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The action of ATP on the hepatic arterial and portal venous vascular networks of the rabbit liver: the role of adenosine

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Abstract

ATP is released from blood vessels during periods of hypoxia and may be responsible for hepatic arterial vasodilatation during instances of reduced hepatic portal venous flow. The role of adenosine in ATP-induced vasodilator and vasoconstrictor responses of the hepatic arterial and portal venous vascular networks respectively was studied in the isolated dual-perfused rabbit liver in vitro to ascertain whether ATP could be catabolised to adenosine during transit through the hepatic parenchyma. Intra-arterial and intra-portal injections of ATP (-10 to -4 log mol/100 g liver) resulted in dose-dependent vasodilatation in the hepatic artery and vasoconstriction in the portal vein. Addition of 8-phenyltheophylline (10 μM), a non-selective P_1-purinoceptor antagonist, to the hepatic arterial and portal venous perfusate significantly inhibited the hepatic arterial ED_{50} for responses to intra-arterial injected ATP from -8.70 ± 0.22 to -7.63 ± 0.28 log mol/100 g liver ($P < 0.001$); it also inhibited hepatic arterial responses to, mid-range, portal venous injections of ATP. The data suggest that the hepatic arterial vasodilatation to ATP is partly mediated via catabolism to adenosine and may be an important mechanism during periods of relative hepatic hypoxia associated with portal flow reduction.

Keywords: Hepatic circulation; Vasodilatation; 8-Phenyltheophylline; ATP; Adenosine; (Rat)

1. Introduction

Adenosine-5′-triphosphate (ATP) has been proposed to play an important role in the control of systemic vascular tone (Burnstock, 1987; Su, 1985) and we have shown that the appropriate purinoceptors reside in the hepatic vasculature of the rabbit (Mathie et al., 1991a; Ralevic et al., 1991). ATP has been shown to be released from blood constituents (Bergfeld and Forrester, 1992) and vascular endothelium (Bodin et al., 1991; Palmer et al., 1987) during hypoxia (Belloni et al., 1985) or altered flow (Ralevic et al., 1992), conditions which may be encountered during reduction or total cessation of portal venous blood flow.

In most microcirculatory vascular networks, ATP has been shown to elicit vasodilatation by stimulation of purinergic P_{2Y} receptors, generally located in the vascular endothelium (Ralevic et al., 1991) although these are located on vascular smooth muscle on the extra-hepatic artery of the rabbit (Brizzolara and Burnstock, 1991). Our earlier studies in the hepatic arterial vascular network of the rabbit in vitro (Ralevic et al., 1991) demonstrated that vasodilatation to ATP occurred following signal transduction to nitric oxide (NO) (Mathie et al., 1991b). A similar mechanism is probably at least partly responsible for the hepatic arterial vasodilatation seen following portal venous injection of ATP in the same model (Browse et al., 1994a). In some vessels, however, ATP may cause vasodilatation via P_1- purinoceptors via catabolism to adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP) and adenosine in endothelial and smooth muscle cells (Kennedy and Burnstock, 1985; Pearson et al., 1980). We previously postulated that this mechanism of action of ATP did not occur in the rabbit liver, because a previous study showed that high doses of intra-arterial ATP were not attenuated by the non-selective adenosine P_1-purinoceptor antagonist 8-phenyltheophylline (Ralevic et al., 1991). However, ATP could be released from the portal venous vasculature as a response to hypoxia, induced by reductions in portal venous flow (Mathie et al., 1991b). The diffusion of ATP across the hepatic parenchyma could increase the potential for catabolism to adenosine before arrival at the hepatic arterial resistance sites.

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The aim of this study, therefore, was to determine whether portal venous-derived ATP could exert a significant proportion of its hepatic arterial vasodilator action following catabolism to adenosine and activation of P_1-purinoceptors and also whether adenosine, produced by ATP catabolism, modulates the portal venous, vasoconstrictor, action of ATP in the intra-hepatic vascular network of the rabbit liver. We also wished to determine whether the role of adenosine in vascular responses elicited by ATP differed according to the site of injection (direct, measured in the injected vasculature; indirect or transhepatic, measured in the adjacent, non-injected, vasculature). All the experiments were conducted in our, in vitro, isolated dual-perfused rabbit liver model which has been described in detail elsewhere (Alexander et al., 1992).

