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Localisation of hepatic vascular resistance sites in the isolated dual-perfused rat liver

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Abstract

The locations of the vascular resistance sites which regulate vascular tone in the hepatic arterial and portal venous vasculatures of the rat liver were identified using a new, in vitro, dual-perfused liver preparation. Twelve livers of male Wistar rats were perfused via the hepatic artery and portal vein at fixed flow and at physiological pressure. Dose-related vasoconstriction to injections or infusions of noradrenaline was measured as transient or sustained increases in perfusion pressure, respectively, in the hepatic arterial and portal venous vasculatures. Direct injections/infusions of noradrenaline refer to those administered into the vasculature from which pressure was recorded, e.g., the effects of hepatic arterial (direct) injections/infusions of noradrenaline upon hepatic arterial perfusion pressure. Indirect injections/infusions of noradrenaline were those administered to the adjacent afferent vasculature, e.g., the effects of portal venous (indirect) injections of noradrenaline upon hepatic arterial perfusion pressure. The converse applies for recordings of portal venous perfusion pressure. The $-\log(M)$ ED_{50} values to direct (hepatic arterial) and indirect (portal venous) injections in the hepatic artery were 4.25 ± 0.20 and 3.40 ± 0.10, respectively, and were significantly different ($P < 0.01$, Student's unpaired *t*-test); the $-\log(M)$ ED_{50} values to direct (portal venous) and indirect (hepatic arterial) injections in the portal vein were 3.91 ± 0.08 and 3.85 ± 0.11, respectively, and were not significantly different ($P > 0.05$, Student's unpaired *t*-test). Similarly, the $-\log(M)$ ED_{50} values to direct (hepatic arterial) and indirect (portal venous) infusions in the hepatic artery were 5.28 ± 0.11 and 3.75 ± 0.12, respectively, and were significantly different ($P < 0.01$, Student's unpaired *t*-test); the $-\log(M)$ ED_{50} values to direct (portal venous) and indirect (hepatic arterial) infusions in the portal vein were 5.31 ± 0.19 and 5.70 ± 0.16, respectively, and were not significantly different ($P > 0.05$, Student's unpaired *t*-test). These results demonstrated that there is little transfer of noradrenaline from the portal venous to the hepatic arterial resistance sites, but significant transfer from the hepatic artery to the portal venous suggesting that; (a) the portal venous resistance sites are located at the sinusoidal or post-sinusoidal level; and (b) the hepatic arterial resistance sites are located at the pre-sinusoidal level.

Keywords: Resistance site, hepatic; Transhepatic effect; Liver in vitro, perfused, rat; Vascular

1. Introduction

Perfused rat livers have been used for physiological and pharmacological studies into the mechanisms which regulate hepatic metabolism and vascular tone for many years (Ross, 1972). However, the mechanisms which regulate hepatic blood flow in the rat liver remain unclear, partially due to the lack of a suitable in vitro dual-perfused, model where the complex interrelationship between the hepatic artery and portal vein can be investigated in detail. It has been known that a complex reciprocal blood flow relationship exists between the two vasculatures and that reductions in portal venous blood flow induce a partial compensatory increase in hepatic arterial blood flow, although total hepatic blood flow was not fully regained in the canine liver (Burton-Opitz, 1911). The magnitude of the increase in hepatic arterial blood flow, later termed the 'buffer response' (Lautt and Legare, 1985), has been correlated to the prognosis of patients who had undergone surgery for portal vein decompression for portal hypertension (Burchell et al., 1976). A detailed understanding of the mechanisms which regulate hepatic vascular tone is essential for development of pharmacological strategies for treatment of pathological conditions such as portal hypertension, cirrhosis and liver failure but also for improvement in transplantation protocols (Gryf-Lowczowski et al., 1997).

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The location and characterisation of the hepatic arterial and portal venous resistance sites is particularly important for the development of pharmacological agents which may modulate hepatic vascular tone. Previous studies in vivo elicited profound changes in systemic haemodynamics and prevented detailed receptor characterisation studies from being conducted (Mathie and Alexander, 1990). An in vitro, dual-perfused rabbit liver preparation was therefore developed where perfusion conditions could be accurately controlled independent of alterations in systemic haemodynamics (Alexander et al., 1992) which was stable for up to 5 h of perfusion (Browse et al., 1995a). The purinoceptor population was characterised (Ralevic et al., 1991) and the interrelationship between purines and nitric oxide in portal venous-induced hepatic arterial vasodilatation described (Browse et al., 1994; Alexander, 1996). However, one limitation of the rabbit model was that it was impractical for studies of the mechanisms which regulate hepatic vascular tone in the diseased liver or following cold preservation for transplantation (Alexander et al., 1992). Dual-perfusion of the isolated rat liver has been reported (Gardemann et al., 1987; Lee and Filkins, 1988; Reichen, 1988) where perfusion of the hepatic artery was approached through cannulation of the descending aorta and exposure of the coeliac axis. The gastroduodenal, gastric, and other relevant arteries, which otherwise diverted blood away from the hepatic artery, were also ligated. However, these models were laborious and left doubts as to whether all the relevant arteries and their collateral vessels had, in fact, been ligated or accidentally divided during excision of the liver. Moreover, these models were unsuitable for pharmacological studies where confirmation that the responses recorded were derived specifically from the hepatic arterial vascular bed was essential.

