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Adenosine-induced dilatation of the rabbit hepatic arterial bed is mediated by A_2-purinoceptors

[1]R.T. Mathie, B. Alexander, *V. Ralevic & *G. Burnstock

Department of Surgery, Royal Postgraduate Medical School, London W12 0NN and *Department of Anatomy & Developmental Biology, University College London, London WC1E 6BT

1 This study was carried out in order to identify the receptor responsible for adenosine-induced dilatation of the hepatic arterial vascular bed.

2 Livers of 10 New Zealand White rabbits were perfused *in vitro* with Krebs-Bülbring buffer via the hepatic artery and the portal vein at constant flows of 26 and 77 ml min^{-1} 100 g^{-1} liver respectively. The tone of the preparation was raised by the presence of noradrenaline in the perfusate (concentration: 10^{-5} M).

3 Dose-response curves for adenosine and its analogues 5'-N-ethyl-carboxamido-adenosine (NECA), the 2-substituted NECA analogue CGS 21680C, and **R**- and S-N^6-phenyl-isopropyl-adenosine (**R**- and S-PIA) were obtained after their injection into the hepatic arterial supply.

4 The order of vasodilator potency of these agents was: NECA > CGS 21680C > adenosine > **R**-PIA > S-PIA. Their potency, expressed relative to that of adenosine, was in the approximate ratio 10:3:1:0.3:0.1, consistent with that resulting from activation of P_1-purinoceptors of the A_2 sub-type (which mediate vasodilatation due to adenosine).

5 The P_1-purinoceptor antagonist 8-phenyltheophylline (10^{-5} M) caused significant attenuation of the vasodilatation to adenosine and analogues.

6 It is concluded that adenosine-induced dilatation of the hepatic arterial vascular bed is mediated by P_1-purinoceptors of the A_2 sub-type.

Keywords: Hepatic artery; portal vein; adenosine; purinoceptors

Introduction

The vasodilator response of the hepatic artery (HA) to portal vein (PV) flow interruption is mediated, at least in part, by adenosine (Lautt *et al.*, 1985; Lautt & Legare, 1985; Mathie & Alexander, 1990). Adenosine therefore plays an important role in the regulation of HA blood flow, though the actual mechanism by which it exerts physiological control of the circulation remains speculative. The release of adenosine may be continuous, so that during PV occlusion it may accumulate and result in HA dilatation (Lautt, 1985; 1988), or its release may be regulated by tissue hypoxia or oxygen supply-to-demand imbalance (Berne *et al.*, 1983; Bardenheuer & Schrader, 1986).

On the basis of studies in other tissues, it may be surmised that adenosine, whatever its mechanism of release, exerts its dilator action in the liver via a purine receptor located within the HA microvasculature (Burnstock & Kennedy, 1986), but little or no information is available in the literature to confirm or refute this supposition. Purine receptors in the cardiovascular system comprise two distinct populations (Burnstock & Kennedy, 1986; Williams, 1987): P_1-purinoceptors, which mediate responses to adenosine and adenosine monophosphate (AMP), and P_2-purinoceptors, which mediate responses to adenosine diphosphate (ADP) and adenosine triphosphate (ATP). P_1-purinoceptors of the A_2 sub-type (which mediate vasodilatation due to adenosine) have been identified in the smooth muscle of the aorta (Collis & Brown, 1983), and of the coronary (Kusachi *et al.*, 1983; 1986; Mustafa & Askar, 1985; Hamilton *et al.*, 1987) and cerebral circulations (Edvinsson & Fredholm, 1983), but this has not been investigated in the HA circulation. The present study was carried out to identify the purine receptor sub-type in the HA vascular bed which may be responsible for adenosine induced dilatation.