2. Materials and methods

New Zealand White rabbits, which were allowed access to water and food ad libitum, were used throughout the study and supplied from a single breeder.

2.1. Surgical protocol

Twelve rabbits were anaesthetised with Hypnovel (midazolam) 1.5 mg/kg i.v., and fentanyl/fluanisone s.c. (Hypnorm, 0.3 ml/kg, Janssen Animal Health) injected i.m. for continued analgesia during the 40 min operative period. The operative technique has been described in detail elsewhere (Alexander et al., 1992). Briefly, the abdomen was opened though a mid-line incision, and the common bile duct cannulated. After administration of heparin i.v. (300 units/kg) the common hepatic artery and the gastroduodenal artery were cannulated (Portex 3FG). 10 ml of heparinised saline (20 units/ml) were infused into the catheters to prevent intrahepatic coagulation. The gastroduodenal vein was ligated, the portal vein cannulated and 40 ml of heparinised saline flushed through the portal venous system. The liver was then rapidly excised from the animal, weighed and placed in an organ bath.

2.2. Liver perfusion

Livers were perfused through the hepatic arterial and portal venous cannulae at constant flow rates of 25 and 75 ml/min/100 g liver, respectively, with Krebs-Bülbring buffer solution (composition mmol/litre: 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 79F polygraph (Grass Instrument). Perfusion under these conditions maintains liver viability for 5 h (Browse et al., 1994b).

2.3. 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. All the drugs were injected as a bolus and the concentrations stated refer to those of the injected boluses and not those within the hepatic arterial and portal venous vasculatures. Two groups of rabbits were studied: ATP injection into the hepatic artery (group 1), and ATP injection into the portal vein (group 2). Dose-response curves were constructed to ATP (10^{-10} to 10^{-6} mol/100 g liver for intra-arterial, and 10^{-8} to 10^{-4} mol/100 g liver for intra-portal injection) and repeated after a 15 min equilibration period following the addition of the adenosine

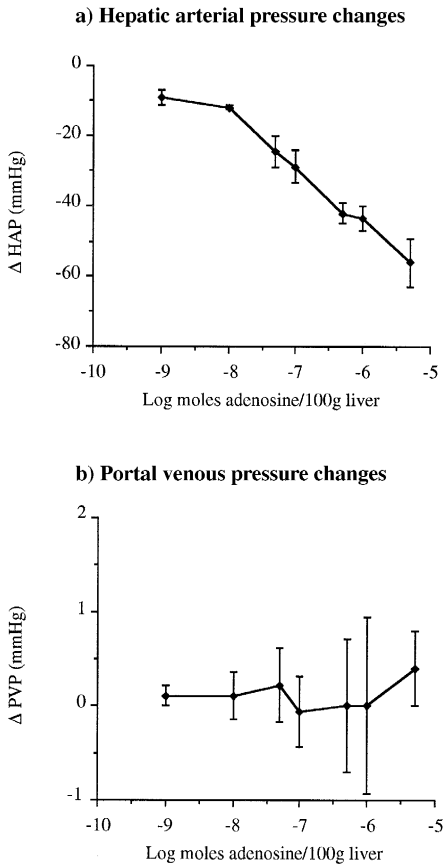

Fig. 1. (a) Hepatic arterial pressure changes (ΔHAP) and (b) portal venous pressure changes (ΔPVP) to hepatic arterial injections of adenosine. Adenosine induced dose-related hepatic arterial vasodilatation but had little effect upon portal venous vascular tone. Constraints of time upon the viability of the preparation prevented this curve to be reproduced in its entirity during this series of experiments and therefore a mid-range dose of 10^{-7} log mol adenosine/100 g liver was chosen to confirm inhibition of adenosine responses for the duration of the perfusions.