The present study reports upon the characterisation of vascular responses to applied noradrenaline in both the hepatic artery and portal vein, in order to elucidate the localisation of hepatic arterial and portal venous resistance sites in the rat liver. This has been achieved using a new simple, in vitro, perfusion model which uses a direct cannulation procedure of the common hepatic artery, based upon previous models in the rabbit (Alexander et al., 1992) and the rat (Alexander et al., 1993, 1995). The effects of both constant infusions of fixed concentrations of nor-

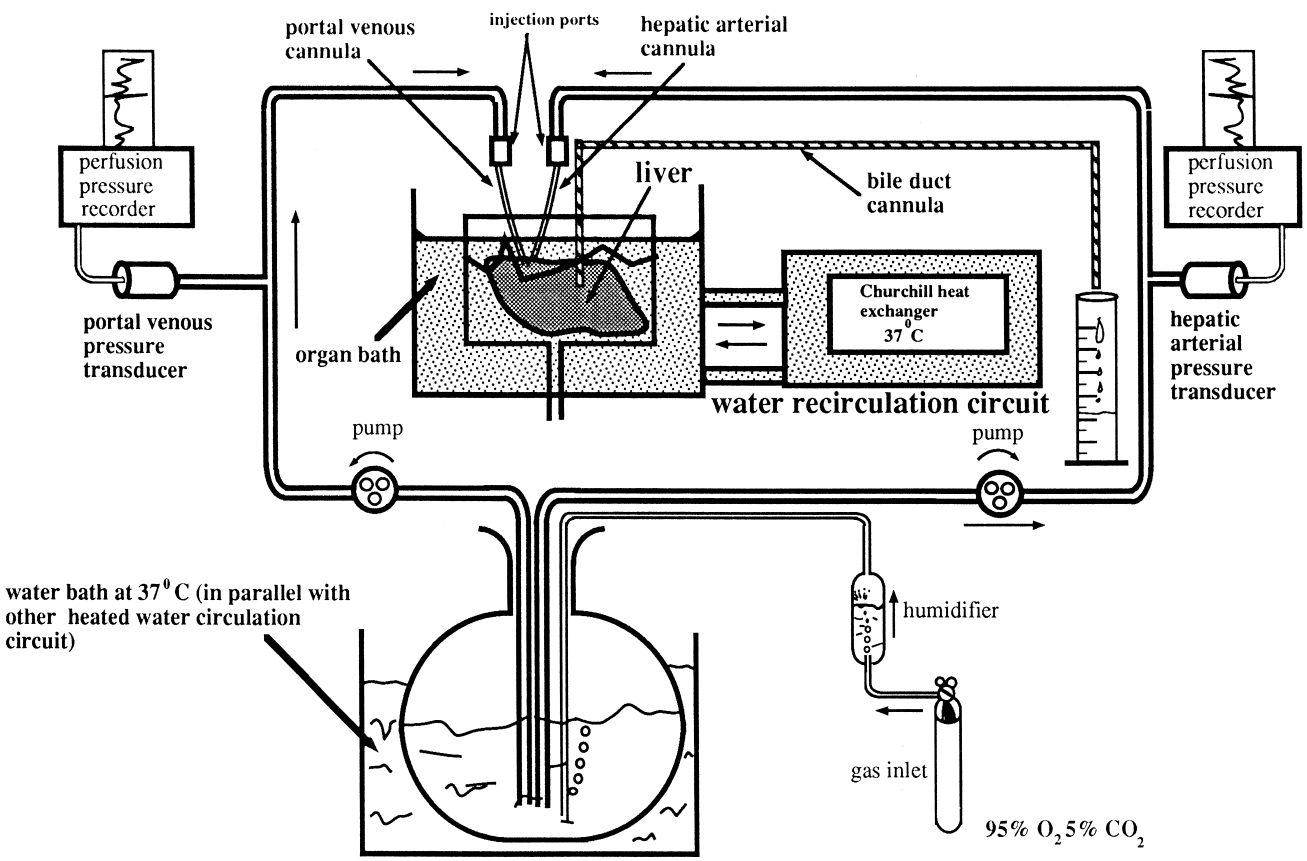

Fig. 1. Schematic diagram of the perfusion circuit. The liver is suspended in perfusate in a specially designed organ bath at 37°C which promotes improved perfusion of the liver extremities. The liver is perfused simultaneously via the hepatic artery and portal vein at constant flow rates of 0.32 ± 0.01 and 0.98 ± 0.03 ml min^{-1} g $liver^{-1}$ respectively from a shared reservoir of perfusate. The perfusion pressures generated by the hepatic arterial and portal venous cannulae alone at each flow rate were 25.8 ± 2.8 and 5.1 ± 0.9 mm Hg, respectively. These were measured prior to the cannulation of the liver and subtracted from the recorded pressure at each respective flow rate, once the liver was placed on perfusion. Injection of noradrenaline into either injection port resulted in transient increases in perfusion pressure which were indicative of vasoconstriction.

adrenaline and bolus injections are described and compared. Basic characteristics of the model such as bile volume production, perfusion pressure and flow rate are also presented.

