al.*, 1991; Braillon *et al.*, 1993; Bomzon *et al.*, 1993). It has been suggested that endotoxaemia associated with cirrhosis induces vascular nitric oxide (NO) synthase, leading to increased synthesis of NO and vasodilatation (Vallance & Moncada, 1991), and there is evidence both for and against this hypothesis. In cirrhotic rats, hyporesponsiveness of mesenteric arteries to potassium chloride (Sieber *et al.*, 1993) and of the aorta to angiotensin II (Castro *et al.*, 1993) was suggested to be at least partially mediated by NO. Claria *et al.* (1992) have shown enhanced sensitivity to the pressor effect of inhibitors of NO in cirrhotic rats although other workers have failed to demonstrate increased NO synthesis (Sogni *et al.*, 1992). Reversal of systemic and splanchnic vasodilatation by inhibition of the formation of NO in cirrhosis and portal hypertension (Pizcueta *et al.*, 1992a,b) has also been described. Increased serum nitrite and nitrate levels are present in patients with cirrhosis (Guarner *et al.*, 1993), although there was no difference in the fall in forearm blood flow by local inhibition of NO synthesis between controls and patients with alcoholic cirrhosis (Calver *et al.*, 1994).

Recently, altered relaxation of arterial smooth muscle mediated via the efflux of K^+ through membrane K^+ channels has been proposed to contribute to cirrhosis-induced vasodilatation, since the ATP-sensitive K^+ channel blocker, glibenclamide, caused a significantly higher inhibition of K^+ outward currents in vascular smooth muscle of cirrhotic rats than in controls (Moreau *et al.*, 1994). These ATP-sensitive K^+ channels may be activated by shear stress since glibenclamide blocked vasodilatation of the pulmonary vasculature in response to increased flow (Hasséssian *et al.*, 1993).

The aim of the current study was to examine the roles of NO and ATP-sensitive K^+ channels as contributors to mesenteric hypotension in cirrhosis. Mesenteric arterial beds isolated from cirrhotic rats (carbon tetrachloride (CCl_4)/phenobarbitone), and from phenobarbitone-control and untreated

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controls were tested for vasoconstrictor responsiveness before and during inhibition of NO synthase with N^G-nitro-L-arginine methyl ester (L-NAME) (Moore *et al.*, 1990). The effects of L-NAME or glibenclamide on pressure changes with increased flow, and constrictor responses to NA at increased flow, were also determined.

Methods

Experimental groups

Cirrhosis was induced in 7 male Wistar rats (140–160 g), by a modification of the method of Proctor & Chatamra (1982) as previously described (Firth *et al.*, 1989). Animals were allowed free access to drinking water containing 350 mg l^{-1} phenobarbitone (to induce liver enzymes), for 2 weeks before treatment with CCl_4 and thereafter. CCl_4 was administered by gavage weekly for 3–4 months, with a starting dose of 40 μl increasing to a maximum of 600 μl. Cirrhosis was judged to have occurred when abdominal ascites was present as indicated by a sudden increase in body weight and by clinical inspection. A further 5 rats were given phenobarbitone alone (phenobarbitone controls), and a group of 5 age-matched animals served as untreated controls. Segments of liver from each of two lobes were excised from each of these animals after killing and were fixed in 4% paraformaldehyde for subsequent histological analysis.

Isolated mesenteric arterial bed preparation

Rats were killed by asphyxiation in CO_2 followed by cervical dislocation. The mesenteric bed was isolated and perfused essentially as described previously (Ralevic *et al.*, 1993). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, the gut dissected away and the preparation mounted on a stainless steel grid (7 × 5 cm) in a humid chamber (custom made at University College London). The preparation was perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, Illinois, U.S.A.). The perfusate was Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2-5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, Mass, U.S.A.). Preparations were allowed to equilibrate for approximately 30 min prior to experimentation.

