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Hypoxia attenuates hepatic arterial vasodilatation and enhances portal venous vasoconstriction to ATP in the perfused rabbit liver

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Abstract

Dose-related responses to acetylcholine, adenosine 5′-triphosphate (ATP), adenosine and sodium nitroprusside were studied in an in vitro perfused rabbit liver gassed with (95% N_2/5% CO_2, Group 1) and without carbon dioxide (100% N_2, Group 2). At raised tone, achieved by addition of methoxamine to the perfusate, significantly attenuated hepatic arterial vasodilatation to sodium nitroprusside, acetylcholine, ATP and adenosine was measured in Group 1 and responses to all but sodium nitroprusside were abolished in Group 2. Portal venous responses to acetylcholine, adenosine and sodium nitroprusside were not significantly altered in either Group 1 or Group 2. However, portal venous vasoconstriction to ATP was significantly enhanced in Group 1 and less so in Group 2. It is concluded that carbon dioxide-free hypoxia attenuated hepatic arterial vasodilatation to acetylcholine and ATP and enhanced vasonstriction to ATP. Both these effects may be characteristic of damage to the microvascular endothelium and may be the result of decreased synthesis of nitric oxide.

Keywords: Hepatic circulation; Vascular tone; Hypoxia; Carbon dioxide; Anoxia

1. Introduction

The liver is uniquely characterised by dual inflow from the hepatic arterial and portal venous networks. The liver may be more vulnerable to hypoxia than other organs because up to 80% of the total hepatic blood flow is derived from the portal venous supply which reaches the liver in a partially oxygenated state. Thus reductions in portal venous flow can dramatically alter the hepatic blood gas environment and induce hypoxia (Alexander, 1996). The liver, in addition to the heart, is also vulnerable to hypoxia during pathological conditions, such as emphysema and congestive heart failure, and, in the latter, it is unknown whether alterations in hepatic vascular tone, which lead to hepatomegaly, may be ameliorated pharmacologically in order to reduce the workload on the failing heart. The roles of adenosine (Koos et al., 1995; Mian and Marshall, 1995, 1996; Skinner and Marshall, 1996), ATP (Paddle and Burnstock, 1974; Dart and Standen, 1995) and nitric oxide (NO) (Iadecola, 1992; Wang et al., 1992; Carr et al., 1993; Reid et al., 1994; Sandor et al., 1994) during hypercapnia and hypoxia have been the subject of several studies. These studies, which have investigated the role of hypoxia upon vascular tone, attempt to maintain relatively constant partial pressures of CO_2 across the tissues, by using 95% N_2/5% CO_2 gas mixtures, presumably to retain a physiological pH. However, none of these studies have actually differentiated the action of 5% CO_2 from that of 100% N_2 in the hypoxic gas mixture upon vascular tone. A few studies have investigated the effects of hypercapnia in the hepatic circulation (Hughes et al., 1979a,b) but none have addressed the modulator role of CO_2 during hypoxia upon the regulation of hepatic vascular tone either in vivo or in vitro where haemodynamic stability is easily maintained (Alexander et al., 1992). A perfused rabbit liver preparation was chosen as the preferred model in the present study because perfused pressurised vessels provide a closer approximation to physiological conditions than wire-mounted, isometric myographs (Buus et al., 1994). Moreover, responses to various vasoactive substances are known to vary according to their origin in the body (Robinson, 1981) and also along the length of vessel under investigation (Altura and Altura, 1970) and we wished to study the activity of the intra- and extrahepatic microvasculature in toto.

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We have previously shown in the dog (Mathie and Alexander, 1990), rabbit (Mathie et al., 1991a), pig (Alexander and Mathie, 1993) and others in the cat liver (Lautt and Legare 1985) that adenosine is important in the regulation of hepatic vascular tone. Adenosine $P_1(A_2)$-(Mathie and Alexander 1990; Mathie et al., 1991b) and ATP P_{2x} and P_{2y} purinoceptors (Ralevic et al., 1991) have been characterised in the hepatic arterial vasculature of the isolated dual-perfused rabbit liver preparation (Alexander et al., 1992) and ATP-induced vasodilatation could be partially mediated by the release of NO (Mathie et al., 1991b; Browse et al., 1994a) and also by catabolism to adenosine (Browse et al., 1997) in the isolated perfused rabbit liver. Although adenosine, ATP or NO may be released during prolonged periods of hypoxia (Mian and Marshall, 1996), their function in the modulation of hepatic microvascular tone during hypoxia is unclear.

The purpose of the present study, therefore, was twofold; firstly, to determine how hypoxia may modulate vascular responses of the endothelium-dependent vasodilators acetylcholine and ATP and the direct smooth muscle vasodilators, sodium nitroprusside and adenosine in the isolated dual-perfused rabbit liver preparation; and secondly, to determine the modulator role of CO_2 during hypoxia on the above responses by comparison of data using a gas mixture of 95% N_2/5% CO_2 and 100% N_2 with no CO_2 present since this has not been ascertained previously.