2. Materials and methods

2.1. Surgical protocol

Twelve Wistar rats (250–300 g) were anaesthetised with sodium pentobarbitone (3 mg 100 g^{-1} i.p.). A midline incision was made and the common bile duct cannulated. The gastroduodenal artery and vein were then carefully dissected and the vein ligated and divided, followed by the gastroduodenal artery. The common hepatic artery was then exposed and carefully dissected away from underlying tissue. Following heparinisation (100 units per kg i.v.), the hepatic artery was cannulated with a 0.75-mm o.d.(3FG) cannula (Portex, Hythe, Kent, England, UK) and the portal vein cannulated with a 1.65-mm o.d. (5FG) cannula (Portex, Hythe, Kent). The liver was then flushed with 15 ml Krebs–Bülbring buffer, excised, weighed, and placed into the organ bath and connected by the cannulae to the perfusion circuit (Fig. 1).

This consisted of two parallel circuits, one of which perfused the hepatic artery and the other, the portal vein, both of which were connected to a shared reservoir containing the perfusate at 37°C. A 'Y-piece' was inserted between the cannulae and the transmission tubing of the circuit, immediately proximal to the point of insertion of the cannulae into each respective vessel, to allow the inclusion of a sealed rubber septum for the injection of vasoactive substances. The livers were perfused in a constant flow, variable pressure mode via perfusion pumps in both the hepatic artery and portal vein circuits such that injection of a vasoconstrictor would induce a transient increase in perfusion pressure and that of a vasodilator a transient reduction in perfusion pressure. Bile was allowed to escape freely from the bile duct cannula into a calibrated collection vessel.

2.2. Liver perfusion

Livers were perfused at constant flow rates via the hepatic artery and portal vein cannulae, at 0.32 ± 0.01 and 0.98 ± 0.03 ml min^{-1} g $liver^{-1}$, respectively. Krebs–Bülbring buffer (pH 7.4, 37°C) containing (mM): NaCl, 133; KCl, 4.7; NaH_2PO_4, 1.35; $NaHCO_3$, 20.0; $MgSO_4$,

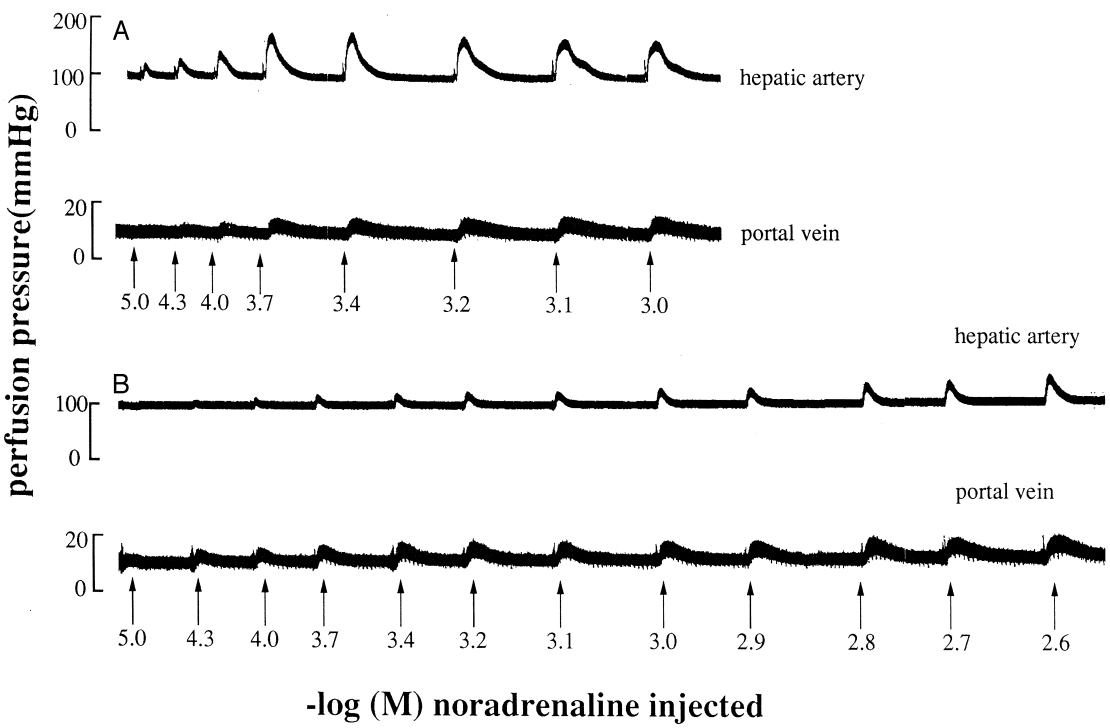

Fig. 2. Hepatic arterial and portal venous responses to bolus injections of noradrenaline. (a) Pressure traces to hepatic arterial bolus injections of noradrenaline recorded from the hepatic arterial and portal venous vasculatures. The upper (hepatic artery) trace represents the direct responses to noradrenaline; the lower (portal vein) trace represents the indirect, transhepatic, response to hepatic arterial injections of noradrenaline. (b) Pressure traces to portal venous bolus injections of noradrenaline recorded from the hepatic arterial and portal venous vasculatures. The upper (hepatic artery) trace represents the indirect, transhepatic, responses to bolus injections of noradrenaline; the lower (portal vein) trace represents the direct responses to portal venous injections of noradrenaline. The portal venous, direct, responses to portal venous injections of noradrenaline (Fig. 2b, portal vein trace) were not significantly different to the indirect, transhepatic, portal venous responses to hepatic arterial injections of noradrenaline (Fig. 2a, portal vein trace). Conversely, hepatic arterial, direct responses to hepatic arterial injections of noradrenaline (Fig. 2a, hepatic artery trace) were significantly greater than hepatic arterial, indirect, transhepatic responses to portal venous injections of noradrenaline (Fig. 2b, hepatic artery trace).