Electrical field stimulation (90V, 1 ms, 30 s) was applied at increasing frequencies (4–32 Hz) to obtain frequency-response curves. Vasoconstrictor responses of preparations were then tested to doses (50 μl bolus injections) of increasing concentration of noradrenaline (NA), methoxamine, ATP and vasopressin. Individual doses were applied at intervals of at least 2 min, but as much as 20 min, depending on the time it took for the tone to return to baseline. Approximately 10 min was allowed between consecutive dose-response curves. Flow-pressure relationships of the preparations were studied by incrementally increasing the perfusion flow rate from 5 to 10, 15 and 20 ml min^{-1}. At each increment responses to a single dose of NA (15 nmol; approximately the ED_{50} dose) were tested as soon as the preparations had stabilized at their new levels of tone. Flow was returned to 5 ml min^{-1} and preparations were equilibrated with L-NAME (30 μM) for 30 min, after which response curves to electrical field stimulation (EFS), NA, methoxamine, ATP and vasopressin were established, followed by a repeat of the step-wise increases in flow. Preparations were then equilibrated with L-arginine (300 μM, for 20 min) (still in the presence of L-NAME) and responses to EFS and NA were re-established. After washout of all drugs preparations were equilibrated with glibenclamide (5 μM, for 15 min) and the flow-pressure relationships of the preparations were reassessed.

Drugs used

All drugs were applied as 50 μl bolus injections into a rubber septum proximal to the preparation. Drug dilutions were made up daily from stock solutions of 10 or 100 mM in distilled water, except for NA which was made up as a stock solution in 0.1 mM ascorbic acid and diluted in distilled water, and glibenclamide, which was made up as a stock solution of 10 mM in dimethylsulphoxide (DMSO). The following drugs were obtained from Sigma: adenosine 5'-triphosphate (disodium salt), L-arginine, glibenclamide, N^G-nitro-L-arginine methyl ester, methoxamine (hydrochloride), noradrenaline bitartrate. Vasopressin was obtained from Cambridge Research Biochemicals (U.K.).

Data analysis

All data are presented as means ± s.e. Statistical analysis was performed by analysis of variance to see if there were differences between the groups taking into account all frequencies/doses, followed by a modified *t* test to see where the differences lay, or by Student's paired *t* test as appropriate. To analyse the effect of flow rate, an unbalanced ANOVA was performed to characterize differences in linear trend, using the restricted maximum likelihood estimation procedure in the Genstat statistical software package. A probability (P) of 0.05 was taken as the level of statistical significance.

Results

Cirrhotic model

Of 7 animals treated with CCl_4 only 6 were used in the present study based on the development of fibrosis and regenerative nodules in the liver, as revealed by histological examination. All 6 rats had mild to moderate ascites on opening the abdomen (5–10 days after the original clinical diagnosis of ascites). The data from one phenobarbitone-treated rat are excluded from the analysis due to technical failure of the experiment after the first period of EFS. Collateral vessels around the superior mesenteric artery were characteristically evident in cirrhotic rats, but not in either of the control groups. Rat final body weights were: 521 ± 41 g (n = 5) controls; 476 ± 26 g (n = 4) phenobarbitone controls; 431 ± 29 g (n = 6) cirrhotics.

Electrical field stimulation

EFS (4–32 Hz, 90V, 1 ms, 30s) elicited frequency-dependent vasoconstrictor responses which were short-lasting and similar between the groups (Figure 1). In the presence of L-NAME (30 μM) responses to EFS were significantly enhanced in each group. At the highest frequencies (24 and 32 Hz) responses of the cirrhotic group were approximately 50 and 100 mmHg less than those of the control and phenobarbitone-treated control groups respectively. The magnitude of the response at 32 Hz in the presence of L-NAME was significantly less in cirrhotics compared with both control groups (P < 0.05) (Figure 1). L-Arginine (300 μM) reversed the potentiation that was induced by L-NAME, as assessed in control and cirrhotic rats (data not shown).

Noradrenaline

Noradrenaline elicited dose-dependent vasoconstrictor responses in the mesenteric arterial preparations which were not different between the groups (Figure 2). At doses of below approximately 50 nmol these responses were typically tran-