inhibitor 8-phenyltheophylline (10 μM) (Sigma UK) to the arterial and venous perfusate. Mid-range doses of acetylcholine (10^{-7} mol/100 g liver) and sodium nitroprusside (10^{-8} mol/100 g liver), established from previous experiments (data not presented) were given at regular intervals throughout the experiment as single bolus injections into the hepatic artery to confirm the maintenance of the vascular responses with time. The degree to which adenosine-mediated vasodilatation could contribute to ATP-induced vasodilatation was established by inhibition of adenosine responses by 8-phenyltheophylline. Establishment of the inhibitory action of 8-phenyltheophylline was achieved by selection of a single, intra-arterial, mid-range dose of 10^{-7} mol/100 g liver adenosine (Fig. 1). Constraints of time upon the viability of the preparation prevented construction of a complete adenosine/8-phenyltheophylline dose-response curve; the inhibitory action of 8-phenyltheophylline upon a complete curve was established in an earlier study using this model (Mathie et al., 1991b).

2.4. Statistical analysis

Student's paired *t*-test was used to test the significance of differences between the magnitude of vascular responses to ATP before and during administration of 8-phenyltheophylline. All data are presented as mean ± S.E.M. $P \leq 0.05$ was accepted as a statistically significant difference between groups.

3. Results

3.1. Group 1. The effect of intra-arterial ATP and adenosine

Livers from 6 rabbits (body weight 2.93 ± 0.14 kg, liver weight 119.2 ± 13.4 g) were perfused at raised tone (hepatic arterial pressure = 146.7 ± 7.7 and portal venous pressure 3.3 ± 0.8 mmHg). 8-Phenyltheophylline (10 μM) significantly inhibited the hepatic arterial response to 10^{-7} mol/100 g liver intra-arterial adenosine from 50.8 ± 6.2 to 31.6 ± 8.1 mmHg ($P < 0.05$), but did not significantly inhibit hepatic arterial responses to 10^{-8} mol/100 g liver intra-arterial acetylcholine (68.9 ± 6.6 compared with 72.2 ± 5.7 mmHg) or to 10^{-8} mol/100 g liver intra-arterial sodium nitroprusside (36.3 ± 4.4 compared with 41.6 ± 9.7 mmHg) (Fig. 2). The hepatic arterial dose-related response curve to intra-arterial ATP was shifted to the right by 8-phenyltheophylline (−log mol/100 g liver ED_{50} 8.70 ± 0.22 compared with 7.63 ± 0.28, $P < 0.001$), indicating inhibition of responses to ATP (Fig. 3a). Hepatic arterial injections of ATP elicited dose-dependent vasoconstriction as a transhepatic (indirect) portal venous response (Fig. 3b). Although there was a trend towards increased vasoconstriction during infusion of 8-phenyltheophylline which may have suggested an adenosine-induced attenuation, this was not statistically significant and was consistent with prolonged periods of perfusion (Browse et al., 1994b).

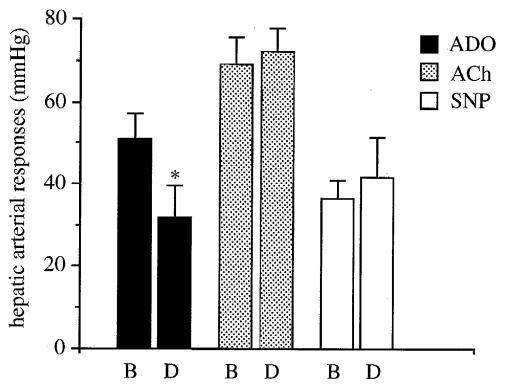

Fig. 2. Hepatic arterial responses to mid-range intra-arterial injections of 10^{-7} mol adenosine (ADO), 10^{-8} mol acetylcholine (ACh) and 10^{-8} mol sodium nitroprusside (SNP) before (B) and during (D) addition of 10 μM 8-phenyltheophylline to the perfusate. * $P < 0.05$ before vs. during perfusion with 8-phenyltheophylline.