0.61; glucose, 7.8; $CaCl_2$, 2.52; and oxygenated with a 95% O_2/5% CO_2 mixture was used as the perfusate. Perfusion pressures were measured with Spectramed (Statham) P23XL physiological pressure transducers from side arms of the perfusion cannulae. Recordings of transient increases in perfusion pressure, indicative of vasoconstriction, were made on a Grass 79F polygraph (Grass Instrument, Quincy, MA, USA). The contribution of each cannula to the recorded perfusion pressure in each vasculature was accurately measured before and after completion of each perfusion. Prior to commencement of each perfusion, the pressure contribution of each cannula was measured over the full range of flows likely to be encountered before cannulation of the liver. Thus, for any applied flow rate, the pressure contributed by each cannula was accurately measured, by reference to a constructed pressure vs. flow graph, and this was deducted from the recorded value to give the true perfusion pressure within the liver as we have reported in our previous models (Alexander et al., 1984, 1992, 1995; Browse et al., 1994). Furthermore the pressure contributed by each cannula during perfusion with the liver was reconfirmed at the termination of perfusion by pressure measurements after detachment of the liver from the perfusion cannulae. This value was always identical to that measured prior to cannulation of the liver.

Bile volume production was measured directly with the calibrated collection vessel from the bile duct cannula. The homogeneity of perfusion was confirmed at termination of perfusion by injection of Evan's Blue into the hepatic artery and finally into the portal vein circuit. The livers were weighed prior to and at the termination of perfusion in order to assess the development of any oedema.

2.3. Drug administration

Noradrenaline bitartrate (Sigma) was dissolved in 0.1 mM ascorbic acid and diluted appropriately to include a dose range from $10^{-5} - 5 \times 10^{-3}(M)$ noradrenaline and each dose administered as a 0.05 ml bolus injection into either hepatic artery or portal vein in Group 1. This range

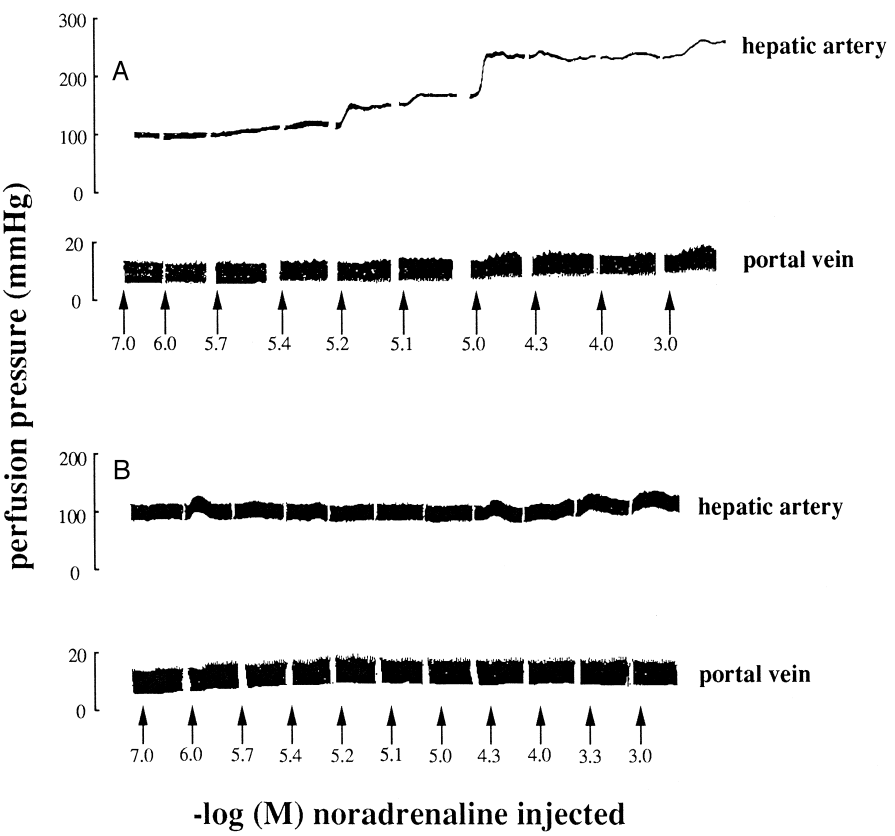

Fig. 3. Hepatic arterial and portal venous responses to infusions of noradrenaline. (a) Pressure traces to hepatic arterial infusions of noradrenaline recorded from the hepatic arterial and portal venous vasculatures. The upper (hepatic artery) trace represents the direct responses to noradrenaline; the lower (portal vein) trace represents the indirect, transhepatic, response to hepatic arterial infusions of noradrenaline. (b): Pressure traces to portal venous infusions of noradrenaline recorded from the hepatic arterial and portal venous vasculatures. The upper (hepatic artery) trace represents the indirect, transhepatic, responses to infusions of noradrenaline; the lower (portal vein) trace represents the direct responses to portal venous infusions of noradrenaline. The portal venous, direct, responses to portal venous infusions of noradrenaline (Fig. 3b, portal vein trace) were not significantly different to the indirect, transhepatic, portal venous responses to hepatic arterial infusions of noradrenaline (Fig. 3a, portal vein trace). Conversely, hepatic arterial, direct responses to hepatic arterial infusions of noradrenaline (Fig. 3a, hepatic artery trace) were significantly greater than hepatic arterial, indirect, transhepatic responses to portal venous infusions of noradrenaline (Fig. 3b, hepatic artery trace).