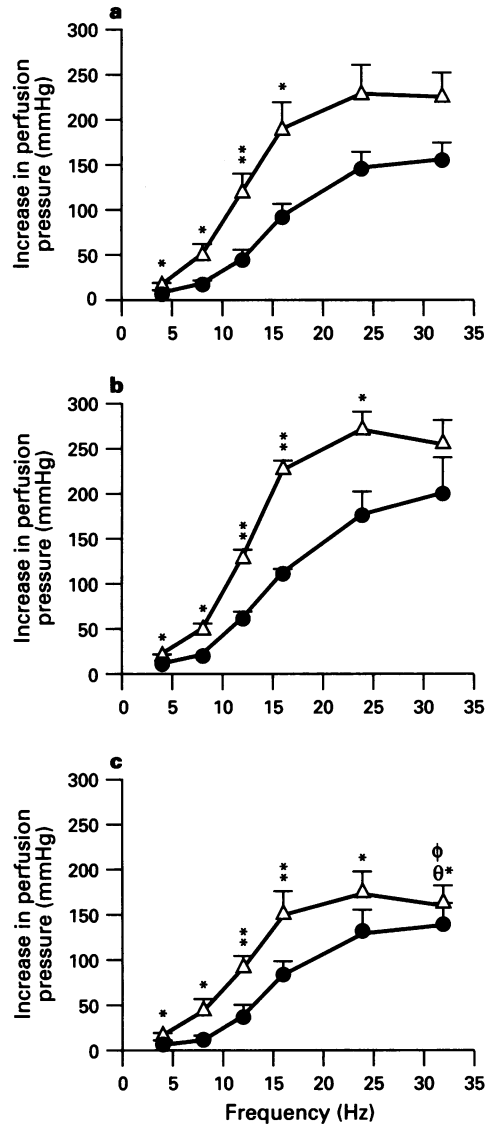

Figure 1 Frequency-dependent constrictor responses to electrical field stimulation (EFS; 4–32 Hz, 90V, 1 ms, 30 s) of mesenteric arterial preparations from (a) control ($n=5$), (b) phenobarbitone control ($n=4$) and (c) cirrhotic ($n=6$) rats. Responses are shown in the absence of agents (●) and in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 30 μM) (△). There was no significant difference between the two control groups and the cirrhotic group under basal conditions. 'Basal' denotes responses in the absence of drugs. Within groups differences (L-NAME versus basal) are denoted by * ($P<0.05$) and ** ($P<0.01$). In the presence of L-NAME a significantly smaller response produced by the cirrhotic group compared to the phenobarbitone control group is denoted by θ ($P<0.05$), and to the untreated controls by ø ($P<0.05$).

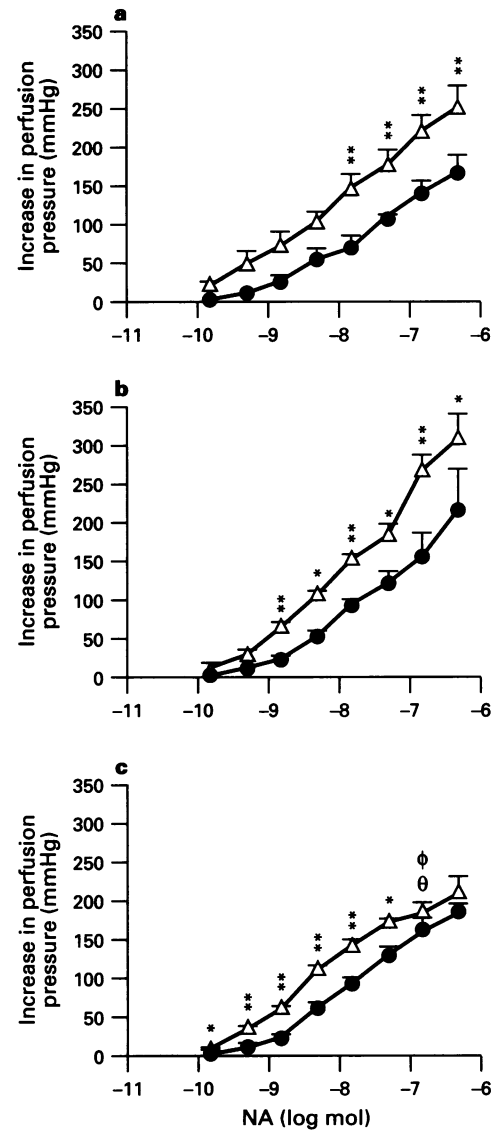

Figure 2 Dose-response curves to noradrenaline (NA) of mesenteric arterial preparations from (a) control ($n=5$), (b) phenobarbitone control ($n=4$) and (c) cirrhotic ($n=6$) rats. Responses are shown in the basal state (●) and in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 30 μM) (△). There was no significant difference between the two control and cirrhotic groups under basal conditions. 'Basal' represents responses in the absence of drugs. Within groups differences (L-NAME versus basal) are denoted by * ($P<0.05$) and ** ($P<0.01$). A significant difference between cirrhotic and phenobarbitone control groups in the presence of L-NAME is denoted by θ ($P<0.05$), and to the untreated controls by ø.

