Comparative Hepatology

Research

The role of ATP and adenosine in the control of hepatic blood flow in the rabbit liver *in vivo*

Dominic J Browse[1], Robert T Mathie[2], Irving S Benjamin[1] and Barry Alexander*[1]

Address: [1]Liver Sciences Unit, Academic Department of Surgery, GKT School of Medicine and Dentistry, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK and [2]Division of Surgery, Imperial College School of Medicine, Hammersmith Hospital, 150 Du Cane Road, London W12 ONN, UK

Email: Dominic J Browse - barry.alexander@kcl.ac.uk; Robert T Mathie - barry.alexander@kcl.ac.uk; Irving S Benjamin - irving.benjamin@kcl.ac.uk; Barry Alexander* - barry.alexander@kcl.ac.uk

* Corresponding author

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Abstract

Background: The role of adenosine and ATP in the regulation of hepatic arterial blood flow in the "buffer response" was studied *in vitro* and in a new *in vivo* model in the rabbit. The model achieves portal-systemic diversion by insertion of a silicone rubber prosthesis between the portal vein and inferior vena cava and avoids alterations in systemic haemodynamics.

Results: Hepatic arterial (HA) blood flow increased in response to reduced portal venous (PV) blood flow, the "buffer response", from 19.4 (3.3) ml min^{-1} 100 g^{-1} to 25.6 (4.3) ml min^{-1} 100 g^{-1} (mean (SE), $p < 0.05$, Student's paired t-test). This represented a buffering capacity of 18.7 (5.2) %. Intra-portal injections of ATP or adenosine (1 micrograms kg^{-1}-0.5 mg kg^{-1}) elicited immediate increases in HA blood flow to give -log ED_{50} values of 2.0 and 1.7 mg kg^{-1} for ATP and adenosine respectively. Injection of ATP and adenosine had no measurable effect on PV flow. *In vitro*, using an isolated dual-perfused rabbit liver preparation, the addition of 8-phenyltheophylline (10 MicroMolar) to the HA and PV perfusate significantly inhibited the HA response to intra-arterial adenosine and to mid-range doses of intra-portal or intra-arterial ATP ($p < 0.001$).

Conclusions: It is suggested that HA vasodilatation elicited by ATP may be partially mediated through activation of P_1-purinoceptors following catabolism of ATP to adenosine.

Background

The hepatic arterial (HA) hyperaemic response to portal vein (PV) occlusion, the hepatic arterial "buffer response" [1], is thought to be mediated by adenosine. Studies conducted in the cat demonstrated both inhibition of the buffer response by the adenosine receptor antagonist, 8-phenyltheophylline, and potentiation by the adenosine uptake inhibitor dipyridamole [2]. Further studies however, suggested that adenosine was not the sole agent responsible in the dog and other species [3-6].

Adenosine-5'-triphosphate (ATP) has been proposed to play an important role in the control of systemic [7,8] and hepatic vascular tone [9] and may therefore be a candidate for a role in the buffer response. ATP has been shown to be released from blood constituents [10] and vascular endothelium [11,12] during hypoxia [13] or altered flow

conditions [14] which may be encountered during reduction or total occlusion of portal venous blood flow.

Defined criteria have been proposed which must be fulfilled for a substance to be considered as a regulator of the buffer response [2]. These included: 1) the substance must dilate the hepatic artery; 2) substances in portal blood must have access to hepatic arterial resistance sites; 3) potentiators of the substance should also potentiate the buffer response; and 4) inhibitors of the substance should inhibit the buffer response. ATP has been shown to dilate the isolated hepatic artery [15] and the hepatic arterial vascular bed of the rabbit *in vitro* [9] and has been shown to act via the release of nitric oxide (NO) [16]. A similar mechanism is at least partly responsible for the hepatic arterial vasodilatation seen following portal venous injection of ATP in the same model [17]. In most vessels, ATP has been shown to elicit vasodilatation by stimulation of purinergic P_{2y} receptors, generally located in the vascular endothelium [9] although they may also be on HA vascular smooth muscle in the rabbit [15]. In some vessels however, ATP, which is rapidly catabolised to adenosine-5'-diphosphate (ADP), adenosine-5'-monophosphate (AMP) and adenosine in endothelial cells and vascular smooth muscle cells [18], causes vasodilatation via P_1-purinoceptors [19]. Total catabolism of ATP to ADP, AMP or adenosine would therefore raise the possibility that all previous findings relating to the buffer response were consistent with release of ATP alone. However, this mechanism of action of ATP is not believed to occur in the rabbit liver [9].

In vivo studies are required to confirm whether ATP is involved in the generation of the buffer response because it cannot be demonstrated in the *in vitro* perfused rabbit liver (Browse and Alexander, unpublished observation). In addition, current Home Office restrictions and economical factors which influence the use of larger animal models for experimentation has restricted *in vivo* studies in the UK although a feasibility study conducted in the Asian hybrid minipig in our laboratories proved unsuccessful [4]. The purpose of the present study therefore, was to develop an *in vivo* model for the assessment of liver blood flow in the rabbit to compare with our *in vitro* dual-perfused rabbit liver model [20] in order to establish whether ATP is involved in the generation of the buffer response.