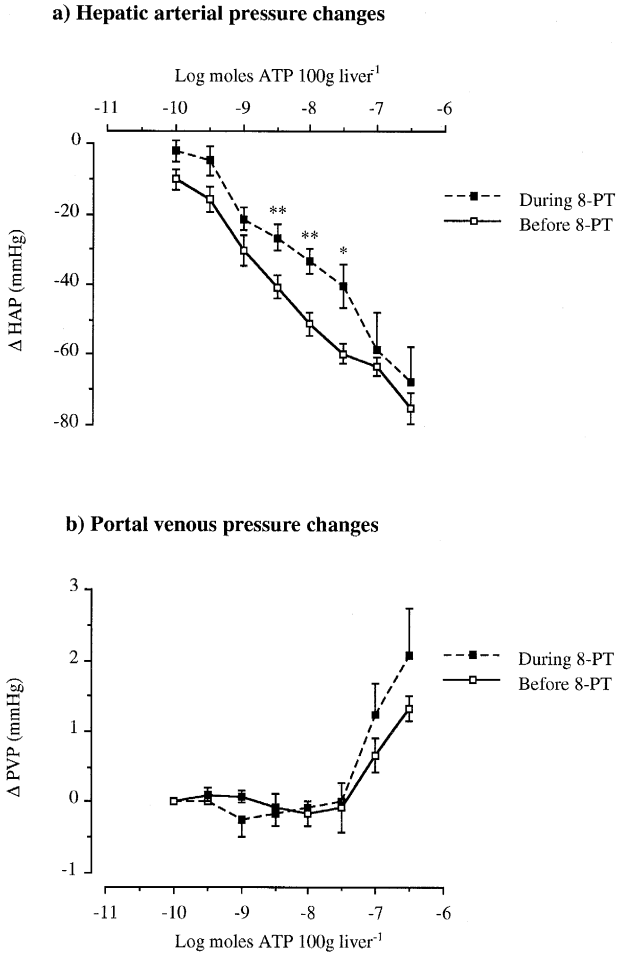

Fig. 3. The changes in (a) hepatic arterial pressure responses (ΔHAP) and (b) portal venous pressure responses (ΔPVP) to intra-arterial injection of ATP. The adenosine receptor antagonist 8-phenyltheophylline (8-PT; 10 μM) significantly decreased hepatic arterial responses to ATP. * $P < 0.05$, ** $P < 0.01$ before (□) vs. during (■) perfusion with 8-PT.

3.2. 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 (hepatic arterial pressure $= 156.2 \pm 4.8$ and portal venous pressure $= 2.3 \pm 0.7$ mmHg). The addition of 8-phenyltheophylline to the hepatic arterial and portal venous perfusate significantly inhibited the hepatic arterial response to 10^{-7} mol/100 g liver intra-arterial adenosine from 33.2 ± 3.5 to 6.5 ± 3.8 mmHg ($P < 0.001$). The hepatic arterial responses to mid-range doses of intra-portal ATP were also significantly reduced by 8-phenyltheophylline (Fig. 4a). The portal venous responses to intra-portal injections of ATP, which were predominantly vasoconstriction, were not significantly altered (Fig. 4b). In neither group did the addition of 8-phenyltheophylline affect the perfusion pressure, although there was occasionally a small transient increase in hepatic arterial pressure which settled after 5–10 min.

