The transhepatic action of ATP on the hepatic arterial and portal venous vascular beds of the rabbit: the role of nitric oxide

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1 The effect of bolus administration of adenosine 5'-triphosphate (ATP) into the portal vein on hepatic arterial pressure (the transhepatic action of ATP) and portal venous pressure, and the contribution of nitric oxide towards these responses, was studied in the *in vitro* dual-perfused rabbit liver.

2 At basal tone, hepatic arterial and portal venous vasoconstriction followed ATP injection, while at a tone raised with methoxamine (10^{-6} – 10^{-5} M) ATP caused hepatic arterial vasodilatation, and a phasic vasodilatation followed by vasoconstriction in the portal venous vascular bed.

3 To determine whether the transhepatic arterial dilatation was due to the diffusion of nitric oxide (NO) from the portal venous vasculature, N^G-nitro-L-arginine methyl ester (L-NAME, 100 µM), an inhibitor of NO synthesis, was infused selectively into the portal vein. L-NAME infusion potentiated portal venous vasoconstriction to ATP ($-\log M ED_{50}$ 5.32 ± 0.31 to 6.51 ± 0.43 , $P < 0.05$, Student's paired *t* test) indicating the possible inhibition of a NO-mediated vasodilator component of the portal venous response to ATP. There was, however, no demonstrable difference in the transhepatic arterial vasodilatation induced by ATP during this infusion.

4 Simultaneous perfusion of both the hepatic arterial and portal venous inflows with L-NAME (100 µM) resulted in a significant decrease in the amplitude of hepatic arterial responses to ATP demonstrating that these responses were ultimately mediated by an NO-dependent mechanism.

5 This study has thus demonstrated a vasodilator component of the portal venous response to ATP that is NO-mediated. It also provides evidence that it is not portally-derived NO, but NO released from the hepatic arterial vascular bed, that accounts for the hepatic arterial vasodilatation to intra-portal administration of ATP. This implies that ATP itself, and not a second messenger, diffuses from the portal venous to hepatic arterial vascular bed to elicit the hepatic arterial response.

Keywords: Hepatic circulation; vasodilatation; vasoconstriction; L-NAME

Introduction

It is widely accepted that adenosine 5'-triphosphate (ATP) has a role in the control of systemic (Su, 1985; Burnstock, 1986) and hepatic vascular tone (Mathie *et al.*, 1991). It has been shown to act as a co-transmitter with noradrenaline in the sympathetic nervous system in the hepatic artery of the rabbit (Brizzolara & Burnstock, 1990), and perhaps with nitric oxide (NO) in the non-adrenergic, non-cholinergic nerves of the longitudinal muscle of the rabbit portal vein (Brizzolara *et al.*, 1993). ATP also affects the spontaneous electrical and mechanical activity of the longitudinal muscle of the rat portal vein (Reilly & Burnstock, 1987). ATP may act directly on the vessel wall to cause either vasoconstriction as in the rabbit's perfused liver (Ralevic *et al.*, 1991) and rat liver (Lee & Filkins, 1988) via P_{2x} receptors on the smooth muscle of the vessel (Kennedy & Burnstock, 1985), or vasodilatation via P_{2y} receptors located either on the endothelium in the hepatic arterial vascular bed (Mathie *et al.*, 1991) or on the smooth muscle in the isolated hepatic artery (Brizzolara & Burnstock, 1991).

It has been recognized for a long time that a complex interrelationship occurs in the liver between the hepatic arterial and portal venous blood flows (Burton-Opitz, 1911). In particular the 'hepatic arterial buffer response' (Lautt, 1985), hepatic arterial dilatation in response to a reduction in portal venous flow, has been shown to be in some way dependent on adenosine (Lautt *et al.*, 1985) although the exact mechanism is not clear. Adenosine may not be the only participant in the control of the buffer response (Mathie &

Alexander, 1990) and it is possible that ATP is also involved, although an extrinsic neural regulation has been excluded (Mathie *et al.*, 1980). Injection of vasoactive agents into either the hepatic artery or the portal vein affects vascular tone in both inflow vessels by a 'transhepatic effect' (Withrington & Richardson, 1990), or 'transvascular' route of action (Lautt *et al.*, 1984). It seems that tissue diffusion is the most likely mechanism for the transfer of action from one vessel to the other, although a direct intraluminal transfer through a portal to arterial communicating vessel has not been completely excluded. The transhepatic effect may be important in the generation of the buffer response because purines, including ATP, may be released from blood constituents (Bergfeld & Forrester, 1992) and endothelium (Palmer *et al.*, 1987; Bodin *et al.*, 1991), during conditions such as hypoxia (Belloni *et al.*, 1985) or altered flow (Ralevic *et al.*, 1992).