Results

In vivo

In a number of experiments irreversible hypotension (n = 2), respiratory depression (n = 2) and acidosis (n = 2) occurred during the temporary occlusion of the portal vein for the insertion of the mesocaval shunt and data from these preparations have therefore not been included. It was imperative that haemodynamic stability should be attained before measurements were conducted and this was achieved in 5 preparations presented here. HA flow (HAF) was 19.4 (3.3) ml min^{-1} 100 g^{-1}, PV flow (PVF) 85.5 (19.3) ml min^{-1} 100 g^{-1} and mean arterial pressure was 80.2 (5.8) mmHg. When the mesocaval shunt was opened and the mesenteric vein occluded PVF decreased to 38.5 (3.7) ml min^{-1} 100 g^{-1} and HAF increased to 25.6 (4.3) ml min^{-1}100 g^{-1} ($p < 0.05$, Figure 2a) a calculated buffering capacity of 18.7 (5.2) % (Table 1, n = 5). During portal venous flow reduction the mean arterial pressure consistently rose to 85.2 (5.2) mmHg, ($p < 0.001$). When the portal venous flow was re-established there was often a small rebound portal "hyperaemia" accompanied by a temporary fall in HA flow and a fall in systemic blood pressure (Figure 2b).

In the 5 experiments described above HAF and PVF were stable for a sufficiently long period to allow the construction of dose-response curves for HA flow responses to intra-portal injection of adenosine or ATP. Intraportal injection of ATP and adenosine both caused immediate increases in HAF (Figure 3) and the -log ED_{50} values (calculated from the graph) for these agents were 2.0 mg kg^{-1} and 1.7 mg kg^{-1} for ATP and adenosine respectively. Injection of ATP and adenosine had no measurable effect on PV flow.

In vitro

Group 1. The effect of intra-arterial ATP

Livers from 6 rabbits [body weight 2.93 (0.14) kg, liver weight 119.2 (13.4) g] were perfused at raised tone [HAP 146.7 (7.7) and PVP 3.3 (0.8) mmHg]. The effect of the addition of 8-SPT to the hepatic arterial and portal venous perfusate was evaluated using previously calculated mid-range doses of adenosine, ACh and sodium nitroprusside [16]. 8-SPT (10 μM) significantly inhibited the HA response to 10^{-7}moles 100 g $liver^{-1}$ intra-arterial adenosine from 50.8 (6.2) to 31.6 (8.1) mmHg ($p < 0.05$), but did not significantly inhibit HA responses to 10^{-8}moles 100 g $liver^{-1}$intra-arterial ACh [68.9 (6.6) to 72.2 (5.7) mmHg] or to 10^{-8}moles 100 g $liver^{-1}$intra-arterial SNP [36.3 (4.4) to 41.6 (9.7) mmHg]. The dose-related response curve to intra-arterial ATP was also shifted to the right by 8-SPT [-log Molar ED_{50} 8.70 (0.22) to 7.63 (0.28), $p < 0.001$] indicating inhibition of responses to ATP (Figure 4a). The amplitude of portal venous responses to intra-arterial ATP correlated with the duration of perfusion (Figure 4b).

Group 2. The effect of intra-portal ATP

Livers from another group of 6 rabbits [body weight 2.60 (0.14) kg, liver weight 98.8 (5.2) g] were perfused at raised tone [HAP 156.2 (4.8) and PVP 2.3 (0.7) mmHg]. The addition of 8-SPT to the hepatic arterial and portal venous