sient, returning to baseline within 1–2 min. At doses of 0.15 and 0.5 μmol the responses were longer in duration requiring up to 10 min to return fully to baseline. At doses of NA between 0.15 and 50 nmol, L-NAME (30 μM) augmented the responses to a similar extent in all groups. At the top two doses of NA (0.15 and 0.5 μmol), however, responses in the cirrhotic group in the presence of L-NAME were smaller than those in either of the control groups, reaching statistical significance at 0.15 μmol NA ($P<0.05$), but not at 0.5 μmol NA ($P=0.07$, ANOVA). However, analysis of variance comparison of the differences in responses at the top two doses of NA caused by adding L-NAME revealed a significantly smaller enhancement in the cirrhotic group compared with either control group ($P<0.05$) (Figure 2). L-Arginine (300 μM) reversed the potentiation to NA in the control and cirrhotic groups (data not shown).

Methoxamine

Bolus injections of methoxamine elicited dose-dependent vasoconstrictor responses that were generally similar between the groups and these were augmented by L-NAME (30 μM) to a similar degree (Figure 3). In the cirrhotics, however, the response to the highest dose of methoxamine (5 μmol) in the presence of L-NAME was significantly less than in the phenobarbitone group ($P<0.05$). Responses were transient at all doses tested, returning to baseline within approximately 2 min, except for the top dose of 5 μmol which took up to 10 min to return to baseline.

Adenosine 5'-triphosphate

ATP elicited similar dose-dependent constrictions in cirrhotic, control and phenobarbitone-control groups (Figure 4). Re-

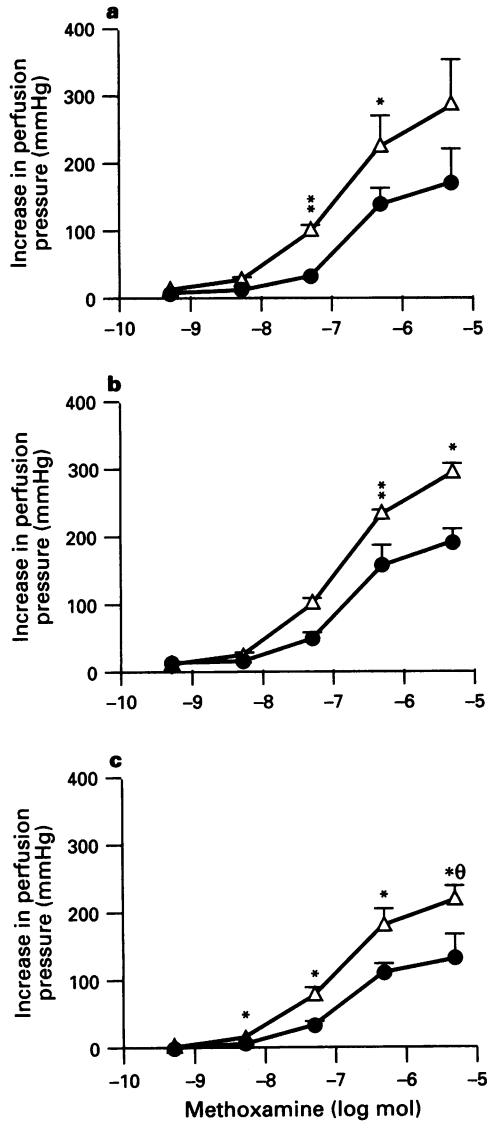

Figure 3 Dose-response curves to methoxamine of mesenteric arterial preparations from (a) control ($n=5$), (b) phenobarbitone control ($n=4$) and (c) cirrhotic ($n=6$) rats. Responses shown are in the basal state (●) and in the presence of N^G-nitro-L-arginine methyl ester (30 μM) (△). There was no significant difference between the two control and cirrhotic groups under basal conditions. 'Basal' represents responses in the absence of drugs. Within groups differences (L-NAME versus basal) are denoted by * ($P<0.05$) and ** ($P<0.01$). A significant difference between cirrhotic and phenobarbitone control groups in the presence of L-NAME is denoted by θ ($P<0.05$).