refers to the concentration of drug in each bolus injected and not the final concentration in the perfusate arriving at the receptor sites since this was obviously not known. Responses, measured as transient increases in perfusion pressure, were allowed to return to the basal perfusion pressure before subsequent injections were given. Group 2 were given infusions of noradrenaline at fixed concentrations, to include a dose range from 10^{-6}–$10^{-2}(M)$ noradrenaline, in order that the hepatic arterial and portal venous resistance sites received identical concentrations of noradrenaline. Infusions of noradrenaline commenced with the lowest concentrations and when the new perfusion pressure had attained a plateau, the next highest concentration was infused by transfer of the input tubing into a freshly-made reservoir of noradrenaline at 37°C.

2.4. Statistics and presentation of data

Responses were recorded as changes in perfusion pressure (mm Hg) in each vessel. Following confirmation that all the data were normally distributed, the results were expressed and presented as mean ± standard error. Statistics were carried out using Student's, two-tailed, unpaired *t*-test.

3. Results

Basal perfusion pressures were within the physiological range of 86.7 ± 2.8 and 7.2 ± 0.9 mm Hg (Group 1) and 81.4 ± 3.4 and 7.0 ± 0.3 mm Hg (Group 2) in the hepatic artery and portal vein, respectively, and remained stable for the duration of perfusion of 3 h. There were no significant differences between the two Groups. Bile volume was produced at mean rates of 0.23 ± 0.06 ml h^{-1} (Group 1) and 0.25 ± 0.02 ml h^{-1} (Group 2) and were not significantly different. The liver weights were 11.38 ± 0.40 and 10.89 ± 0.49 g (Group 1) and 10.4 ± 0.2 and 10.1 ± 0.3 g (Group 2) pre- and post-perfusion, respectively, and were not significantly different.

In Group 1, 0.05 ml bolus injections of noradrenaline into the hepatic arterial and portal venous vasculatures (Fig. 2a and b, respectively) elicited dose-related vasoconstriction which was measured as transient increases in hepatic artery and portal vein pressure. Consecutive infusions of noradrenaline into the hepatic artery and portal vein (Fig. 3a and b, respectively) induced further dose-related vasoconstriction in Group 2. Injections (Group 1, Fig. 4) and infusions (Group 2, Fig. 5) of noradrenaline produced dose-related vasoconstriction in both the hepatic artery and portal vein in response to both direct (measured in the vessel injected/infused) and indirect (measured in the non-injected/non-infused vessel) applications.

The mean $-\log(M)$ ED_{50} for hepatic arterial responses to hepatic arterial (direct) and portal venous (indirect) injections of noradrenaline were 4.25 ± 0.20 and 3.40 ± 0.10, respectively, and were significantly different ($P < 0.01$, Fig. 4a). The mean $-\log(M)$ ED_{50} for portal venous responses to hepatic arterial (indirect) and portal venous (direct) injections were 3.91 ± 0.08 and 3.85 ± 0.11, respectively, and not significantly different ($P > 0.05$, Fig. 4b). The mean $-\log(M)$ ED_{50} for hepatic arterial responses to hepatic arterial (direct) and portal venous (indirect) infusions of noradrenaline were 5.28 ± 0.11 and 3.75 ± 0.12, respectively, and were significantly different ($P < 0.01$, Fig. 4aFig. 5a, respectively). The mean $-\log(M)$ ED_{50} for portal venous responses to hepatic arterial (indirect) and portal venous (direct) infusions of noradrenaline were 5.31 ± 0.19 and 5.70 ± 0.16, respectively, and not significantly different ($P > 0.05$, Fig. 4bFig. 5b, respectively).

The maximum increases in hepatic artery perfusion pressure were 57.5 ± 7.7 and 29.3 ± 5.1 mm Hg to hepatic arterial and portal venous injections of noradrenaline, respectively, and were significantly different ($P < 0.01$). However, the maximum increases in portal venous perfusion pressure to portal venous and hepatic arterial injections of noradrenaline, respectively, of 5.32 ± 0.7 and

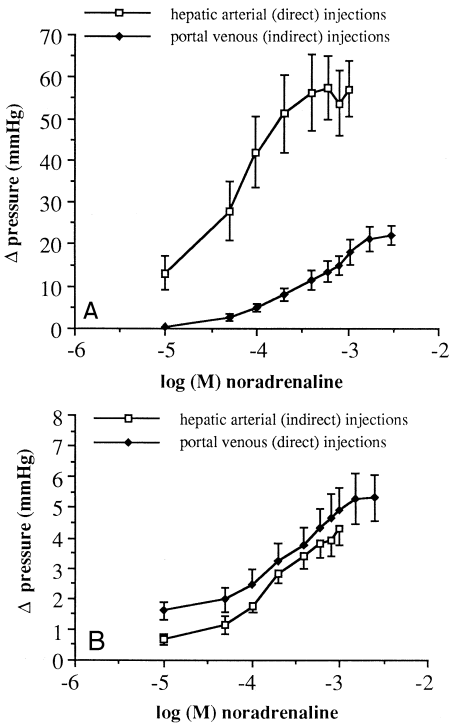

Fig. 4. Hepatic arterial and portal venous responses to bolus injections of noradrenaline. (a) Dose–response curves to noradrenaline measured as transient changes in perfusion pressure in the hepatic arterial vascular bed during direct, hepatic arterial or, indirect (transhepatic) portal venous injections of noradrenaline. Results shown are expressed as mean ± SE, $n = 6$ per dose. (b) Portal venous dose-response curves to noradrenaline measured as transient changes in portal venous pressure in the portal venous vascular bed during direct, portal venous or indirect (transhepatic) hepatic arterial injections of noradrenaline. Results shown are expressed as mean ± SE, $n = 6$ per dose.