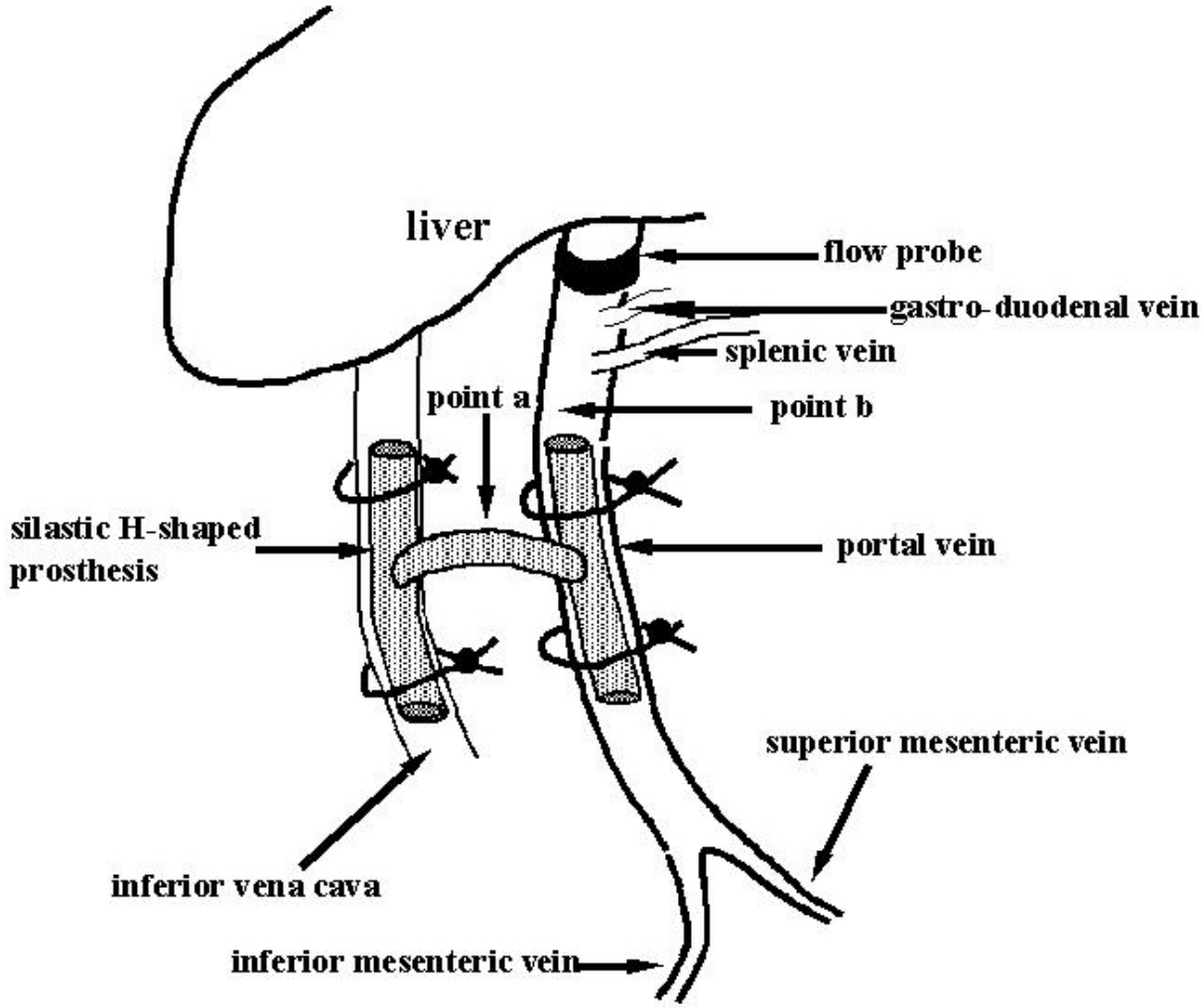

Figure 1
Diagram of silastic H-shaped prosthesis inserted into the portal vein and the inferior vena cava of the *in vivo* rabbit model. During control conditions, the prosthesis is clamped across the horizontal limb at "a". Portal-systemic diversion is achieved by removal of the clamp from "a" and cross-clamping at point "b", distal to the point of entry of the splenic vein into the portal vein.

perfusate significantly inhibited the HA response to 10^{-8} moles 100 g $liver^{-1}$ intra-arterial adenosine from 33.2 (3.5) to 6.5 (3.8) mmHg ($p < 0.001$). The HA dose-related responses to mid-range doses of intra-portal ATP were also significantly reduced by 8-SPT, causing a non-significant right shift of the dose-response curve to ATP from -log Molar ED_{50} 5.08 (0.15) to 4.97 (0.12) ($p = 0.05$) (Figure 5a). The portal venous responses to intra-portal injections of ATP were not significantly altered by 8-SPT (Figure 5b).

Discussion

A new model for the study of liver blood flow in the rabbit has been presented, based on a concept developed in the dog [6,21]. The preparation employed a mesocaval shunt to divert blood to the systemic circulation during portal venous occlusion to prevent the fall in systemic blood pressure due to mesenteric pooling of portal blood [22]. This model is also closer to physiological portal venous flow conditions than models where splenectomy is necessary [2,23]. The insertion of the prosthetic mesocaval

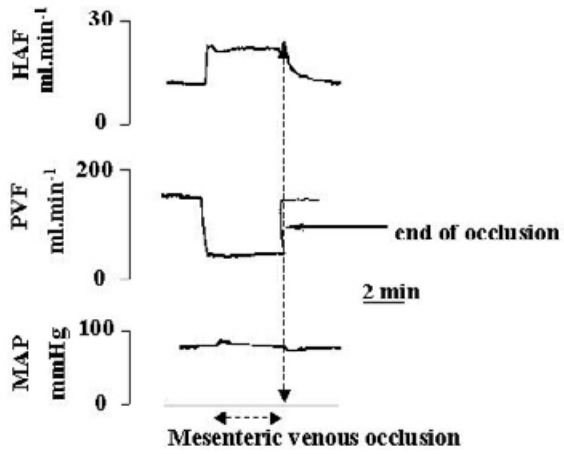

Figure 2
The hepatic arterial buffer response during portal venous occlusion. There was a significant increase in hepatic arterial flow during portal venous occlusion (* $p < 0.05$) compared to basal hepatic arterial flow. HAF = hepatic arterial flow, PVF = portal venous flow and MAP = mean arterial pressure.

shunt, which required a brief period of PV occlusion, can cause irreversible systemic hypotension, and this reduced the success rate. Experiments are in progress to improve this model further by the surgical construction of a meso-caval shunt, although this is difficult due to the fragility of the rabbit portal vein. Nevertheless a hepatic arterial buffer response could be clearly demonstrated in all the successful preparations. During portal venous occlusion the mean arterial blood pressure also increased but this was insufficient to account for the increase in hepatic arterial flow. This model, if further developed, may therefore prove to be an alternative to experimental models in the cat and dog for investigations of this nature.