Recently Falcone & Bohlen (1990) showed that perivenular injection of acetylcholine (ACh) caused dilatation of the paired arteriole and that this was not due to diffusion of ACh but of NO, following release of NO by the venule. The present study was designed to test the hypothesis that the hepatic arterial vasodilator action of portally injected ATP was due to diffusion of NO, released from the portal vascular bed and acting on the hepatic arterial resistance sites, rather than transfer of the primary agent itself. ATP was chosen as the prime agent because of its importance in the regulation of hepatic arterial vascular tone. This investigation, therefore, studied the effect of portally administered N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthesis inhibitor (Moore *et al.*, 1990), on the hepatic arterial vasodilator res-

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ponses to intraportal injections of ATP. The results further characterize hepatic arterial vasodilatation and portal venous vasoconstriction to ATP and provide evidence which suggests that it is ATP itself, and not NO, that diffuses from the portal vein to the hepatic arterial resistance sites to produce the transhepatic effect of ATP.

Methods

Operative procedures

Male New Zealand White rabbits, weighing 2.2–2.9 kg, were initially sedated with subcutaneous fentanyl-fluanisone (Hypnorm, 0.3 ml kg⁻¹), and 15 min later anaesthetized with intravenous midazolam (Hypnovel, 1.5 mg kg⁻¹) through a cannulated marginal ear vein. A further 0.3 ml kg⁻¹ Hypnorm was injected i.m. for continued analgesia during the 40 min operative period. The operative technique has been described elsewhere (Alexander *et al.*, 1992) but will be outlined in brief here.

The abdomen was opened through a mid-line incision, and the common bile duct cannulated. After administration of heparin i.v. (300 units kg⁻¹) the common hepatic artery, and the gastroduodenal artery when present, were cannulated (Portex 3FG) and the gastroduodenal vein ligated. Heparinised saline, 10 ml of a 20 units ml⁻¹ solution, was infused into the catheters to prevent blood coagulation in the intra-hepatic hepatic arterial vasculature. The portal vein was then cannulated and 40 ml of heparinised saline were flushed through the portal venous system. The liver was then rapidly excised from the animal, weighed and placed in an organ bath.

Liver perfusion

Livers were perfused via the hepatic arterial and portal venous cannulae at constant flow rates of 25 and 75 ml min⁻¹ 100 g⁻¹ respectively. The perfusate used was Krebs-Bülbring buffer solution (composition mM: 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 an even beige colour. 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 Co., Quincy, Mass., U.S.A.). Perfusion under these conditions has been shown to maintain liver viability for 5 h (Browse *et al.*, 1994a).

Drug administration

Methoxamine (hydrochloride), ATP (disodium salt) and L-NAME (hydrochloride) were obtained from Sigma Chemical Company. All were dissolved in distilled water. Methoxamine was added to the perfusate at a $-\log M$ dose of 5.31 ± 0.06 (mean $\pm$ s.e.mean) to raise the tone in the hepatic vascular bed. ATP was injected into the hepatic artery and portal vein as a 0.1 ml bolus to give doses over the range 10^{-10} to 10^{-4} mol 100 g liver⁻¹ (doses were adjusted for liver weight), further injections being given when the pressures had returned to normal. L-NAME was administered either as an infusion into the portal inflow via a syringe pump to a portal venous perfusate concentration of 100 μ M, or was added to the perfusate reservoir to enter both inflow circuits (arterial and venous) at the same perfusate concentration (100 μ M). A 30 min equilibration period followed any addition of L-NAME.

Statistics and presentation of data

Responses were recorded as changes in perfusion pressure (mmHg). All results are presented as mean $\pm$ s.e. Student's paired or unpaired *t* test was used, as appropriate, to test for differences between responses, $P < 0.05$ being taken as significant.