2. Materials and methods

2.1. Surgical protocol

Twelve male New Zealand White rabbits, allowed food and water ad libitum, were used throughout the study and were obtained from a single breeder. Each rabbit was initially sedated with subcutaneous fentanyl–fluanisone ('Hypnorm', 0.3 ml kg^{-1}) and 15 min later anaesthetised with intravenous midazolam ('Hypnovel', 1.5 mg kg^{-1}) through a marginal ear vein. A further 0.3 ml kg^{-1} Hypnorm was injected i.m. for continued analgesia during the 40-min operative period. A ventral midline incision was made to exposure the viscera and the gut was carefully retracted to the right hand side of the animal and placed in a warm, saline-moistened swab. The bile duct was dissected, cannulated and bile collected for the remainder of the dissection procedure. The oesophagus was then exposed, ligated and divided to provide additional access to the common and proper hepatic arteries. The gastroduodenal artery was ligated and divided and the common hepatic artery cannulated and perfused with 10 ml warm, heparinised Krebs' buffer. The portal vein was then exposed, ligated and cannulated and perfused with 30 ml of warm Krebs' buffer and the liver excised and placed upon the perfusion circuit. Further details of the operative procedure have been published elsewhere (Alexander et al., 1992; Browse et al., 1994a).

2.2. Liver perfusion

Livers were perfused via the hepatic artery and portal vein cannulae at constant flow rates of 25 and 75 ml min^{-1} 100 g $liver^{-1}$, respectively. The perfusate used was Krebs–Bülbring buffer solution (composition (mM) NaCl 133, KCl 4.7, NaH_2PO4 1.35, $NaHCO_3$ 20.0, $MgSO_4$ 0.61, Glucose 7.8, and $CaCl_2$ 2.52) at 37°C, from a common oxygenated reservoir, gassed with the appropriate mixture (see Section 2.4). Homogeneous liver perfusion was confirmed by all sections of the liver changing to an even beige colour. Following an equilibration period of 10–15 min, vascular responses to intra-arterial injections of vasoactive substances were measured as a transient reduction, indicative of vasodilatation, or increase, indicative of vasoconstriction, in perfusion pressure (Alexander et al., 1992). Perfusion under these conditions has been shown to maintain liver viability for over 5 h (Browse et al., 1994b). Perfusion pressures were measured with Spectramed (Statham) P23XL physiological pressure transducers from side arms of the perfusion circuit and from the gastroduodenal cannula. Recordings were made on a Grass 79F Polygraph (Grass Instrument, Quincy, MA, USA). Hepatic arterial responses were recorded directly from the common hepatic artery and portal venous responses as an indirect, transhepatic, recording from the portal vein. Gas tensions in the perfusate were measured upon a Corning 158 blood–gas analyser.

2.3. Drug administration

Methoxamine (hydrochloride), ATP (disodium salt), adenosine, acetylcholine and sodium nitroprusside (all from Sigma) were dissolved in distilled water. Methoxamine was added to the perfusate at a $-\log$ (M) concentration of 5.31 ± 0.06 to raise the tone in the hepatic vascular bed to 75% of the maximum attainable pressure. All drugs were injected into the hepatic artery as 0.1 ml boluses to cover the dose range 10^{-11} to 10^{-5} mol 100 g $liver^{-1}$ (doses were adjusted for liver weight). Each subsequent injection was given when the perfusion pressure had returned to normal. An additional 15-min equilibration period was allowed following any change of gas mixture. Drugs were injected in the following sequence to avoid tachyphylaxis and desensitisation of purinoceptors (Ralevic et al., 1991): sodium nitroprusside, adenosine, acetylcholine and ATP.

2.4. Experimental plan

A perfusion time of 120 min was required for the construction of all the dose–response curves under each experimental condition. It was therefore necessary to divide each experimental condition into 2 groups in order to account for alterations in vascular reactivity which could be attributed to the long duration of the perfusions follow-

ing the initial 2 h perfusion under control conditions (Browse et al., 1994a). Livers from 12 male New Zealand White rabbits (2.5–3.5 kg) were divided into 2 groups and perfused upon an isolated, dual-perfused, rabbit liver circuit described above. Dose-related response curves to acetylcholine, ATP, sodium nitroprusside and adenosine in each group in the hepatic artery and portal vein were then compared in order to determine the effects of hypoxic perfusion upon endothelium-mediated and direct smooth muscle vascular responses.

2.4.1. Normoxic perfusions (95% O_2/5% CO_2): conducted in groups 1 and 2

Livers were perfused under control conditions using a standard gas mixture of 95% O_2/5% CO_2. Direct (hepatic arterial) and indirect (transhepatic, portal venous) changes in perfusion pressure were measured to hepatic arterial injections of sodium nitroprusside, adenosine, acetylcholine and ATP before changing the perfusate to one gassed with one of the hypoxic gas mixtures. These data served as controls to those obtained during hypoxic perfusions in Groups 1 and 2.

2.4.2. Group 1 (n = 6), hypoxia (95% N_2/5% CO_2)

Livers were perfused with (95% N_2/ 5% CO_2) to produce hypoxic, conditions which retained balanced pCO_2 and physiological pre-hepatic pH (Table 1).