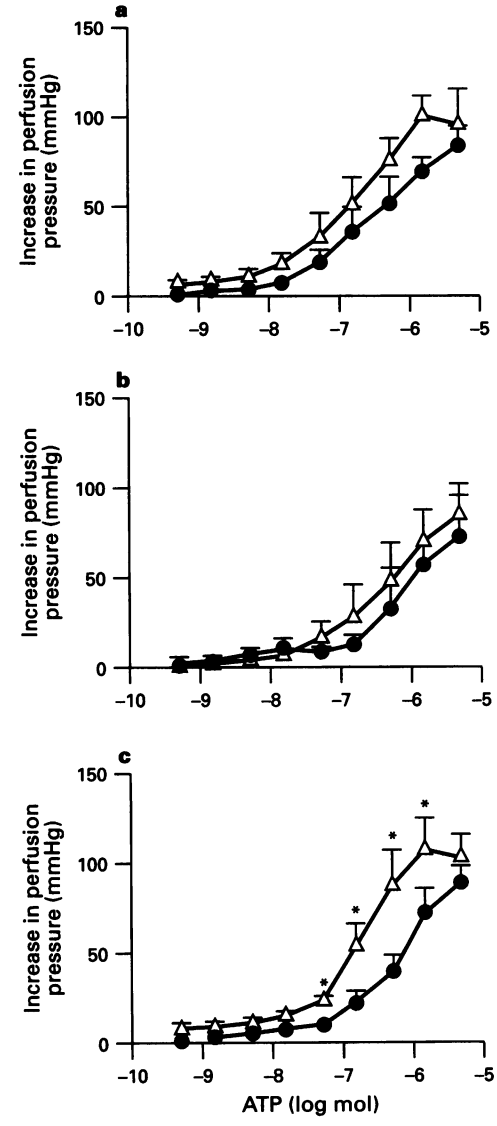

Figure 4 Dose-response curves to adenosine 5'-triphosphate (ATP) in mesenteric arterial preparations from (a) control ($n=5$), (b) phenobarbitone control ($n=3$) and (c) cirrhotic ($n=6$) rats. Responses shown are in the basal state (●) and in the presence of N^G-nitro-L-arginine methyl ester (30 μM) (△). There was no difference between the two control and the cirrhotic groups under basal conditions. 'Basal' represents responses in the absence of drugs. Within groups differences ($P<0.05$) are denoted by * (L-NAME versus basal).

sponses to ATP were transient at all doses tested. L-NAME (30 μM) augmented responses to ATP, but this reached statistical significance only in the cirrhotic group. One phenobarbitone-treated preparation did not respond to ATP due to a period of instability of the preparation at the time of ATP injection.

Vasopressin

Vasopressin elicited long-lasting constrictor responses which took up to 15 min to return to baseline at the highest dose tested (0.5 nmol). Dose-dependent vasoconstrictor responses to vasopressin were similar between the groups and these were augmented to a similar degree by L-NAME (30 μM) in the control and cirrhotic groups (data not shown).

Flow

At basal flow (5 ml min^{-1}) there was no significant difference in perfusion pressure between the groups. Stepwise increases in flow rate (up to 20 ml min^{-1}) produced an increase in perfusion pressure within each group that was marginally but significantly greater for the phenobarbitone group than either of the other two groups ($P<0.05$) independently of the type of type (glibenclamide, 5 μM or L-NAME, 30 μM) in the perfusate (ANOVA for linear trend).

Noradrenaline with flow

Comparing responses to NA at increasing flow rates, there was a progressive decrease in responsiveness to NA in the cirrhotic