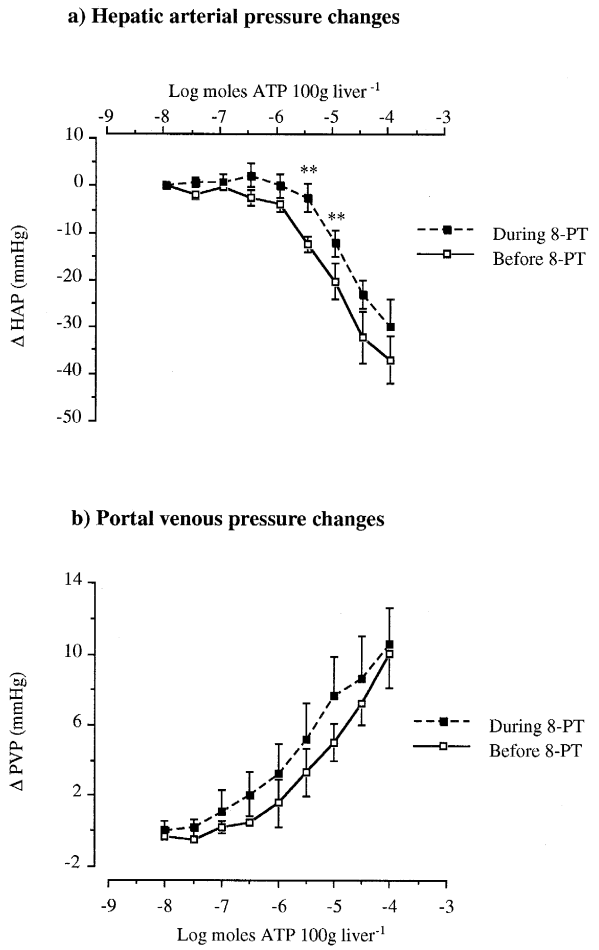

Fig. 4. The changes in (a) hepatic arterial pressure responses (ΔHAP) and (b) portal venous pressure responses (ΔPVP) to intra-portal injection of ATP. The adenosine receptor antagonist 8-phenyltheophylline (8-PT) (10 μM) significantly decreased hepatic arterial responses to ATP (** $P < 0.01$ before (□) vs. during (■) perfusion with 8-PT), while portal venous responses were not significantly altered.

4. Discussion

We have previously shown that intra-portal or intra-arterial injection of ATP can induce vasodilatation of the hepatic arterial vascular network in the isolated dual-perfused rabbit liver via NO release following P_{2Y}-purinoceptor activation (Mathie et al., 1991a). Moreover, we have shown that portal venous injected ATP may diffuse across the hepatic parenchyma, possibly in a manner similar to noradrenaline (Browse et al., 1995), to elicit vasodilatation at the hepatic arterial resistance sites as a result of signal transduction via NO (Browse et al., 1994a). However, in some vessels ATP has been shown to induce smooth muscle relaxation via adenosine acting on P_1-purinoceptors (Kennedy and Burnstock, 1985). We therefore wished to test the hypothesis that some of the hepatic arterial dilatation induced by portal venous-injected ATP was due to catabolism to adenosine, following diffusion across the hepatic parenchyma, by using the non-selective P_1-purinoceptor antagonist 8-phenyltheophylline (Griffith et al., 1981).

Our results have demonstrated that both intra-arterial and intra-portal injection of ATP elicit vasodilatation of the hepatic arterial vascular network that can be inhibited by 8-phenyltheophylline, indicating the involvement of P_1-purinoceptors in its mechanism of action. The manner in which 8-phenyltheophylline inhibited responses to ATP was of interest. The responses to lower doses of ATP were unaffected, the mid-range doses of ATP were inhibited substantially, while higher doses were not. These data confirm our earlier findings, where hepatic arterial vasodilatation to high doses of ATP was not affected by 8-phenyltheophylline (Ralevic et al., 1991). This lack of attenuation at maximum doses of ATP may have been due to the overwhelming competitive inhibition at high doses or have been indicative of a different mechanism of ATP and/or adenosine action, perhaps through an alternative receptor. Another possibility is that the highest doses of ATP used may have released sufficiently large quantities of adenosine to elicit the release of NO since it has been suggested that adenosine may act via A_2-purinoceptors to release NO during hypoxia (Vials and Burnstock, 1993) and possibly during reactive hyperaemia (Gryglewski et al., 1995) in the guinea-pig heart. In addition, adenosine may elicit vasodilatation via an endothelium-dependent mechanism consistent with effects shown in human cultured endothelial cells (Sobrevia et al., 1996) and from the human forearm (Smits et al., 1995). The design of the present experiments did not allow us to determine whether adenosine releases NO in the hepatic arterial vasculature of the rabbit.