2.4.3. Group 2 (n = 6), hypoxia (100% N_2)

The modulator action of CO_2 during hypoxia was studied by omitting CO_2 from the gas mixture and gassing the perfusate with 100% N_2 to retain a physiological, non-acidotic post-hepatic pH (Table 1).

2.4.4. Effects of prolonged hypoxic perfusion

A time period of 120 ± 22 min was required to complete all of the control dose-response curves in Groups 1 and 2 and it has previously been shown that responses may deteriorate following perfusion periods prolonged beyond 5 h under normoxic conditions using this preparation (Browse et al., 1994a). It was therefore necessary to ascertain whether the vascular changes recorded after this period of perfusion were characteristic of the changes due to hypoxia or deterioration of the preparation after prolonged hypoxic perfusion. This was conducted by injection of a single mid-range dose of each vasoactive agent into the hepatic artery at the initiation and after 10, 60 and 120 min of hypoxic perfusion during the construction of the

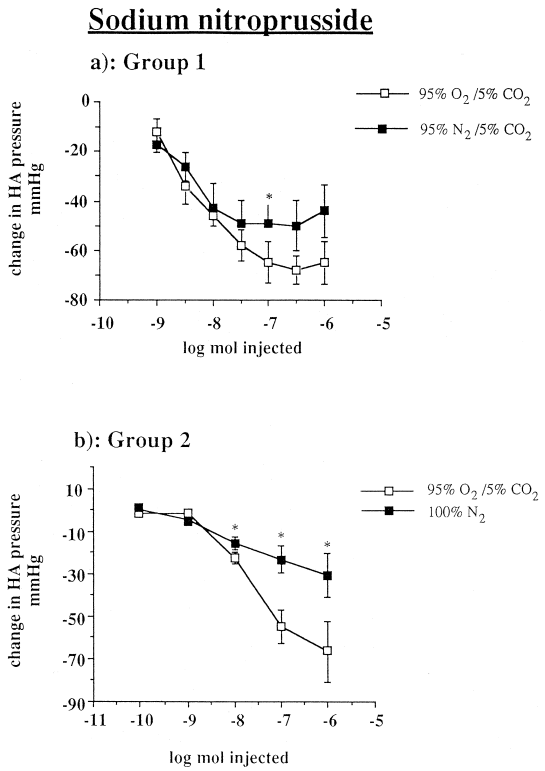

Fig. 1. Changes in HA perfusion pressure elicited by HA injections of sodium nitroprusside in (a) Group 1 and (b) Group 2. $^*P < 0.05$, oxygenated vs. hypoxic perfusion, Student's paired *t*-test.

Table 1
Table of physiological values measured during the perfusions. Pre and post = pre- and post-hepatic perfusate samples, respectively

Perfusion pressure (mmHg)	Control (95% O_2/5% CO_2)		Group 1 (95% N_2/5% CO_2)		Group 2 (100% N_2)	
HA	150.0 ± 14.2		181.3 ± 3.3		165.8 ± 22.6	
PV	7.6 ± 1.9		4.5 ± 1.5		6.2 ± 1.4	
Bile volume production ml h^{-1}	11.57 ± 2.35[a]		7.89 ± 1.61		2.37 ± 0.93	
	pre	post	pre	post	pre	post
pO_2	502.9 ± 17.3[b]	224.7 ± 28.2	47.7 ± 6.2[c]	38.1 ± 5.0	54.28 ± 3.02	29.8 ± 2.0
pCO_2	30.7 ± 1.3[b]	47.1 ± 2.04	28.3 ± 1.2[d]	38.4 ± 1.9	3.7 ± 0.6	34.4 ± 7.0
pH	7.25 ± 0.01[b]	7.05 ± 0.02	7.26 ± 0.01[d]	7.10 ± 0.02	7.99 ± 0.01	7.30 ± 0.05
Liver weight (g)						
Before	–		90.6 ± 9.3		95.1 ± 5.5	
After	–		76.6 ± 7.8		80.6 ± 5.2	

[a] $P < 0.01$.
[b] $P < 0.001$, oxygenated vs. 100% N_2.
[c] $P < 0.001$, oxygenated vs.95% N_2/5% CO_2, Student's unpaired *t*-test.
[d] $P < 0.001$, 95% N_2/5% CO_2 vs. 100% N_2.

dose response curves to the vasoactive agents in Group 1 (95% N_2/5% CO_2) and Group 2 (100% N_2). Mid-range doses of acetylcholine, ATP, adenosine and sodium nitroprusside were calculated by analysis of dose–response curves obtained from Groups 1 and 2 above perfused under normoxic conditions.

2.5. Statistics and presentation of data

Responses were recorded as changes in perfusion pressure (mmHg). Direct responses refer to those recorded in the hepatic artery, the vasculature in which the substances were injected. Indirect (transhepatic) responses refer to those recorded in the adjacent, afferent, portal venous, vasculature as an effect from hepatic arterial injections. All results are presented as mean ± S.E. Newman–Keuls analysis of variance technique was applied to the data before Student's paired or unpaired *t*-test was used as appropriate, to determine the level of significance between responses, $P < 0.05$ being taken as significant.