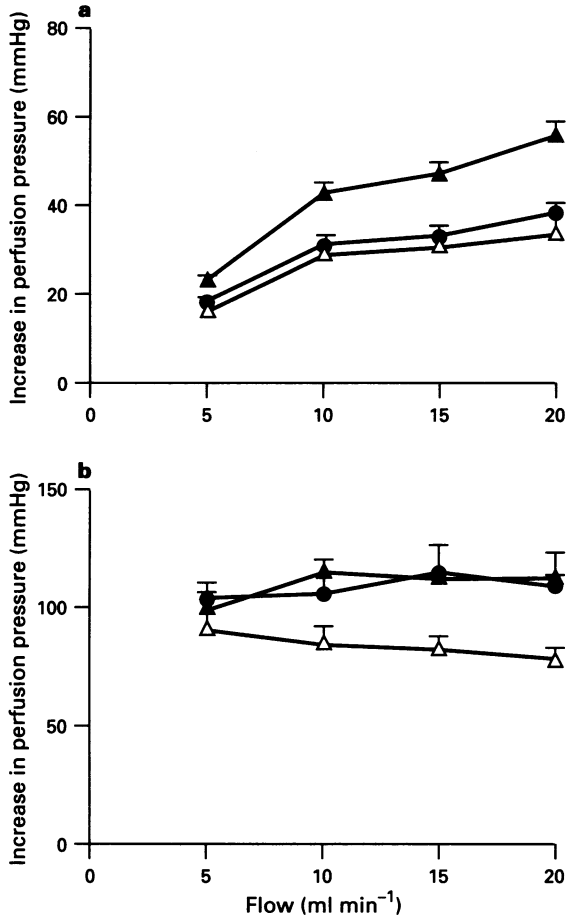

Figure 5 (a) Increase in perfusion pressure (mmHg) with step-wise increases in perfusate flow rate from 5 to 20 ml min^{-1} in mesenteric arterial preparations from control (●, $n=5$), phenobarbitone control (▲, $n=4$) and cirrhotic rats (△, $n=6$) in the absence of L-NAME or glibenclamide. The increase in flow with perfusion pressure was significantly greater in the phenobarbitone group. (b) Vasoconstrictor responses (increase in perfusion pressure, mmHg) due to bolus injections of doses of NA (15 nmol) applied at increasing flow rates in mesenteric arterial preparations of control (●, $n=5$), phenobarbitone control (▲, $n=4$) and cirrhotic (△, $n=6$) rats in the absence of L-NAME or glibenclamide. The cirrhotic group were significantly less responsive to NA than the phenobarbitone controls ($P<0.05$).

group which was significantly different from a progressive increase in responsiveness with flow, noted in the phenobarbitone-treated animals ($P<0.05$) though not in the untreated controls (Figure 5). There was no significant difference between the effect of NA in the two control groups. There was no significant effect of type of drug (glibenclamide, 5 μM or L-NAME, 30 μM) in the perfusate on these changes (ANOVA for linear trend).

Discussion

The present results show that at a flow rate of 5 ml min^{-1} the mesenteric arterial vasculature of cirrhotic rats elicits similar constrictor responses to EFS, or bolus injections of NA, methoxamine, ATP and vasopressin compared with controls. This finding was unexpected since we and others have previously demonstrated hyporesponsiveness to continuous perfusion of methoxamine (as opposed to bolus injections) in the cirrhotic mesenteric arterial bed (Sieber & Groszmann, 1992; Mathie *et al.*, 1992). The reason for this difference is not known but may reflect changes in the utilization of calcium for vasoconstriction in the cirrhotic vascular bed. Mesenteric resistance vessels of the rat have been shown to respond to prolonged exposure to NA by an α_1-adrenoceptor-mediated phasic constriction followed by tonic constriction (Cauvin & Malik, 1984). The phasic component is primarily dependent on intracellular Ca^{2+} release, while the tonic component is completely dependent on Ca^{2+} influx (Cauvin & Malik, 1984). The utilization of intra- and extracellular Ca^{2+} for NA-mediated phasic and tonic responses respectively has also been described in smooth muscle of the rabbit mesenteric artery (Itoh *et al.*, 1983; Kanmura *et al.*, 1983). Transient constrictions to doses of methoxamine and NA may be analogous to the phasic component of constriction, requiring intracellular Ca^{2+}, while maintained constriction to continuous perfusion with methoxamine may be analogous to the tonic component and require extracellular Ca^{2+}. An implication of this observation is that mesenteric arteries of cirrhotic rats are defective with respect to influx or utilization of extracellular Ca^{2+}. This suggestion is consistent with the finding of Hartleb *et al.* (1993) who showed that a limited opening of L-type calcium channels contributed to vascular hyporeactivity to endothelin-1 in cirrhotic rats, and suggested that this was more important than overproduction of NO. Further studies with calcium-entry blockers are required to examine this hypothesis in rat mesenteric arteries.