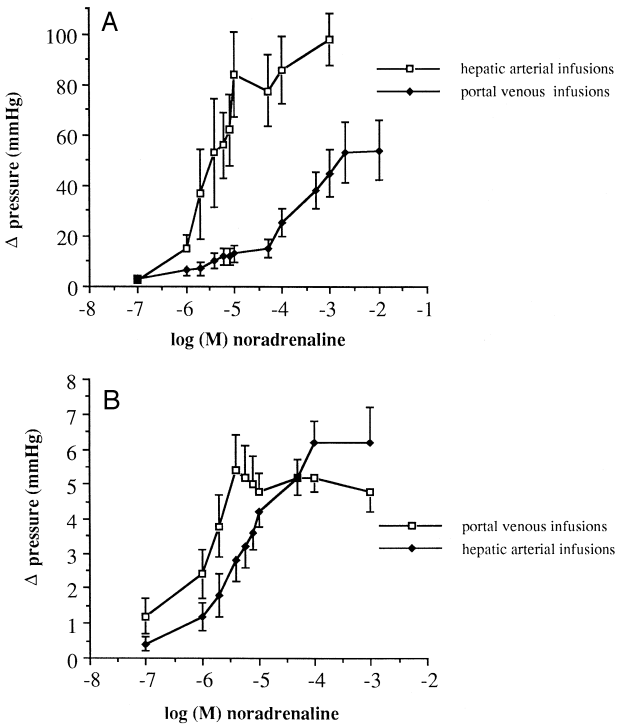

Fig. 5. Hepatic arterial and portal venous responses to infusions of noradrenaline. (a) Dose–response curves to noradrenaline measured as sustained changes in perfusion pressure in the hepatic arterial vascular bed during direct, hepatic arterial or indirect (transhepatic) portal venous infusions of noradrenaline. Results shown are expressed as mean ± SE, $n = 6$ per dose. There was a highly significant difference between the $-\log(M)$ ED_{50} of these two curves and this substantiated observations with bolus injections of noradrenaline (Fig. 4a). (b) Portal venous dose–response curves to noradrenaline measured as sustained changes in portal venous pressure in the portal venous vasculature during direct, portal venous or indirect (transhepatic) hepatic arterial infusions of noradrenaline. Results shown are expressed as mean ± SE, $n = 6$ per dose.

4.3 ± 0.5 mm Hg were not significantly different and were further substantiated by the $-\log(M)$ ED_{50} values of 3.91 ± 0.08 and 3.85 ± 0.11, respectively. The maximum hepatic arterial responses to direct, hepatic arterial, injections were 4.3 times greater than the indirect (transhepatic) responses to portal venous injections for the same dose of 600 mM ($-\log(M)$ $ED_{50} = 4.25 \pm 0.20$) of noradrenaline ($P < 0.01$). Similarly, the maximum hepatic arterial responses to direct, hepatic arterial, infusions were 3.4 times greater than the indirect (transhepatic) responses to portal venous infusions for the same dose of 600 mM ($-\log(M)$ $ED_{50} = 5.28 \pm 0.11$) of noradrenaline ($P < 0.01$). The maximum portal venous responses to portal venous, direct, injections and infusions of noradrenaline were only slightly greater than transhepatic, indirect responses of hepatic arterial injections and infusions noradrenaline, and were not significantly different.

Injection of Evan's Blue dye into the hepatic artery and portal vein circuits at the termination of each experiment confirmed that all lobes of the liver had been perfused homogeneously. No evidence of oedema was seen in any of the livers following perfusion according to liver weight pre- and post-perfusion.

4. Discussion

The rate at which substances can diffuse across the hepatic arterial and portal venous vasculatures has been shown to be modulated by the magnitude of the arterioportal pressure gradient and therefore this was maintained at a physiological level established previously in vitro (Browse et al., 1995b). High flow rates, which are non-physiological, are frequently a common feature of liver perfusions that do not incorporate an oxygen carrier in the perfusate (Gores et al., 1986). The flow rates of 0.32 ml min^{-1} g $liver^{-1}$ (hepatic artery) and 0.98 ml min^{-1} g $liver^{-1}$ (portal vein) used in the present study were comparable to the physiological average of 25 ml min^{-1} 100 g $liver^{-1}$ (hepatic artery) and 75 ml min^{-1} 100 g $liver^{-1}$ (portal vein) (Greenway and Stark, 1971) and resulted in physiological perfusion pressures following subtraction of the pressures contributed by the cannulae. It is appreciated that the perfusion pressures are slightly lower than physiological values and this was due to the absence of sympathetic innervation. Thus, on addition of injections of noradrenaline and particularly, infusions of noradrenaline, the perfusion pressures were restored to physiological limits. These flow rates have been used previously in the dual-perfused rabbit liver preparation and, although the hepatic oxygen uptake of the preparation is lower than one which contains an oxygen carrier in the perfusate (Alexander et al., 1994a), they do not depress the pharmacological reactivity of the preparation for perfusion periods of up to 5 h (Browse et al., 1995a). In addition, the vascular reactivity of the preparation, expressed as the ED_{50} or G_{max} was significantly higher than hypoxic perfusions where a gas mixture of 95% N_2/5% CO_2 was used to gas the perfusate (Alexander et al., 1994b).