3. Results

3.1. Perfusion indices

There were no significant differences in hepatic arterial or portal venous perfusion pressures between any of the groups in either the hepatic arterial or portal venous vascular networks (Table 1). A progressive and significant reduction was observed in bile volume production during hypoxia without CO_2 ($P < 0.01$). There were no significant differences between hypoxic perfusions with CO_2 (Group 1) and normoxic (control) perfusions. Perfusate pre-hepatic pO_2 pressures were significantly reduced following the transfer to hypoxic perfusion but there was no significant difference between groups 1 and 2 (Table 1). Also, there were no significant differences in pre-hepatic pCO_2 between control and Group 1 perfusions but a significant reduction in Group 2 ($P < 0.001$). Pre-hepatic pH values in control perfusions were significantly more acidotic than those in Group 2 ($P < 0.001$). Post-hepatic perfusate pH values in the control and Group 1 perfusions were significantly more acidotic than Group 2 ($P < 0.001$).

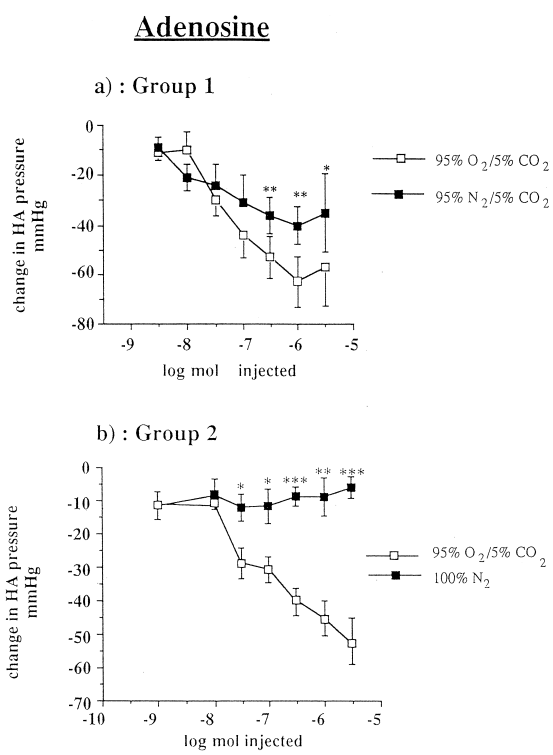

Fig. 2. Changes in HA perfusion pressure elicited by HA injections of adenosine in (a) Group 1 and (b) Group 2. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, oxygenated vs. hypoxic perfusion, Student's paired *t*-test.

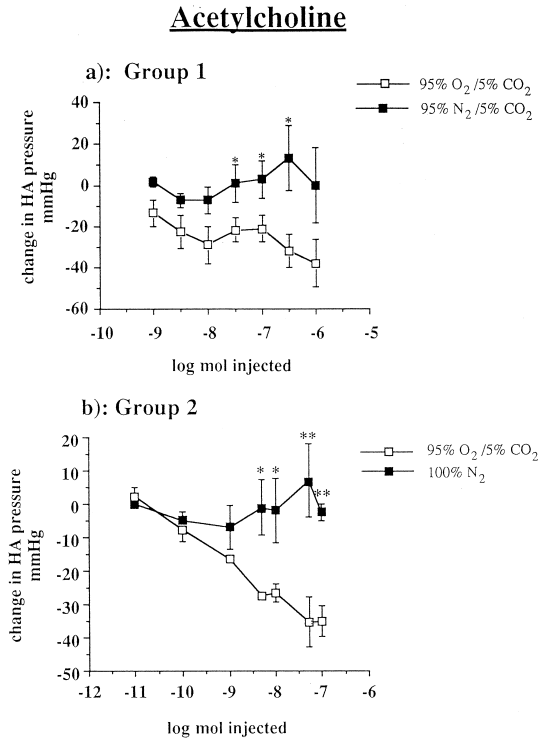

Fig. 3. Changes in HA perfusion pressure elicited by HA injections of acetylcholine in (a) Group 1 and (b) Group 2. $^{*}P < 0.05$, $^{**}P < 0.01$, oxygenated vs. hypoxic perfusion, Student's paired *t*-test.

3.2. Hepatic arterial responses

All 4 substances tested elicited vasodilatation which was measured as transient decreases in perfusion pressure.

3.2.1. Direct smooth muscle responses

Responses to sodium nitroprusside were only significantly attenuated in Group 1 at a single dose (Fig. 1a). However, a highly significant attenuation of responses was