There was no apparent difference in the degree of inhibition of hepatic arterial responses to ATP by 8-phenyltheophylline when comparing 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 a significant proportion of the adenosine produced from ATP catabolism was taken up effectively by the endothelium and vascular smooth muscle (Pearson et al., 1978) 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 probable higher concentration of adenosine, produced by ATP catabolism, in the liver following intra-portal ATP (10^{-8}–10^{-4} log mol/100 g liver) compared with intra-arterial injections (10^{-10}–10^{-6} log mol/100 g liver).

Since the portal venous flow rate is 3 times greater than the hepatic arterial flow rate in our preparation, the exposure time of substances in the portal venous system would be expected to be much less than that in the hepatic arterial vasculature. Therefore the degree of hepatic arterial vasodilatation elicited by portal venous (indirect) injections of ATP may be smaller compared to hepatic arterial (direct) injections because there is little likelihood for similar quantities of ATP to diffuse across the hepatic parenchyma from the portal venous to the hepatic arterial vascular network to elicit a comparable degree of vasodilatation to direct (hepatic arterial) injections. Therefore higher doses of ATP/adenosine would be required to be injected into the portal vein to elicit a similar response to hepatic arterial injections. This could explain why a higher dose range was required in the portal venous vasculature assuming that the vasoactive potential of ATP remains unaltered by diffusion from the portal venous vasculature, across the hepatic parenchyma and into the hepatic arterial vasculature.

In addition, the reduced vasoactive potential of portal venous-injected ATP to elicit hepatic arterial vasodilatation is unlikely to have been due to increased catabolism to adenosine because: (i) adenosine and ATP are equipotent hepatic arterial vasodilators (Browse et al., 1994b) and therefore one would have expected the direct and indirect curves to be similar if all of the ATP-derived adenosine reached the hepatic arterial resistance sites; and (ii) our previous study showed that parenchymal diffusion of ATP and not NO elicits NO-dependent vasodilatation in the hepatic artery (Browse et al., 1994a). The possibility remains that ATP may diffuse from the portal venous to the hepatic arterial vascular network to elicit vasodilatation by activation of NO-dependent P_{2Y}- and A_2-purinoceptors via catabolism to adenosine on arrival at the hepatic artery.

The vasoconstrictor action of ATP in the portal venous vasculature was consistent with our earlier observations of P_{2X}-purinoceptor activation at basal tone (Ralevic et al., 1991; Browse et al., 1994b). Portal venous responses to ATP were indicative of predominant vasoconstriction and not vasodilatation even though the tone of the preparation was raised by the addition of methoxamine. The amplitude of portal venous responses to intra-arterial ATP (Fig. 3b) slightly increased with the duration of perfusion, but not significantly, and was consistent with the effects of prolonged perfusion on the preparation (Browse et al., 1994a). It is unlikely that the increased vasoconstrictor action of ATP was due to decreased catabolism to adenosine because adenosine or ATP-induced portal venous vasodilatation has not been demonstrated in this preparation (Browse et al., 1994b). This absence of vasodilatation therefore suggests that there are few, if any, A_2 or P_{2Y}-purinoceptors in the portal venous vascular bed of the rabbit. The fact that the responses were not significantly altered after the addition of 8-phenyltheophylline suggested that ATP catabolism to adenosine either exerted very little effect upon the vasoconstrictor potential of ATP and that P_1-purinoceptors do not exist in the rabbit portal venous vasculature (implied by our previous findings; Browse et al., 1994b), or that there was very little catabolism of ATP in the portal venous vascular network. Neither of these possibilities appears likely.

In conclusion, we have demonstrated that hepatic arterial vasodilatation elicited by ATP may be not solely mediated by P_{2Y}-purinoceptor activation and be partly mediated through P_1-purinoceptors via the catabolism of ATP to adenosine. In addition, portal venous-induced hepatic arterial vasodilatation may, in fact, be mediated through the concomitant receptor-mediated release of NO by adenosine formed from ATP. This dual action of ATP may be important for maintenance of vasodilatation, and therefore vascular tone, during periods of reduced portal venous flow and/or endothelial damage if A_2-purinoceptors are located on vascular smooth muscle.

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