The action of ATP in this *in vivo* rabbit liver model was also demonstrated. Intra-portal injection of ATP or adenosine elicited a potent vasodilatation of the hepatic artery. This action occurred over a similar dose range to that observed in our *in vitro* perfused rabbit liver model [17]. The HA dilator action of intra-portal ATP fulfilled the first two criteria defined by Lautt [2], equivalent to the first criterion originally proposed by Dale [24], in order to be considered as a regulator of the buffer response, namely that the addition of ATP elicited the appropriate response (vasodilatation of the HA) and portal injection of ATP permitted access to the arterial resistance sites. In addition, antagonists of adenosine, the catabolite of ATP, although indirect, partially attenuated the response, thus fulfilling the second of Dale's postulates. However, further experiments using inhibitors of these agents have proved difficult in the past and often resulted in haemodynamic instability [6] or prolonged hepatic arterial vasospasm [25]. Thus we used our comparable *in vitro* model as an alternative preparation for these investigations.

We have previously shown that intra-portal or intra-arterial injection of ATP dilated the rabbit HA vascular bed, and that this was mediated, at least in part, by NO [16,17]. However, in other vessels ATP has been shown to act via adenosine receptors [19]. We therefore tested whether some of the HA dilatation to ATP was attributable to catabolism to adenosine by using the non-selective P_1-purinoceptor antagonist 8-SPT [26].

Our results demonstrated that both intra-arterial and intra-portal injection of ATP caused HA vasodilatation, at least in part, through activation of P_1-purinoceptors. The way in which 8-SPT inhibited responses to ATP was of interest. The responses to lower doses of ATP were unaffected, as expected, because ATP is a more potent vasodilator than adenosine, but the 'middle range' doses of ATP were certainly inhibited, while higher doses were not. These data do not contradict our earlier findings where HA vasodilatation to ATP did not appear to be affected by 8-SPT [9]. The previous study only reported the action of 8-SPT at the two highest doses of ATP used, due to constraints of time upon the viability of the preparation, since characterised in greater detail by Browse et al [27]. The data points at the two highest doses used in this study conformed with these since only responses to mid-range doses of ATP were significantly attenuated. This may have been due to the overwhelming of competitive inhibition at high doses or have been indicative of a different mechanism of ATP and/or adenosine action [28].

There was no apparent difference in the degree of inhibition of HA responses by 8-SPT between intra-arterial and intra-portal injection of ATP despite a longer lag-time between injection and response following intra-portal injection of ATP. This might suggest that nearly all the adenosine produced from ATP catabolism was taken up effectively by the endothelium and vascular smooth muscle [18] as soon as the adenosine was formed, and that only the adenosine formed in the hepatic arterial vasculature from ATP catabolism contributed to the hepatic arterial response to ATP. This occurred despite the presumably higher concentration of adenosine in the liver as a whole following intra-portal (10^{-8} - 10^{-4} log moles ATP 100 g liver^{-1}) compared with intra-arterial injections (10^{-10} - 10^{-6} log moles ATP 100 g liver^{-1}) of ATP.

This 8-SPT-induced inhibition of responses to ATP raises the possibility that, in studies where 8-SPT reduced the

