Research

The role of ATP and adenosine in the control of hepatic blood flow in the rabbit liver in vivo
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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 ED₅₀ 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 P₁-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.
purinergic P2y receptors, generally located in the vascular has been shown to elicit vasodilatation by stimulation of ATP in the same model [17]. In most vessels, ATP is believed to act via the release of nitric oxide (NO) [16]. A similar mechanism is at least partly responsible for the hepatic arterial 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 P2x 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 P1'-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 ED₅₀ 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 midrange doses of adenosine, ACh and sodium nitroprusside [16]. 8-SPT (10 µM) significantly inhibited the HA response to 10⁻⁷moles 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 10⁻⁸moles 100 g liver⁻¹ intra-arterial ACh [68.9 (6.6) to 72.2 (5.7) mmHg] or to 10⁻⁸moles 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 ED₅₀ 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 toxification (Figure 4b).
perfusate significantly inhibited the HA response to $10^{-8}$ moles $100 \text{ g liver}^{-1}$ 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 $-\log \text{ Molar ED}_{50}$ 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
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.

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 P1-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 P1-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...
The effect of intra-portal injection of (a) ATP and (b) adenosine on changes in hepatic arterial flow ($\Delta$ HAF) in vivo. Both agents increased hepatic arterial flow in a dose-dependent manner. The error bars in the graphs represent the SE.
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 P1-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).

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

| Exp. no. | n | HAF (ml min⁻¹100 g⁻¹) | PVF (ml min⁻¹100 g⁻¹) | MAP (mmHg) | HAF (ml min⁻¹100 g⁻¹) | PVF (ml min⁻¹100 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).

### 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 PO2 and PCO2 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-
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 4
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.

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:

\[
\text{[Increase in HA flow / Decrease in PV flow]} \times 100
\]

**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 through 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. (500 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, NaH₂PO₄ 1.35, NaHCO₃ 20.0, MgSO₄ 0.61, Glucose 7.8, and CaCl₂ 2.52) at 37 °C, from a common oxygenated reservoir (95% O₂: 5% CO₂). 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 10⁻⁶ 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 α = 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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