The lack of differences in constrictor responses to EFS between the groups under control conditions suggests that mesenteric arterial sympathetic nerves are not impaired in cirrhosis and do not contribute to a hyperdynamic mesenteric circulation. However, in the presence of L-NAME, maximal constriction of the cirrhotic preparations to EFS as well as to NA and methoxamine was significantly less than in the control groups. It is possible that this difference does not involve NO *per se* since hyporesponsiveness was not shown across the frequency- and dose-response curves. It may be that this impaired ability of the cirrhotic vasculature to achieve maximal constriction is a result of structural and/or functional changes in the vascular smooth muscle. Folkow *et al.* (1992) have suggested that in perfused rat mesenteric arteries a decrease in maximal constrictor responsiveness is a consequence of lower wall thickness. Gastric vascular ectasia (dilatation and thinning) is a recognised feature associated with cirrhosis (Lowes & Rode, 1989; Payen *et al.*, 1995). Alternatively, as suggested to explain hyporesponsiveness during maintained constriction to methoxamine, there may be a more limited opening of calcium channels which comes apparent during pronounced constriction in cirrhosis. On the other hand, the pronounced constrictor responses to vasopressin in the absence and presence of L-NAME were similar between the groups, suggesting that other mechanisms may be involved.

Endogenous NO, released continuously under basal conditions, is an important modulator of vascular function in both control and cirrhotic preparations as shown by the augmentation by L-NAME of constrictor responses to EFS, NA, methoxamine and vasopressin. The reversal of potentiation by L-arginine, the substrate for NO synthase, is consistent with an NO-mediated response. It is not clear why NOS inhibition with L-NAME did not augment responses to ATP under control conditions. In rat mesenteric arteries ATP elicits constriction via P_{2X}-purinoceptors on the smooth muscle and vasodilatation via endothelial P_{2Y}-purinoceptors (Ralevic & Burnstock, 1988). P_{2X}-purinoceptors mediate vasoconstriction by acting as non-selective cation channels, whereas vasoconstrictor responses to NA, vasopressin and methoxamine are mediated by receptors acting as ligand-gated cation channels. It is possible that these receptors are differently modulated by smooth muscle NOS.

Significant augmentation of responses to ATP in the presence of L-NAME, however, did occur in the cirrhotic group. This is consistent with our previous findings in the rat mesenteric arterial bed in cirrhosis, which showed that in raised-tone preparations responses to ATP were converted from vasodilatation to vasoconstriction in the presence of L-NAME only in cirrhosis group (Mathie *et al.*, 1996). L-NAME clearly implicates NO in this effect both in the present and in our previous study, although it is intriguing that there are no obvious differences in responses to ATP between the groups in the absence of L-NAME.

Assessment of the perfusion pressure at different flow rates revealed a similar increase in perfusion pressure with step-wise increases in flow in all the groups, though the increase in the phenobarbitone group showed evidence of a statistically significant greater effect. More importantly, however, in the cirrhotic group responses to NA were hyporesponsive at higher flow rates compared to those of the control groups (significantly so in the case of the phenobarbitone-treated rats). It is possible that this is due to an increase in distensibility as a consequence of a thinner mesenteric arterial wall in cirrhosis, or due to differences in the utilization of calcium at these different flow rates. Clearly, there is an important relationship between flow, perfusion pressure and responsiveness which needs to be considered in studies of hypotension when perfusion systems are involved. It must also be noted that phenobarbitone *per se* may have some effect on haemodynamics, as indicated by mild portal hypertension and increased portal flow (Yates *et al.*, 1979).

A recent report suggested that abnormal activation of K^+ currents may contribute to the relaxation of aortic smooth muscle cells in cirrhotic rats since glibenclamide had a greater inhibitory effect on outward K^+ currents in cirrhosis than in controls (Moreau *et al.*, 1994). It is well known that NO release is increased with enhanced flow or shear stress (Kelm *et al.*, 1991; Davies & Tripathi, 1993), raising the possibility that greater levels of endothelial NO in cirrhosis may also be involved. However, these suggestions are not supported by the finding that perfusion pressure and responses to NA at different flow rates were not modified by L-NAME or glibenclamide. These negative results do not exlcude a role for shear stress-activated endothelial Ca^{2+} channels (Lansman *et al.*, 1987) or K^+ currents (Olesen *et al.*, 1988).

In summary, constrictor responses of cirrhotic preparations to doses of methoxamine were similar to those in controls, in contrast to the hyporesponsiveness reported during continuous perfusion. However, maximum constriction of the mesenteric vasculature of cirrhotic rats, at high frequencies of EFS and high doses of NA and methoxamine in the presence of L-NAME, was significantly smaller than in the controls. Changes in endogenous NO in cirrhosis may be involved in the greater augmentation of constrictor responses to ATP during NO synthesis inhibition, and possibly in the hyporesponsiveness to NA at increased flow.

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