Comparison between the direct responses (injection and response in same vascular bed) and transhepatic responses (injection and response in opposite vascular beds) to ATP was made by the method described by Withrington (1992). A 'transhepatic' ratio was calculated by dividing the arterial molar ED₅₀ by the molar portal dose required to give a response equivalent to that seen with the arterial molar ED₅₀. In practice this was performed by subtracting the logarithms of these doses to give a log-transhepatic ratio.

Experimental protocol

Experiments were divided into three groups, with five rabbits in each.

Group 1 To define the dose-related responses of the hepatic arterial and portal venous vascular beds to ATP, injected via the hepatic artery and the portal vein, at both basal and raised tone.

Group 2(a) To demonstrate the effect of L-NAME administration via the portal vein on the hepatic arterial and portal venous responses to portally injected ATP at raised tone. First, dose response curves to ATP for portal venous injection were constructed (phase 1); L-NAME was then infused into the portal venous circuit and the dose-response curves to ATP repeated (phase 2); finally, further dose-response curves were obtained after addition of L-NAME to the perfusate reservoir, common to both hepatic arterial and portal venous inflows (phase 3). Single hepatic arterial doses of 10^{-7} mol 100 g⁻¹ liver ATP and adenosine were also given into the hepatic artery at regular intervals throughout the experiment to monitor the effect of portal venous L-NAME on direct responses of the hepatic vascular bed, and to confirm the stability of hepatic arterial responses with time.

Group 2(b) To act as control experiments to allow for the changes seen during a long duration of perfusion and for changes associated with the vehicle for L-NAME. Three ATP dose-response curves were constructed following portal venous injection. These experiments were time-matched with those in group 2a and used the vehicle for L-NAME (distilled water) as a control portal venous infusion during phase 2, and as an addition to the reservoir in phase 3. Single hepatic arterial doses of ATP and adenosine were given at intervals similar to those in group 2(a).

Results

Perfusion indices

Group 1 Five rabbits (2.65 ± 0.10 kg), with liver weight 80.4 ± 5.7 g, were perfused at basal pressure (hepatic arterial and portal venous pressures were 33.6 ± 2.5 , and 0.8 ± 0.6 mmHg respectively) while dose-response curves to ATP were constructed for both hepatic arterial and portal venous injection. After addition of methoxamine, further dose-response curves were obtained at raised pressure (hepatic arterial pressure = 142.0 ± 10.4 , portal venous pressure = 3.0 ± 1.3 mmHg).

Group 2a and 2b In group 2a, 5 rabbits (2.58 ± 0.10 kg), liver weight 96.0 ± 0.2 g, were perfused at raised tone (hepatic arterial pressure = 158.8 ± 5.2 , portal venous pressure = 2.7 ± 0.8 mmHg) for the duration of the experiment.

In group 2b, 5 rabbits (2.59 ± 0.12 kg), liver weight 89.8 ± 4.1 g, were perfused in the same manner (hepatic arterial pressure = 152.0 ± 4.9 , portal venous pressure = 1.9 ± 0.7 mmHg). There were no significant differences in perfusion pressure between these groups.

The rate of bile production fell during the perfusion period in all groups (group 2a, 7.0 ± 2.2 to 2.9 ± 0.7 , group 2b, 7.5 ± 1.2 to 3.7 ± 0.8 ml h⁻¹), consistent with interruption of the entero-hepatic circulation of bile salts. This fall was not correlated with the administration of L-NAME and was not investigated further.

ATP responses

Group 1 Hepatic arterial and portal venous dose-response curves at basal and raised tone The hepatic arterial and portal venous responses to ATP, following hepatic arterial and portal venous injection, at basal tone are shown in Figure 1a and 2a respectively. In all cases vasoconstriction was the demonstrated response. There was a significant difference between direct and indirect hepatic arterial responses to ATP above 10^{-7} mol 100 g⁻¹ liver. Although the responses were small, hepatic arterial vasoconstriction was seen at high intraportal doses with a threshold to elicit a measurable response of 5.40 ± 0.37 - log mol 100 g⁻¹ liver (maximal transmitted response = 3.2 ± 0.5 mmHg, resolution limit approximately = 1 mmHg pressure change). The portal venous responses were comparable following direct and indirect injection of ATP, differing significantly only at low

Figure 1 Responses of hepatic arterial pressure (HAP) to increasing doses of ATP injected direct (into the hepatic artery) and indirectly (into the portal vein) at basal tone (a) and at a tone raised with methoxamine (b). There is a significant difference between direct (■) and indirect (□) injections above 10^{-7} and 3×10^{-10} mol 100 g⁻¹ liver in (a) and (b) respectively. (Group 1).