The present study confirmed the existence of adrenergic receptors in the rat liver and that they are present in both the hepatic arterial and portal venous vascular beds. These have been previously isolated in the hepatic vasculature of the cat, (Lautt and Greenway, 1987), dog (Richardson and Withrington, 1978; Alexander et al., 1989) and pig (Alexander and Mathie, 1993). Noradrenaline has been shown to be twice as effective in eliciting vasoconstriction in the hepatic artery than in the portal vein in the canine liver (Richardson and Withrington, 1977). The data from the present study demonstrated that the maximum pressor response of the hepatic artery to hepatic arterial injections of noradrenaline was over ten times greater than the maximum pressor response of the portal vein to portal venous injections of noradrenaline. The maximum pressor response of the hepatic artery to hepatic arterial infusions of noradrenaline, however, was just below 20 times greater

than the maximum pressor response of the portal vein to portal venous infusions of noradrenaline. The relatively unreactive nature of the portal vein compared to the hepatic arterial vasculature, at equimolar concentrations of noradrenaline, may probably be related to a paucity of smooth muscle or may be due to the presence of fewer adrenoceptors in the portal venous vasculature (Alexander et al., 1992).

It was possible that the difference in flow rates between the hepatic arterial and portal venous vasculatures may have accounted for the relative unreactive nature of the portal venous vasculature to injections of noradrenaline and this may have induced a larger differential between the maximum portal vein pressures generated by portal venous injections and portal venous infusions of noradrenaline. Bolus injections of any vasoactive substance would have a much shorter, i.e., faster, transit time through the portal vein compared with the hepatic artery because of the much higher proportion of the total hepatic blood flow that is directed through the portal venous vasculature. It was originally anticipated that a maximum response, in whichever vasculature, elicited by a bolus injection of noradrenaline would inevitably induce the maximum increase in perfusion pressure and that this, therefore, was the maximum theoretical pressure attainable. However, since portal vein flow is much larger than hepatic artery flow, the possibility existed that perhaps portal vein-derived vasoactive substances would encounter much fewer of the relevant receptors in the portal vein than a substance injected in the hepatic arterial vasculature. Thus the lower flow rate in the hepatic artery would promote a greater proportion of the injected substance to bind with the appropriate receptors. Constant infusions of vasoactive substances, which in this instance was noradrenaline, would obviate such difficulties in data interpretation, particularly in relation to the calculation of concentrations of vasoactive substances encountering the appropriate receptors, which is obviously impossible to calculate accurately with bolus injections of a drug. Nonetheless, both bolus injections and infusions of noradrenaline produced very similar data profiles in the portal venous vasculature. Interestingly, the major differences occurred with the maximum responses attainable to hepatic arterial injections and infusions rather than portal vein-derived substances. Infused noradrenaline in the hepatic arterial vasculature induced a maximum response which was significantly higher than that induced by hepatic arterial bolus injections. In addition, hepatic artery-infused noradrenaline induced significantly higher portal venous (transhepatic) maximum responses than bolus-injected noradrenaline in the hepatic artery. This, therefore, provided additional evidence for the location of portal vein resistance sites being located at the sinusoidal or post-sinusoidal level and that the hepatic arterial vasculature may coalesce within the first third of the portal venous vasculature or possibly at the pre-sinusoidal level (Watanabe et al., 1994). Moreover, no significant differences were measured in maximum (direct) portal venous responses or (transhepatic) responses between portal vein-infused and portal vein-injected noradrenaline.

A transhepatic signal transfer mechanism has been demonstrated between the portal vein and hepatic artery in the cat (Lautt et al., 1984), dog (Richardson and Withrington, 1981), pig (Alexander and Mathie, 1993) and rabbit (Alexander et al., 1992; Browse et al., 1994). Studies conducted in the cat (Lautt et al., 1984) and dog (Richardson and Withrington, 1978) showed significant differences in the magnitude of hepatic arterial responses but a similarity in the magnitude of portal venous responses between direct (portal venous) and indirect (hepatic arterial) injections of noradrenaline. Thus it was concluded that the hepatic arterial resistance sites may be located at the pre-sinusoidal level and the portal venous resistance sites at the post-sinusoidal level in the cat and dog liver (Richardson and Withrington, 1978; Lautt et al., 1984). However, other studies demonstrated substantial differences in PV responses between hepatic arterial and portal venous injections of equimolar concentrations of noradrenaline in the rabbit liver, and provided evidence to suggest that the portal venous resistance sites were not located at the post-sinusoidal level in this species. Therefore, it is likely that, at least in the rabbit liver, pre-sinusoidal portal venous resistance sites exist, in addition to the hepatic arterial pre-sinusoidal resistance sites, and possibly represent the only active mechanism by which the liver normally controls total flow through the parenchymal cell mass (Lautt et al., 1984).