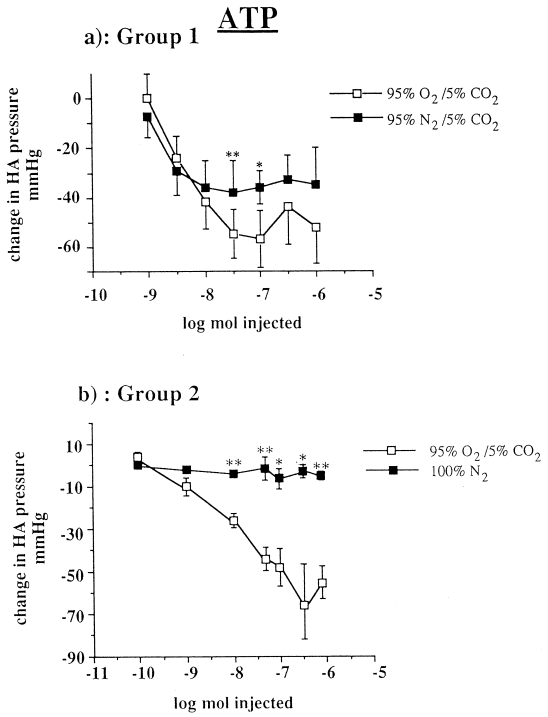

Fig. 4. Changes in HA perfusion pressure elicited by HA injections of ATP in (a) Group 1 and (b) Group 2. $^{*}P < 0.05$, $^{**}P < 0.01$, oxygenated vs. hypoxic perfusion, Student's paired *t*-test.

observed in Group 2 (Fig. 1b). This suggested that alterations in pre-hepatic pH significantly attenuate vascular responses to sodium nitroprusside rather than the effects of hypoxia alone. There were no significant differences in $p\mathrm{EC}_{50}$s between control and Group 1 perfusions (8.38 ± 0.11 vs. 8.36 ± 0.11 −log mol, respectively). Similarly, Group 2 perfusions did not induce a significant alteration in the $p\mathrm{EC}_{50}$s of these curves when compared to control conditions (8.12 ± 0.33 vs. 7.73 ± 0.11 −log mol, Student's paired *t*-test, control vs. Group 2 perfusions).

Responses to adenosine were significantly attenuated in Group 1 perfusions (Fig. 2a) and were abolished by Group 2 perfusions (Fig. 2b). The data, therefore, again suggest that although hypoxia may attenuate vascular responses to adenosine, the effects of pH appear to have, at least, equal effects. Hypoxia in the presence of CO_2, however, did not induce a significant alteration in the $p\mathrm{EC}_{50}$s when compared to control perfusions (7.42 ± 0.02 vs. 7.39 ± 0.18 −log mol, respectively). By contrast, Group 2 perfusions produced a highly significant difference in $p\mathrm{EC}_{50}$s when compared to oxygenated conditions (6.23 ± 0.31 vs. 7.19 ± 0.11 −log mol, respectively)*** ($^{***}P < 0.001$, Student's paired *t*-test, control vs. Group 2 perfusions).

3.2.2. Endothelium-dependent responses

Responses to acetylcholine were significantly attenuated in Group 1 (Fig. 3a). There were no significant differences in $p\mathrm{EC}_{50}$s between control and Group 1 perfusions (8.62 ± 0.51 vs. 8.69 ± 0.58 −log mol, respectively). Responses to acetylcholine were also significantly attenuated in Group 2 (Fig. 3b). Again, there were no significant differences in $p\mathrm{EC}_{50}$s between control and Group 2 perfusions (8.82 ± 0.23 vs. 8.94 ± 0.30 −log mol, respectively).

Responses to ATP were significantly attenuated in Group 1 perfusions (Fig. 4a) but there were no significant differences in $p\mathrm{EC}_{50}$s between control and Group 1 perfusions, however, (8.22 ± 0.16 vs. 8.35 ± 0.19 −log mol ATP, control vs. Group 1 perfusions). Group 2 perfusions produced an almost total abolition of relaxant responses to ATP (Fig. 4b). No significant differences were measured in $p\mathrm{EC}_{50}$s between control and Group 2 perfusions (8.09 ± 0.29 vs. 8.30 ± 0.59 −log mol, respectively). Prolonged Group 1 perfusions did not produce any consistent attenuation in hepatic arterial responses to any of the four substances tested (Table 3). However, portal venous (transhepatic) responses to ATP were significantly attenuated after 60* and 120* min of perfusion compared to control conditions ($^{*}P < 0.05$, Student's paired *t*-test). No significant attenuation was observed in hepatic arterial or portal venous (transhepatic) responses to sodium nitroprusside, adenosine or acetylcholine during prolonged, Group 2 perfusions. However, hepatic arterial responses to ATP were significantly attenuated after 10 ($P < 0.001$), 60 ($P < 0.01$) and 120 ($P < 0.001$) min Group 2 perfusions (Table 3, Group 2).

Table 2
$p\mathrm{EC}_{50}$ and E_{max} values from portal venous responses to hepatic arterial injections of the above named vasoactive substances. No significant differences were measured between Groups 1 and 2 in either $p\mathrm{EC}_{50}$ or E_{max} values. Measurements are marked '+', were indicative of a vasoconstrictor response