Figure 2 Responses of portal venous pressure to increasing doses of ATP injected direct (into the portal vein ■) and indirectly (into the hepatic artery □) at basal tone (a) and at a tone raised with methoxamine (b). There is a significantly lower threshold for vasoconstriction following direct injection at basal tone. * $P < 0.05$ between direct and indirect responses. Numbers in parentheses = n ; these decrease as the dose injected increases due to duration of hepatic arterial response following supramaximal injection. (Group 1).

doses (3×10^{-7} – 3×10^{-6} mol 100 g⁻¹ liver⁻¹, threshold 6.40 ± 0.25 and 5.50 ± 0.16 - log mol 100 g⁻¹ liver⁻¹ for direct and indirect injection respectively, $P < 0.05$). Maximal responses were not attained at the doses administered.

At raised tone, dilatation was the predominant response to ATP in the hepatic arterial vascular bed (Figure 1b) with a - log molar ED₅₀ of 7.72 ± 0.27 for direct hepatic arterial injection and 4.06 ± 0.58 for indirect portal venous injection, giving a log-transhepatic ratio of 3.66 ± 0.56 . In the portal venous bed constriction remained the dominant response (Figure 2b). The venous constriction to indirect (hepatic arterial) injection was similar to that at basal tone although the responses to direct injection were of greater amplitude than at the lower tone ($P < 0.05$ for doses 3×10^{-5} – 3×10^{-4} mol 100 g⁻¹ liver). A modest degree of vasodilatation to ATP was seen at raised tone in the portal bed and had a much lower threshold than vasoconstriction (8.90 ± 0.50 and 7.30 ± 0.30 - log mol 100 g⁻¹ liver for vasodilatation and vasoconstriction respectively, $P < 0.05$). It was, however, overridden by the strong constrictor response at higher doses (above 7.11 ± 0.29 - log mol 100 g liver⁻¹, Figure 2b). Lag times between injection and the initiation of response were of short duration (<5 s) for ATP although this was not the case for hepatic arterial responses to portal venous injection. In this latter situation the lag time was prolonged to 10–20 s, with a slow gradual rise to the maximal response (cf. direct hepatic arterial responses) (Figure 3).

Figure 3 Hepatic arterial pressure responses to ATP following intraportal (a) and intra-arterial injection (b). The effect of 100 mm N^{G} -nitro-L-arginine methyl ester in the perfusate is shown in (c) and (d). (Group 2).

Group 2. The effect of L-NAME on transhepatic responses

Hepatic arterial responses to portal venous injection of ATP Hepatic arterial perfusion pressure was unaffected by the addition of 100 μM L-NAME, or its vehicle.

The arterial responses to intra-portal ATP (phase 1) were unaffected by portal venous L-NAME (phase 2); however, the addition of L-NAME to the perfusate reservoir (phase 3) significantly reduced responses at most doses (Figure 3 and 4a). The vehicle for L-NAME had no effect on the hepatic arterial responses (Figure 4b).

Portal venous responses to direct injection of ATP The addition of 100 μM L-NAME to the portal circulation resulted in an increase in portal venous pressure (by 3.9 ± 0.8 mmHg, $P < 0.001$). Subsequent addition of L-NAME to the perfusate reservoir did not alter portal perfusion pressure further.

L-NAME, 100 μM , in the portal venous circulation, enhanced vasoconstriction and caused a left shift of the direct portal venous dose-response curve to ATP, (Figure 5a) ($-\log$ molar ED_{50} from 5.32 ± 0.37 to 6.51 ± 0.43 for phase 1 and 2 respectively, $P = 0.014$). This value was calculated by assuming that the response at maximal dose was the maximal responsible possible. It was possible that a similar maximal response to that in phase 2 and 3 could have been reached at higher doses but this would have only increased the ED_{50} further and subsequently increase the left shift of the curve between phase 1 and 2. However, when this change in ED_{50} was compared to control results ($-\log$ molar ED_{50} changing from 5.32 ± 0.31 to 5.75 ± 0.27 for phase 1 and phase 2 respectively, $P = 0.13$, (Figure 5b) the left shift did not reach significance ($P = 0.068$). However, analysis of responses to individual ATP doses, with L-NAME in the portal circulation, against controls showed a significant difference in response at the doses of 10^{-7} and 3×10^{-7} mol 100 g^{-1} liver. These results confirmed that L-NAME did have an effect over and above the changes seen during control experiments.