The data from the present study confirmed that a transhepatic mechanism exists in the rat liver and that there was a highly significant transfer of noradrenaline from the hepatic arterial to the portal venous resistance sites. This implied a one-way transfer of noradrenaline from the hepatic artery to portal vein, since there were no significant differences between the maximum portal venous pressor responses to portal venous- (direct) and hepatic arterial- (indirect) administered noradrenaline and therefore virtually all of the noradrenaline in the hepatic artery penetrated and effected all the portal venous resistance sites and resting tone. However, the much diminished hepatic arterial responses to portal venous- (indirect) administered noradrenaline suggested that portal venous-administered noradrenaline could not access all the hepatic arterial resistance sites in the hepatic arterial vascular bed. The possibility existed, of course, that portal venous-administered noradrenaline effected the resting hepatic arterial tone by sinusoidal downstream exposure to noradrenaline at the hepatic parenchyma or at the post-sinusoidal level. We believe this highly unlikely because portal venous-administered noradrenaline did not elicit comparable pressor responses in the hepatic artery and portal vein. However, the fact that the maximal hepatic arterial pressor response cannot be attained by portal venous-administered

noradrenaline, clearly indicates that portal venous blood/perfusate cannot access all the hepatic arterial resistance sites. The most likely reason that portal venous blood cannot access the hepatic arterial resistance sites can only be because these are located proximal to the hepatic sinusoids before the two vasculatures coalesce. In addition, because there were no significant differences in maximum portal venous pressor responses to portal venous- (direct) and hepatic arterial- (indirect) administered noradrenaline this suggested that hepatic arterial blood/perfusate can access the all the portal venous resistance sites. Therefore, the portal venous resistance sites cannot be located at a similar pre-sinusoidal site to the hepatic arterial resistance sites and it is implicit that they must reside at either the sinusoidal or post-sinusoidal level where there is total access to hepatic arterial blood/perfusate. If noradrenaline did not access the hepatic arterial resistance sites and the hepatic arterial pressor responses recorded from portal venous- (indirect) administration of noradrenaline were simply due to downstream exposure of noradrenaline to the hepatic sinusoids, the maximum hepatic arterial pressor response to (indirect) portal venous-administration of noradrenaline would be similar to those recorded in the portal vein since sinusoidal exposure would be comparable. This would especially be the case with infusions of noradrenaline. Our data clearly show this was not the case and the maximum hepatic arterial pressor responses to (indirect) portal venous-injected noradrenaline were 4–5 times greater and those to portal venous-infused noradrenaline were nearly 10 times greater than those recorded from the portal venous vasculature. These observations can therefore only be explained by the fact that some portal venous blood may access some of the hepatic arterial resistance sites which were proximal to the hepatic sinusoids or post-sinusoidal resistance sites. Furthermore, to our knowledge, no smooth muscle cells have been observed in the hepatic sinusoids. Moreover, we have previously shown in the isolated dual-perfused rabbit liver model that hepatic arterial (indirect) vasodilator responses to portal venous-injected ATP, sodium nitroprusside and acetylcholine are much greater than (direct) portal venous responses (Browse et al., 1994, 1995a, 1997). There were no significant differences between direct and indirect portal venous responses. This was again explained by the transhepatic transfer of substances from the portal vein to the hepatic artery, originally described in the dog (Richardson and Withrington, 1978) and cat liver (Lautt et al., 1984).

The nature of this transhepatic response and the precise mechanisms which regulate it remain unclear. Some anatomical studies (Ohtani, 1979; Grisham and Nopanitaya, 1981) have suggested that an intravascular route, probably at the pre-sinusoidal level, may exist between the hepatic artery and portal vein in the rat liver but it was unlikely to carry a large proportion of the hepatic arterial supply, since most of the hepatic arterial blood supply entered the sinusoids directly. Moreover, if this mechanism forced a significant diversion of hepatic arterial blood through to the portal venous vascular bed, this would have resulted in a much attenuated hepatic arterial dose–response curve compared to other species whereas in fact, the rat liver hepatic arterial vasculature appeared to be more sensitive to noradrenaline than other species such as the rabbit or dog (Alexander et al., 1992; Richardson and Withrington, 1977) on an equimolar basis. Furthermore, it is likely that a valve mechanism of some description may exist in these arterioportal presinusoidal anastomoses to restrict the direct transfer of signal in the opposite direction from the portal venous to hepatic arterial vasculature, particularly since this is of considerable proportions in the rabbit, but, at the present time, these have not been located or reported. The reduced diameter of these anastomotic vessels would also tend to exclude any pressure gradient effect between the hepatic arterial and portal venous vasculature to play a role in the signal transfer. Therefore convective diffusion through the hepatic parenchyma, from the hepatic artery to the portal vein at the sinusoidal, or post-sinusoidal level, is the most likely mechanism to explain the transhepatic effect in the rat liver although the possibility of transfer through these anastomotic vessels should not be precluded.

Thus, in the present report, the one-way transfer of stimulus from hepatic artery to portal vein therefore suggested that the resistance sites in the portal venous vasculature of the rat liver may all be located at the post-sinusoidal level and the hepatic arterial resistance sites pre-sinusoidally, similar to those in the dog and cat and perhaps are a closer representation of the anatomical structure in man. It has also established the in vitro isolated dual-perfused rat liver preparation as a potentially useful model for the study of pharmacological mechanisms which control hepatic vascular tone. Therefore receptors responsible for the control of hepatic vascular tone and blood flow in the rat liver may now be fully characterised under close-to-physiological conditions of dual hepatic arterial and portal venous perfusion.

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