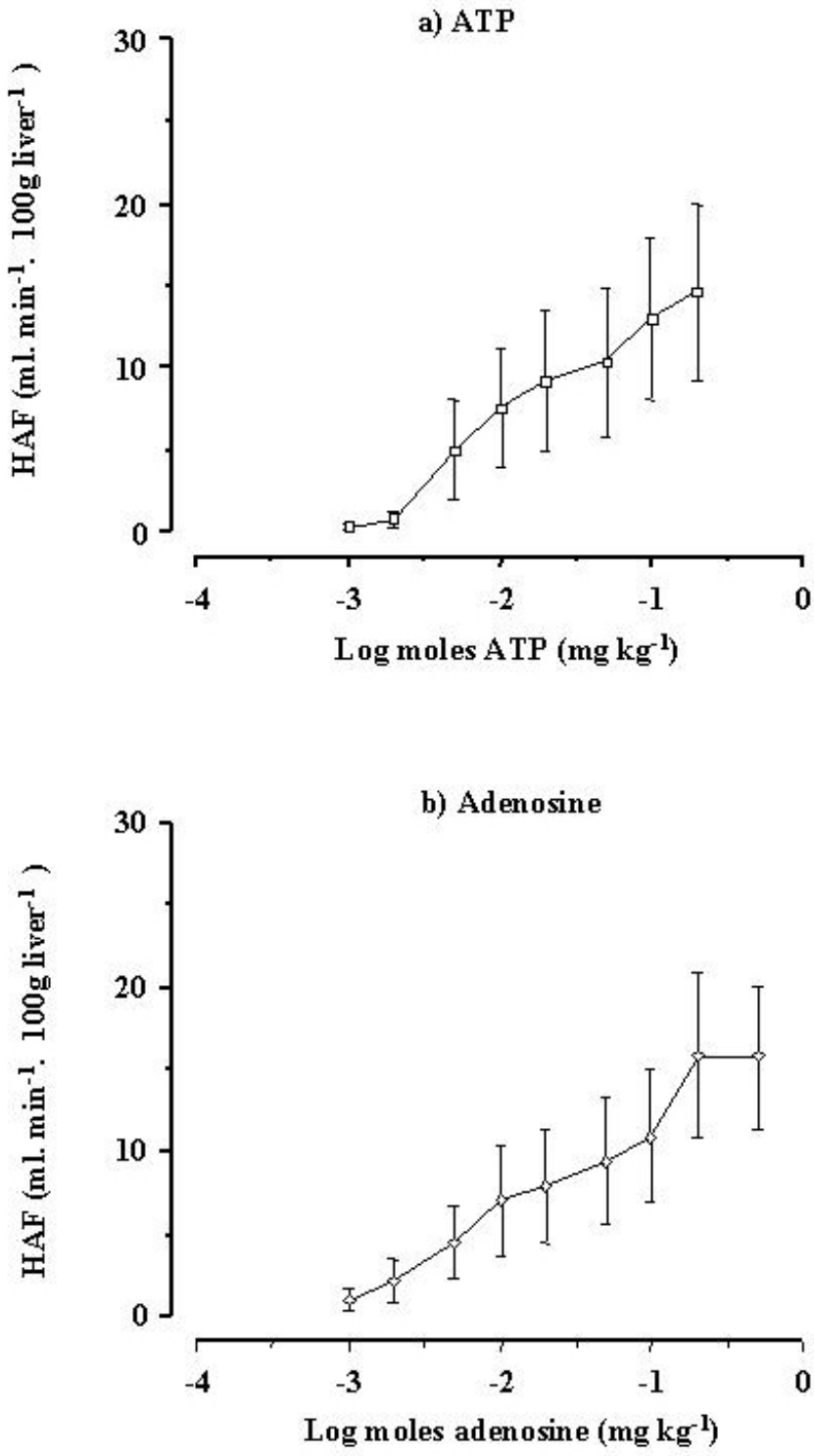

Figure 3
The effect of intra-portal injection of (a) ATP and (b) adenosine on changes in hepatic arterial flow (Δ HAF) *in vivo*. Both agents increased hepatic arterial flow in a dose-dependent manner. The error bars in the graphs represent the SE.

Table 1: The effect of portal venous flow (PVF) reduction on hepatic arterial flow (HAF) and mean arterial blood pressure (MAP).

	BEFORE PORTAL VENOUS OCCLUSION				AFTER PORTAL VENOUS OCCLUSION				
Exp. no.	n	HAF (ml. min^{-1}.100 g^{-1})	PVF (ml. min^{-1}.100 g^{-1})	MAP (mmHg)	HAF (ml. min^{-1}.100 g^{-1})	PVF (ml. min^{-1}.100 g^{-1})	MAP (mmHg)	HAF increase (%)	Buffering capacity (%)
1	4	16.9	143.2	95.0	25.3	39.1	96.8	66.8	8.9
2	2	17.5	64.2	67.5	22.5	35.0	72.5	28.5	17.6
3	2	9.4	63.5	70.0	11.4	48.5	75.0	22.0	15.1
4	6	25.4	70.9	75.0	36.7	31.5	84.2	40.3	33.2
5	3	27.8	-	93.3	32.1	-	97.3	15.3	-
Mean (SE)	-	19.4 (3.3)	85.5 (19.3)*	80.2 (5.8)*	25.6 (4.3)	38.5 (3.7)	85.2 (5.2)	34.6 (9.1)	18.7 (5.2)

Each value is the mean of the number of observations stated. Both HAF and MAP increased significantly during PV occlusion (* $p < 0.05$, $n = 5$).

magnitude of the buffer response [2,6], the primary agent responsible for the buffer response could have been ATP and not adenosine. Further studies will be required to distinguish between these two agents. Firstly, the inhibition by 8-SPT of the ATP induced HA vasodilatation must be shown to occur *in vivo*. Secondly, if ATP is the primary agent, the buffer response may also be, at least partially, inhibited by an NO synthesis inhibitor because we have previously reported that ATP-induced but not adenosine-induced HA vasodilatation is attenuated by such an inhibitor in the rabbit liver [6]; and thirdly, vascular responses to adenosine must be shown to be independent of NO, because recent evidence from the hypoxic guinea-pig heart has suggested that adenosine may act via A2-purinoceptors to release nitric oxide [29] and this point should be considered in this model.

Conclusions

In summary, a new *in vivo* rabbit liver model for the investigation of liver blood flow has been presented which, although at an early stage of development, may prove to be a useful model. The hepatic arterial buffer response and the hepatic arterial vasodilatation elicited by ATP and by adenosine have been consistently and reproducibly demonstrated. In an established *in vitro* model, hepatic arterial vasodilatation elicited by ATP has been shown to be partly mediated through P_1-purinoceptors suggesting that ATP could have a role in the generation of the buffer response in the rabbit liver.