The substitution of portal L-NAME with L-NAME in both inflows did not alter the ED_{50} of the portal dose-response curve further ($-\log$ molar $\text{ED}_{50} = 6.25 \pm 0.39$ and 5.78 ± 0.30 for addition of L-NAME and vehicle control respectively).

Figure 4 Responses of hepatic arterial pressure (HAP) to increasing intraportal doses of ATP, before (phase 1, $\square$), during infusion alone (phase 2, $\circ$) and during arterial and portal perfusion (phase 3, $\triangle$) of 100 μM N^{G} -nitro-L-arginine methyl ester (a) and (b) vehicle. Statistical significance is denoted by $\dagger P < 0.05$ for phase 1 vs phase 3; $\S P < 0.05$, $\S\S P < 0.01$, $\S\S\S P < 0.001$ for phase 2 vs phase 3 respectively. (Group 2).

Figure 5 Responses of portal venous pressure (PVP) to increasing intraportal doses of ATP, before (phase 1, $\square$), during portal infusion alone (phase 2, $\circ$) and during arterial and portal perfusion (phase 3, Δ) of 100 mM N^G -nitro-L-arginine methyl ester (a) and vehicle (b). Statistical significance is denoted by * $P < 0.05$, ** $P < 0.01$ for phase 1 vs. phase 2; and † $P < 0.05$ for phase 1 vs. phase 3. (Group 2).

Hepatic arterial and portal venous responses to hepatic arterial injection of ATP

The direct hepatic arterial vasodilator responses to intra-arterial injection of ATP, 10^{-7} mol 100 g^{-1} liver, were unaffected by portal venous administration of L-NAME, but were reduced with L-NAME in the hepatic arterial and portal venous inflows ($P = 0.058$). The responses to adenosine were not affected by L-NAME. The amplitude of portal venous responses to arterially injected doses of ATP and adenosine (vasoconstriction and vasodilatation respectively) tended to increase in the presence of L-NAME but not in the control experiments.

Discussion

L-NAME perfusion of the portal circulation did not affect the transhepatic arterial vasodilator responses to ATP. It did, however, enhance portal venous vasoconstriction to ATP, presumably by inhibiting a co-existing NO-mediated vasodilatation of the portal venous vascular bed. The subsequent infusion of L-NAME into both portal and arterial inflows inhibited hepatic arterial dilatation to portal venous injections of ATP, demonstrating that intra-portal administered ATP was ultimately acting through a NO-mediated mechanism in the artery. L-NAME would have been expected to

inhibit the transhepatic action of ATP during portal venous infusion of L-NAME if the arterial response was due to NO released by ATP in the portal venous bed. The lack of effect of L-NAME on arterial responses during this phase of the experiment also indicated that L-NAME did not reach the arterial resistance sites in sufficient concentration to block the action of ATP. These results therefore do not substantiate the hypothesis that NO diffuses to the hepatic arterial resistance sites to elicit the transhepatic action of ATP.

Falcone & Bohlen (1990) produced substantial evidence, in the mesentery and skeletal muscle of the rat, that NO could act as a diffusible secondary messenger in the transfer of vasodilatation from a venule to its paired arteriole. Injection of acetylcholine (ACh) into a venular wall caused dilatation of the paired arteriole, but injection into the parenchyma at a similar distance from the arteriole did not result in dilatation. Therefore, a second messenger produced by the venule, which could be blocked by the addition of L-NAME, caused the arteriole to react. Some possible differences between this study and the present one which may explain the difference in results are: (1) The liver may have better diffusion characteristics than the tissues used by Falcone & Bohlen, perhaps as a result of endothelial fenestration of the sinusoids; (2) ACh and ATP differ in their size and electrical charge (ATP is a molecule approximately three times larger than ACh with a greater electrical charge). We chose to use ATP because of its apparent importance in the hepatic circulation and also because of recent evidence that L-NAME acted as a muscarinic receptor antagonist (Buxton *et al.*, 1993); (3) the anatomical distance between the vessels in the inflow tract of the liver may be significantly different from those in the rat mesentery/trapezius muscle, although this seems unlikely. The distance between the ACh injection site and the arteriole in Falcone & Bohlen's work was approx. $200 \mu\text{m}$; in the liver the distance between the pre-sinusoidal vessels in the inflow tract is of the same order (approx. $100 \mu\text{m}$) (Grisham & Nopanitaya, 1981); (4) the extra-venular injection used by Falcone & Bohlen may behave differently from the intravascular injection of an agent, perhaps due to the release of NO from tissues other than the endothelium. Alternatively, the ability to metabolize NO in tissues outside the vessel wall may be less than that in the smooth muscle layer, thereby allowing more NO to diffuse away.