Vasoactive substance	$p\mathrm{EC}_{50}$				E_{max}			
	Group 1		Group 2		Group 1		Group 2	
	Oxygenated	Hypoxia	Oxygenated	Hypoxia (no CO_2)	Oxygenated	Hypoxia	Oxygenated	Hypoxia (no CO_2)
Sodium nitroprusside	7.54 ± 0.28	8.00 ± 0.24	7.42 ± 0.25	6.34 ± 0.32	1.44 ± 0.24	0.52 ± 0.39*	2.17 ± 0.98	8.33 ± 3.14
Adenosine	7.75 ± 0.21	6.75 ± 0.64	6.86 ± 0.55	6.34 ± 0.32	0.10 ± 0.10	0.71 ± 0.44	0.40 ± 0.40	$+3.17 \pm 2.43$
Acetylcholine	8.69 ± 0.58	7.50 ± 0.51[a]	7.92 ± 0.28	7.94 ± 0.11	$+2.80 \pm 0.66$	$+3.20 \pm 0.66$	$+5.28 \pm 1.38$	$+7.5 \pm 2.33$
ATP	6.66 ± 0.05	6.98 ± 0.15	6.56 ± 0.23	7.03 ± 0.20	$+4.20 \pm 1.28$	$+5.10 \pm 1.10$	$+3.20 \pm 1.43$	$+8.40 \pm 4.37$

[a] $P < 0.05$, oxygenated vs. hypoxia, Student's paired *t*-test.

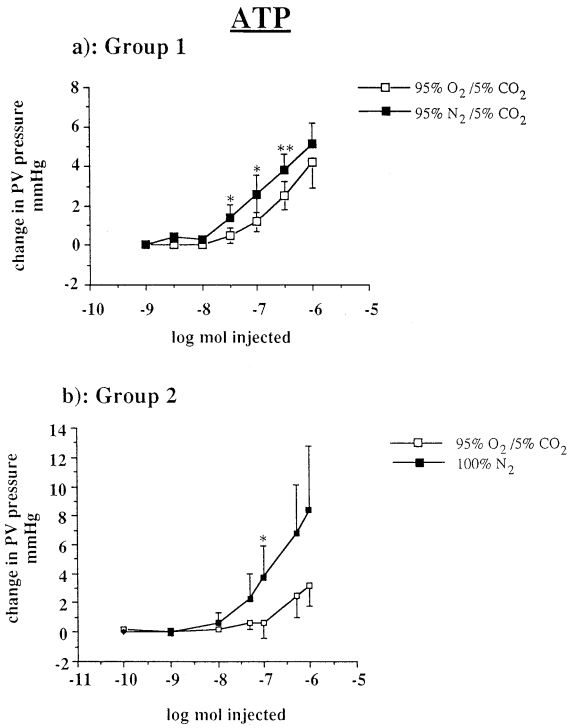

Fig. 5. Changes in PV perfusion pressure elicited by HA injections of ATP in (a) Group 1 and (b) Group 2. $^{*}P < 0.05$, $^{**}P < 0.01$, oxygenated vs. hypoxic perfusion, Student's paired *t*-test.

3.3. Portal venous responses (transhepatic)

The direct smooth muscle vasodilators sodium nitroprusside and adenosine elicited vasodilatation but this was much reduced compared to hepatic arterial responses. No significant differences were measured between hypoxic or oxygenated perfusion at any of the doses tested. Moreover, no significant differences were measured between E_{max}s or $p\mathrm{EC}_{50}$s during oxygenated or hypoxic conditions in Group 2 (Table 2) in any of the 4 substances tested. However, some statistically significant differences between oxygenated and hypoxic perfusions were observed in Group 1.

The predominant portal venous response to acetylcholine during the present series of perfusions was vasoconstriction. A highly significant difference was measured in $p\mathrm{EC}_{50}$s between control and Group 1 perfusions (Table 2). However, no significant differences were measured in E_{max} values during Group 1 or Group 2 perfusions.

The predominant response to ATP during the present series of experiments was also vasoconstriction although we have previously demonstrated a modest vasodilator component (Browse et al., 1994a,b). Group 1 perfusions caused a statistically significant increase in responses to ATP (Fig. 5a) and a significant difference in E_{max} was also measured in responses to sodium nitroprusside between control and Group 1 perfusions (Table 2). Group 2 perfusions only significantly increased vasoconstrictor responses to ATP at one dose.

3.4. Effects of prolonged perfusion

The major changes in Group 1 which could have been attributed to deterioration of responses due to prolonged perfusion occurred with acetylcholine and ATP. Hepatic arterial responses to both substances were significantly decreased after 60 min of perfusion when compared to basal responses recorded at the initiation of perfusion under oxygenated conditions (Table 3). Only portal venous (vasoconstrictor) responses to ATP after 120 min of hypoxic perfusion in Group 1 remained significantly higher than those recorded during oxygenated (control) conditions at the initiation of perfusion. Hepatic arterial responses to ATP remained significantly attenuated compared to values obtained under basal, oxygenated conditions, at the initiation of perfusion in Group 2 (Table 3). Similarly, hepatic arterial responses to adenosine in Group 2 were also

Table 3
Hepatic arterial and portal venous (transhepatic) responses to mid-range doses of sodium nitroprusside, adenosine, acetylcholine and ATP injected via the hepatic artery. Measurements marked '+' or '−' are indicative of vasoconstriction or vasodilatation, respectively