Methods

Experiments were carried out in a total of 27 male New Zealand white rabbits weighing 2.2 – 3.4 kg, fed and permitted access to water *ad libitum*. The experimental protocols were approved by the guidelines and legislative procedures outlined by the Home Office of the United Kingdom in the Animal Scientific Procedures Act 1986. Pre-operative sedation was with fentanyl/fluanisone s.c. ('Hypnorm', 0.3 ml kg^{-1}, Janssen Animal Health).

In vivo *experiments (n = 15)*

Anaesthesia was induced in rabbits [2.8 (0.1) kg] with midazolam ('Hypnovel', 0.3 ml kg^{-1}, Roche Products Limited) and maintained with a continuous infusion of 'Hypnorm' (0.1 – 0.3 ml kg^{-1} hr^{-1}) through a cannulated marginal ear vein. The rabbits were intubated but allowed to breathe spontaneously. The inspired oxygen was adjusted to maintain arterial PO_2 and PCO_2 at normal levels (approximately 100 mmHg and 40 mmHg, respectively) and body temperature was kept at 36–38°C by operating table heating elements. Fluid balance was achieved by intravenous infusion of 150 mM sodium chloride and acid-base balance maintained by injection of sodium bicarbonate as required.

Operative procedure

The experimental preparation was based upon a model we have previously established in the dog [21,30]. After cannulation of the carotid artery for blood pressure monitoring, a midline laparotomy was performed and the inflow vessels to the liver dissected. The gastroduodenal artery and vein were ligated and divided. A prosthetic (H-shaped) mesocaval shunt, constructed from 3.0 mm internal diameter silicone rubber tubing, was inserted proximal to the splenic vein after heparinisation (300 iu. kg^{-1} i.v.). This allowed diversion of mesenteric blood flow to the systemic circulation as required. A clamp was placed on the cross limb of the "H" to restore portal flow. Pre-calibrated electromagnetic flow probes (Statham) were applied to the common hepatic artery and portal vein (1 and 3 mm diameter respectively) (Figure 1).

Experimental protocol

After 1 hour equilibration, the effect of a reduction in PV flow on HA flow (i.e. the buffer response) was tested. PV flow was reduced by clamping the mesenteric vein, proximal to the insertion of the splenic vein, and opening the mesocaval shunt for 3 min. This procedure, which diverts mesenteric flow into the systemic circulation reduces portal flow to that of splenic vein flow was con-