The ability of ATP to diffuse to the artery was further substantiated by the transmission of a vasoconstrictor hepatic arterial response to ATP at a basal tone in this series and the more substantial transmitted vasoconstrictor response to noradrenaline which has been shown to be dependent on the magnitude of the arteriportal pressure gradient (Browse *et al.*, 1994b). Consequently, the threshold for bolus doses of noradrenaline to elicit an hepatic arterial responses was 500–1000 times greater if injection was into the portal vein. At raised tone the arteriportal pressure gradient and the transhepatic ratio for hepatic arterial responses was increased. However, transmission of responses could still be detected although at raised tone, in the current experiments with ATP, the predominant response was vasodilatation. In the present experiments L-NAME infusion into the portal vein did not affect hepatic arterial responses to portal venous injection or hepatic arterial injection of ATP. This implies that either L-NAME diffusion is poor in the liver or more likely that diffusion against the hepatic arterial to portal venous pressure gradient allows too small a concentration to reach the hepatic arterial vascular bed.

This work only excludes NO as a diffusible secondary messenger in the transmission of responses to ATP. ATP has also been shown to release prostacyclin (PGI_2) from the endothelium of rabbit aorta and pulmonary artery (Boeynaems & Galand, 1983) although it is not known whether this happens in the venous system. ATP is also rapidly taken up or metabolized to adenosine by ecto-enzymes (Pearson *et al.*, 1978; 1980). This is so effective that in the guinea-pig heart only 1% of injected ATP was found in the venous

effluent (Paddle & Burnstock, 1974). It may be that another metabolite of ATP is responsible for its transhepatic arterial vasodilatation; however, the agent(s) in question must act, to a large extent, through NO because the hepatic arterial responses to intra-portal ATP were reduced by the addition of L-NAME to the perfusate.

These experiments have also further characterized the responses of the hepatic vascular bed to ATP. ATP is known to be present as a neurotransmitter in the hepatic artery (Brizzolara & Burnstock, 1990) and portal vein (Reilly *et al.*, 1987; Brizzolara *et al.*, 1993). It acts in the vascular bed through smooth muscle P_{2X} receptors mediating arterial constriction and P_{2Y} receptors mediating arterial vasodilatation (Ralevic *et al.*, 1991) although these may be endothelial or on the smooth muscle (Brizzolara & Burnstock, 1991). At basal tone, we have reaffirmed the vasoconstrictor arterial responses showing a similar threshold for direct injection as shown previously (Ralevic *et al.*, 1991) but by using higher doses we have demonstrated that only the lower portion of the dose-dependent response curve had been characterized. It seems therefore that the $-\log$ molar ED_{50} value for P_{2X} receptors is unlikely to be greater than 4.5, a factor of 100 different from that previously reported (Ralevic *et al.*, 1991). We have shown that the artery does constrict to intra-portal administration of high doses of ATP, although a maximal response was not obtained. At raised tone the vasodilator effect of ATP compares well with that seen previously (pD_2 8.3 ± 0.1 (Ralevic *et al.*, 1991) vs 7.72 ± 0.27 in these experiments) although the amplitude of responses and the smaller dose of pre-constriction agent required would suggest that our preparation is more sensitive. The transhepatic action of ATP on the hepatic arterial vascular bed has not been demonstrated before in the *in vitro* perfused rabbit liver and it shows that there is a comparable arterial response threshold (10^{-7} mol 100 g $^{-1}$ liver) to that seen *in vivo* in our laboratory (unpublished observation).