Vasoactive substance (−log mol)	Minutes of perfusion							
	Hepatic arterial responses				Portal venous responses			
	0	10	60	120	0	10	60	120
Sodium nitroprusside (7.5)	−58.0 ± 6.4	−54.0 ± 11.7	−49.0 ± 9.1	−32.0 ± 12.3	−1.2 ± 0.5	−0.6 ± 0.4	−0.6 ± 0.4	−0.4 ± 0.2
Adenosine (7.0)	−44.0 ± 6.2	−44.2 ± 8.6	−31.2 ± 11.1	−30.0 ± 9.5	−0.1 ± 0.1	0.0 ± 0.0	+0.3 ± 0.3	+0.2 ± 0.0
Acetylcholine (8.5)	−21.6 ± 6.2	−11.0 ± 3.3	+1.0 ± 9.3[a]	−9.0 ± 4.3	+0.6 ± 0.2	+2.6 ± 0.4[b]	+1.25 ± 0.5	+0.4 ± 0.2
ATP (7.5)	−55.0 ± 10.0	−49.0 ± 12.1	−38.0 ± 12.9[a]	−27.0 ± 13.0	+0.5 ± 0.4	+1.0 ± 0.5	+1.4 ± 0.7[a]	+1.4 ± 0.7[a]
Sodium nitroprusside (8.0)	−23.6 ± 3.7	−23.7 ± 8.0	−12.5 ± 5.2[a]	−13.7 ± 9.0	−0.7 ± 0.7	−0.7 ± 0.7	−5.2 ± 2.3	−5.0 ± 2.0
Adenosine (7.0)	−33.0 ± 5.6	−22.5 ± 7.8	−13.7 ± 8.0	−5.0 ± 2.9[a]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	+1.5 ± 1.7
Acetylcholine (8.5)	−17.5 ± 10.9	−8.7 ± 2.4	+3.7 ± 9.4	+5.0 ± 8.7	+0.7 ± 0.5	+2.7 ± 0.5	+0.7 ± 1.4	+1.0 ± 0.4
ATP (7.5)	−41.2 ± 7.2	−22.5 ± 5.9[c]	−2.5 ± 8.3[b]	−1.25 ± 5.9[c]	0.0 ± 0.0	+1.7 ± 0.7	+1.0 ± 0.8	+1.5 ± 0.6

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$, test vs. value during oxygenated perfusion at $t = 0$, Student's paired *t*-test.

significantly attenuated after 120 min of perfusion. Thus, a physiological pre-hepatic pH appeared to be critical for the maintenance of responses to adenosine and ATP. Thus, any differences in responses to ATP or adenosine during perfusion in Group 1 were unlikely to be due to prolonged perfusion or pH since this was maintained in Group 1.

4. Discussion

Adequate oxygenation is required for optimal liver function in isolated perfused liver preparations where an oxygen carrier is omitted from the perfusate (Alexander et al., 1993, 1998). It is known that hypoxia may diminish hepatic function (Angus et al., 1990; Chawla and Jones, 1991; Audibert et al., 1993) but the modulator role of CO_2 in experimental hypoxic gas mixtures, where it may be used in concentrations of up to 10%, upon hepatic vascular tone has never been addressed. The present study attempted to address the modulator action of CO_2 upon hepatic vascular tone during hypoxia by comparison of the three gas mixtures. The use of 100% N_2 (Group 2) resulted in pre-hepatic hypocapnic alkalosis combined with hypoxia and was not originally intended. However, the pH of the post-hepatic perfusate samples of Group 2, unlike those of the controls and Group 1, approximated physiological values. It is questionable whether the post-hepatic perfusate samples could be assumed to be representative of the pH and gas tensions at the cellular level but, in the present series of experiments, these measurements were the closest obtainable using the resources available. The data from the present study showed that bile volume production, a recognised dynamic indicator of hepatic function (Alexander et al., 1995), was significantly reduced in Group 2 but not in Group 1 compared to controls. Pre-hepatic pH was increased in Group 2 and therefore the retention of a physiological pre-hepatic pH and pCO_2, as measured in Group 1 and controls, appeared to be more important for maintenance of bile volume production than a decreased pO_2. It would therefore appear that an alkalotic pre-hepatic pH, as observed in Group 2, diminished bile volume production compared to controls and Group 1, although the pH of the post-hepatic perfusate samples in Group 2 approached physiological values compared to those in controls and Group 1 which were acidotic (Table 1). In general, the trends seen in the bile volume production data were also reflected in the dose–response curves to the majority of the vasoactive substances tested, i.e., a diminution in Group 2 perfusions compared to controls.

In addition to bile volume production, the results from the present study suggested that the addition of CO_2 to the perfusate gas mixture was also critical for the maintenance of vasoactivity during hypoxia. Hepatic arterial responses in Group 1 to sodium nitroprusside were not significantly attenuated compared to controls other than at one mid-range dose. Responses to adenosine, ATP and acetylcholine were attenuated at the highest to mid-range doses in Group 1. However, omission of CO_2 in the hypoxic gas mixture (Group 2) virtually abolished hepatic arterial responses to ATP, adenosine and acetylcholine although a residual, significantly attenuated response to sodium nitroprusside remained. It is possible that acetylcholine, ATP and adenosine being, at least in part, endothelium-dependent vasodilators were more affected by hypoxia in the absence of CO_2 because they may exert some of their activity via the vascular endothelium and this may have rendered their activity more vulnerable to the effects of prolonged perfusion and/or hypoxia (Browse et al., 1994b; Yang et al., 1998).