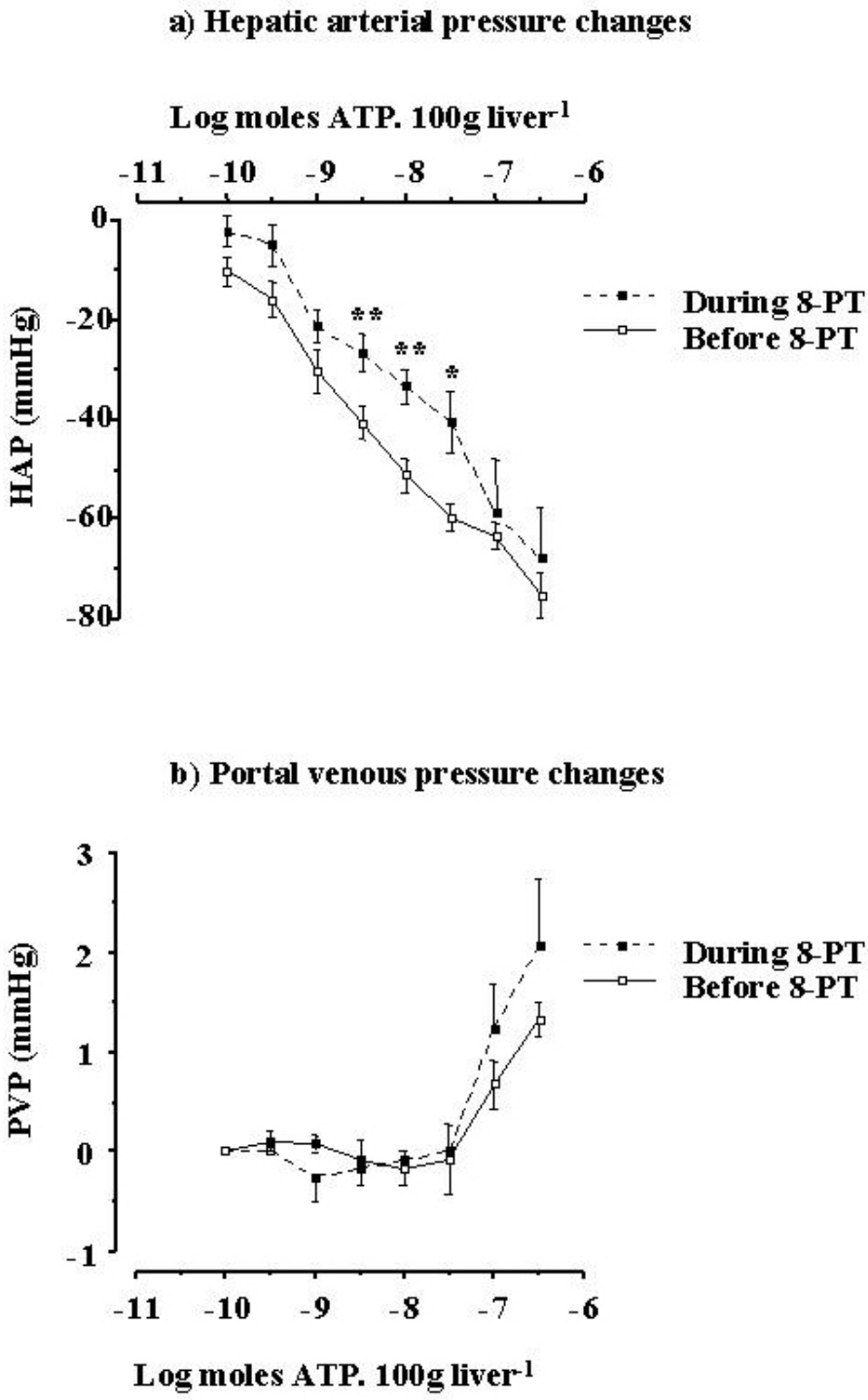

Figure 4
The changes in (a) hepatic arterial pressure responses (Δ HAP) and (b) portal venous pressure responses (Δ PVP) to intra-arterial injection of ATP. The adenosine receptor antagonist 8-phenyltheophylline (10 μM) significantly decreased hepatic arterial responses to ATP, while portal venous responses were unaffected (* $p < 0.05$, ** $p < 0.01$, compared with before 8-SPT). The error bars in the graphs represent the SE.

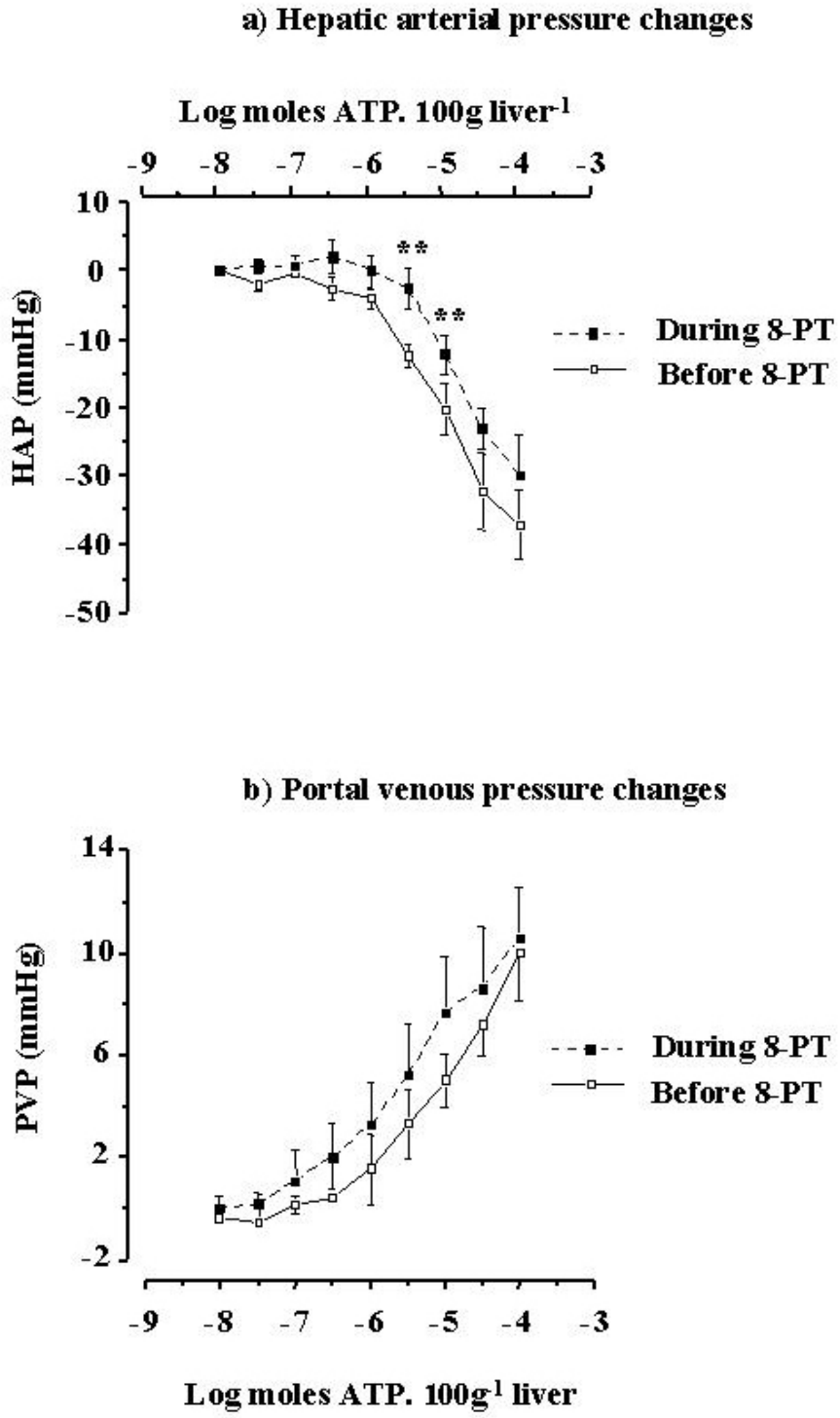

Figure 5
The changes in (a) hepatic arterial pressure responses (Δ HAP) and (b) portal venous pressure responses (Δ PVP) to intra-portal injection of ATP. The adenosine receptor antagonist 8-phenyltheophylline (10 μM) significantly decreased hepatic arterial responses to ATP, while portal venous responses were unaffected (** $p < 0.01$, compared with before 8-SPT). The error bars in the graphs represent the SE.