The effect of ATP on the portal venous bed has not been well documented before, although the constrictor effect of an infusion of ATP at basal tone has been demonstrated in the perfused rat liver (Lee & Filkins, 1988). In our experiments the portal venous responses to direct injection, at basal or raised tone, are similar to the transhepatic responses following hepatic arterial injection, but direct injections have a lower threshold. This suggests that either more ATP is metabolized during passage through the hepatic arterial vascular bed than through the portal system, or that portal venous resistance sites are, at least partly, pre-sinusoidal. In addition the hepatic arterial and portal venous vascular beds are perfused at different flow rates. This could ultimately result in different quantities of drug arriving at the receptors (per unit time) and could contribute to the difference in responses between the 2 vascular beds.

At raised tone, the dominant portal venous response was, again, vasoconstriction with a threshold level similar to that at basal tone. There was, however, a larger amplitude of response at raised tone. This difference can be explained by the order of construction of the dose-response curves in these experiments. Increasing duration of liver perfusion was shown to potentiate venous vasoconstriction in the control experiments, and dose-response curves were constructed at basal and then raised tone. It is also possible that vasoconstriction was facilitated by an increased tone. At lower doses of ATP there was obvious, although small, portal vasodilatation, a feature not noted previously in the portal venous bed. This vasodilatation had a lower threshold than constriction

(8.9 vs $7.3 - \log$ mol 100 g $^{-1}$ liver) but was overcome by constriction at higher doses. There was a marked lag time before constriction and often there was a noticeable dilatation even after the portal pressure/resistance had risen. Certain features of the preparation employed in these experiments, in particular free-flotation of the liver, and the use of methoxamine in preference to noradrenaline as a pre-constriction agent, tend to produce a low portal perfusion pressure, which probably results in an underestimate of the amplitude of the portal vasodilatation. It is quite clear, however, that using L-NAME causes a left shift of the portal venous vasoconstriction-response curve suggesting that a marked vasodilator element, dependent on NO (probably acting via P_{2Y} purinoceptors) has been inhibited. Further physiological limitations in this model include the use of the same perfusate for the portal venous and hepatic arterial vascular beds but this has not been shown to alter the vascular responses to ATP over those using more acidic (hypercapnic) portal venous perfusates (Browse and Alexander, unpublished observation). Experiments on the transhepatic effects of drugs have also been hampered, particularly *in vivo* by the presence of the buffer response (hepatic arterial vasodilatation in response to reductions in portal venous blood flow) which may alter vascular resistance; this preparation does not exhibit a demonstrable buffer response.

The role of NO in the portal venous response to arterial injection of ATP is unclear. Following the addition of intra-portal L-NAME, portal vasoconstriction in response to 10^{-7} mol ATP injected into the artery did not increase significantly; however, the tendency for greater responses was apparent and may be due to an inhibition of the same NO-mediated vasodilatation in the portal venous bed, although the potentiating effect of the increasing duration of perfusion cannot be excluded. Further examination/characterization of these vasodilator responses would require inhibition of P_{2X} receptors and the increase of portal venous pressure. The introduction of L-NAME into the portal circulation also caused a significant increase in portal venous pressure suggesting that there is a basal NO release, a feature which has been noted before in a rabbit isolated portal vein preparation (Brizzolara *et al.*, 1993). This was not seen in the hepatic arterial vascular bed. It may well be that the more important physiological response of the portal circulation to raised ATP levels, which may reach as high as 200 μ M in shock (Gordon, 1986), is vasodilatation.

In summary, we have further characterized the hepatic arterial and portal venous vascular responses to ATP, in particular demonstrating that the portal venous bed exhibits basal NO release, and that portal responses at raised tone are a mixture of vasodilatation and vasoconstriction. The vasoconstrictor response is enhanced by L-NAME, presumably by inhibiting NO-mediated vasodilatation. The lack of effect of portal venous L-NAME on the vasodilator hepatic arterial responses to ATP suggests that NO from the portal venous vascular bed does not have a role in the transhepatic action of ATP, implying that ATP itself is probably responsible for this action. This mechanism of action may contribute to the fine control of hepatic arterial tone by portally-borne intestinal vasoactive agents, and may play a role in the production of the hepatic arterial 'buffer response'.

The authors wish to thank Dr Vera Ralevic for her invaluable suggestions in the preparation of this manuscript. This work was funded by the Joint Research Committee of King's College London.

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(Received January 12, 1994

Revised June 22, 1994

Accepted June 23, 1994)