We have previously shown that ATP and acetylcholine may induce hepatic arterial vasodilatation via the release of NO (Mathie et al., 1991b; Browse et al., 1994a) and it has also previously been demonstrated that adenosine may induce vasodilatation independently of the vascular endothelium (Kennedy et al., 1985; Mathie et al., 1991a). However, adenosine may also elicit vasodilatation via the release of NO in the isolated perfused guinea pig heart (Vials and Burnstock, 1993) and on arterioles in the rat cremaster muscle (Baker and Sutton, 1993). Thus, adenosine may readily elicit vasodilatation by endothelium-dependent and -independent mechanisms, the variability of which appears to depend upon the microvasculature under investigation. Only hepatic arterial responses to acetylcholine and ATP showed a transient, though significant, attenuation after 60 min of perfusion in Group 1. These responses, in conformity with responses to adenosine and sodium nitroprusside, were not significantly attenuated after 120 min of perfusion. Therefore, the attenuation of hepatic arterial responses in Group 1 can possibly be ascribed to the effects of hypoxia per se and that sodium nitroprusside, being an endothelium-independent vasodilator was the least affected.

Attenuation of hepatic arterial responses to adenosine and ATP in Group 2, however, coincided with the effects of prolonged perfusion to adenosine- but not ATP-mediated responses where a significant attenuation to a mid-range dose was apparent at the initiation of perfusion (Table 3). These observations therefore suggested that; (i) hepatic arterial responses to ATP were the most affected by hypoxia without CO_2 at the initiation of hypoxic perfusion and that only adenosine-mediated responses degenerated following prolonged CO_2-free, hypoxic perfusion for 120 min; (ii) pre-hepatic pH may have a greater impact upon vasoactivity than adequate oxygenation during endothelium-dependent vasodilatation to ATP; (iii) adequate oxygenation for vasoactivity was maintained although it was possible that this may be insufficient for optimal hepatic function (Yang et al., 1997, 1998); and (iv) endothelium-independent vasodilatation was the least affected by CO_2-free hypoxic perfusion.

Enhanced vasoconstrictor responses to ATP in Group 1 but not in Group 2 suggested that hypoxia enhanced

vasoconstriction in the portal venous microvasaculature. We have previously reported a significant enhancement of vasoconstriction to acetylcholine and ATP in the presence of the NO synthase inhibitor, N^G-nitro-L-arginine monomethyl ester (L-NAME) (Ralevic et al., 1992; Browse et al 1994a). The similar enhancement of the vasoconstrictor responses to acetylcholine and ATP during the present study may also have been due to inhibition of NO synthesis during Group 1 perfusions although this remains to be proven and is beyond the scope of the present study. It has been reported that P_{2x} purinoceptors are pH-sensitive and that their degree of activation is dependent upon their structural moiety (Stoop et al., 1997). Thus the comparative lack of enhancement of vasoconstriction in Group 2 may have been due to the alkalotic nature of the perfusate desensitising P_{2x} purinoceptors. At the present time, we are uncertain of the nature of P_{2x}-purinoceptor located in the portal venous microvasculature and therefore this aspect remains to be further clarified.

The major difference between hepatic arterial and portal venous responses to the four vasoactive agents used was that acetylcholine and ATP exhibited predominantly vasoconstrictor activity in the portal venous microvasculature in contrast to vasodilatation in the hepatic arterial microvasculature. The precise explanation for this phenomenon is subject to debate but, at basal tone, ATP acts as a vasoconstrictor in the rat (Lee and Filkins, 1988) and rabbit portal venous vasculature (Browse et al., 1994a). This was previously ascribed to a relatively low portal venous flow and pressure in vitro compared to in vivo conditions. However, responses to sodium nitroprusside are readily demonstrable in the rat portal venous vasculature at these pressures and flows (Yang and Alexander, unpublished observations). One remaining possibility is that nitric oxide synthase in the rat portal venous endothelium may be operating at maximal rate thus preventing any exogenous stimulation, but this is speculative and remains to be proven.

The only other significant change observed in portal venous responses was a significant attenuation in the $p\mathrm{EC}_{50}$ values to acetylcholine in Group 1 and was, again, probably due to retention of physiological pre-hepatic pH values (Table 2). This suggested that a higher dose of acetylcholine was required to produce the maximal response, but the actual maximum response ($E_{\max}$) remained unaltered by either hypoxia or hypoxia without CO_2. This therefore suggested that although a higher concentration of acetylcholine was required to elicit a maximum response, the actual magnitude of the response remained unaltered and was unaffected by hypoxia.

It is concluded that acetylcholine and ATP act as vasodilators in the hepatic arterial microvasculature and as vasoconstrictors in the portal venous microvasculature using the present preparation. Prolonged hypoxia, with 5% CO_2, attenuated hepatic arterial vasodilator and enhanced portal venous vasoconstrictor actions to ATP. Omission of CO_2 from the hypoxic gas mixture abolished hepatic arterial vasodilatation.

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