ducted at least twice per experiment (see Table 1). Measurement of the buffer response was then recorded as absolute flow values from the precalibrated electromagnetic flow probes. Hepatic blood flow was then restored by removal of the vascular clamp on the PV, and reapplication of the anastomotic clamp to the cross-limb of the H-shunt.

When haemodynamic stability had been achieved, incremental doses of ATP or adenosine (1 μg kg^{-1} – 0.5 mg kg^{-1}) (Sigma U.K. Ltd), dissolved in saline, were injected into the portal vein and changes in HA or PV blood flow could again be recorded as absolute values from the precalibrated electromagnetic flow meters. Dose-response curves of changes in blood flow vs dose of drug injected were then constructed.

Calculations

Blood flows were recorded on the flowmeters in ml min^{-1} and subsequently recalculated in ml min^{-1} 100 g^{-1} by relating the readings to the wet weight of the liver, determined at the end of each experiment. The "buffering capacity" of the HA was expressed in % as:

[Increase in HA flow / Decrease in PV flow] × 100

In vitro *experiments (n = 12)*

Twelve rabbits were anaesthetised with Hypnovel (midazolam) 1.5 mg kg^{-1} i.v., and a further 0.3 ml kg^{-1} Hypnorm was injected i.m. for continued analgesia during the 40 minute operative period. The operative technique has been described in detail elsewhere [20] but will be outlined in brief here. The abdomen was opened though a mid-line incision, and the common bile duct cannulated to facilitate exposure and cannulation of the common hepatic and gastroduodenal artery in addition for the collection of bile during perfusion. After administration of heparin i.v. (300 units kg^{-1}) the common hepatic artery and the gastroduodenal artery were cannulated (Portex 3FG). Ten ml of heparinised saline (20 units ml^{-1}) were infused into the catheters to prevent intrahepatic coagulation. The gastroduodenal vein was ligated, the PV cannulated and 40 ml of heparinised saline flushed through the PV system. The liver was then rapidly excised from the animal, weighed and placed in an organ bath.

Liver perfusion

Livers were perfused via the HA and PV 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 mmoles L^{-1}: NaCl 133, KCl 4.7, NaH_2PO_4 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 (95% O_2: 5% CO_2). Homogeneous liver perfusion was indicated by all sections of the liver changing to a uniform colour. Changes in vascular tone were recorded as changes in perfusion pressure measured with Spectramed (Statham) P23XL physiological pressure transducers from side arms of the perfusion circuit and from the gastroduodenal artery cannula. These were recorded on a Grass 79 F polygraph (Grass Instrument Co., Quincy, Mass., USA). Perfusion under these conditions maintains liver viability for 5 hours [27].

Experimental protocol

Methoxamine was added to the perfusate at a -log Molar concentration of 5.27 (0.05) to raise the tone of the preparation. Two groups of rabbits were studied: ATP injection into the HA (Group 1), and ATP injection into the PV (Group 2). Dose response curves were constructed to ATP (10^{-10} to 10^{-6} moles 100 g $liver^{-1}$ for intra-arterial, and 10^{-8} to 10^{-4} moles 100 g $liver^{-1}$ for intra-portal injection) and repeated after a 15 minute equilibration period following the addition of the water soluble derivative of 8-phenyltheophylline (8-PT, 8-(*p*-sulphophenyl)-theophylline (8-SPT) (Research Biochemicals Inc.), to the arterial and venous perfusate. Single HA doses of acetylcholine (ACh, 10^{-7} moles 100 g $liver^{-1}$) and/or sodium nitroprusside (SNP, 10^{-8} moles 100 g $liver^{-1}$) were given at regular intervals throughout the experiment to confirm the maintenance of the vascular responses with time, while intra-arterial doses of 10^{-7} moles 100 g $liver^{-1}$ adenosine (the catabolite of ATP) were given to confirm inhibition by 8-SPT [6,16]. All drugs were made up in saline.

Statistical analysis

The data was confirmed to be normally distributed using Kolmogorov-Smirnov test and also that the variances of the data were not significantly different using Graphpad, copyright 1994–1996 by GraphPad Software Inc. Student's paired t-test was therefore used to test the significance of differences between observations before and after PV occlusion, and the magnitude of vascular responses to ATP before and during administration of 8-SPT. Significance level was always taken at $\alpha = 0.05$. All data are presented as mean (SE).

Authors' contributions

Dominic Browse and Robert Mathie with help from Barry Alexander conducted the laboratory experiments. Barry Alexander and Dominic Browse co-wrote the manuscript and Irving Benjamin co-edited the manuscript with Barry Alexander. All authors have read and approved the manuscript.

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