We have adopted a pharmacological technique for characterizing the hepatic P_1-purinoceptor population by establishing the rank order of vasodilator potency of adenosine and its analogues 5'-N-ethyl-carboxamido-adenosine (NECA) and the **R**- and S- stereoisomers of PIA (N^6-phenyl-isopropyl-adenosine) (Bruns *et al.*, 1986; Daly *et al.*, 1986; Oei *et al.*, 1988). (In keeping with current usage, we have employed the Cahn-Ingold-Prelog convention of describing stereoisomers by **R**- and **S**- prefixes instead of L- and D- respectively, while retaining the acronym PIA for the substance designated in IUPAC nomenclature as N^6-[1-methyl-2-phenylethyl]-adenosine (Bruns *et al.*, 1986).) At A_2 receptors, it is well established that NECA is 1–2 orders of magnitude more potent than **R**-PIA, and that **R**-PIA is only 2–5 times more potent than S-PIA (Collis & Brown, 1983; Kusachi *et al.*, 1983; Collis, 1985; 1989). In addition, we have investigated the vasodilator potency of the recently developed, high-affinity A_2 agonist CGS 21680C (a 2-substituted analogue of NECA: Balwierczak *et al.*, 1989; Hutchison *et al.*, 1989) in comparison to adenosine and NECA. The receptors were further characterized by use of the P_1-purinoceptor antagonist, 8-phenyltheophylline.

A novel, *in vitro*, dual-perfused, rabbit liver model has been adopted for the investigation. The HA and PV are perfused simultaneously with Krebs-Bülbring buffer at constant, physiological flow rates (Alexander *et al.*, 1991), an approach that has enabled us to investigate in detail the responses of the HA bed to pharmacological stimulation in the presence of an unchanging, normal PV flow rate.

Methods

Operative procedures

Experiments were carried out on a total of 10 New Zealand White rabbits of either sex, weighing 2.5–3.8 kg (mean 2.9 kg). The operative technique has been described elsewhere (Alexander *et al.*, 1991), but will be outlined in brief here. The rabbits were initially sedated with fentanyl-fluanisone i.p. ('Hypnorm', 0.25 ml kg^{-1}), and then anaesthetized with a

[1] Author for correspondence.

mixture of 1 part Hypnorm (0.3 ml kg^{-1}) and 1 part midazolam ('Hypnovel', 1.5 mg kg^{-1}) in 2 parts water i.p. (total: 1.20 ml kg^{-1}) (Flecknell, 1987). A marginal ear vein was cannulated for subsequent i.v., administration of the Hypnorm/midazolam/water mixture (0.25–0.5 ml kg^{-1} h^{-1}).

The abdomen was opened through a mid-line incision, and the common bile duct cannulated. The gastroduodenal artery was cannulated (Portex 3FG), and the catheter advanced to the junction of the common and proper hepatic arteries; the common hepatic artery was then ligated and divided, and 4–5 ml saline infused into the catheter to prevent blood coagulation in the intrahepatic HA vasculature. After administration of heparin i.v. (100 units kg^{-1}), the PV was cannulated and 40–50 ml saline infused into the catheter to prevent accumulation of blood in the intrahepatic PV system. The liver was then rapidly, but carefully, excised from the animal, weighed and placed in an organ bath.

Liver perfusion

The liver was perfused at constant flow rates via the HA and the PV from a common reservoir of oxygenated (95% O_2/5% CO_2) Krebs-Bülbring buffer solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52 at 37°C. All livers appeared evenly perfused, and this was confirmed at the end of each experiment by the local injection of Patent Blue into both the HA and the PV. Mean flow rates for all experiments were 26 ml min^{-1} 100 g^{-1} (HA) and 77 ml min^{-1} 100 g^{-1} (PV). Perfusion pressures were measured with Gould P23 pressure transducers on side-arms of the perfusion circuit, and recordings made on a Grass 79D polygraph. Bile was collected for the duration of perfusion. No evidence of oedema was observed in any liver.

After an equilibration period of 5–10 min, the tone of the preparation was raised by adding to the perfusate noradrenaline to a final concentration of 10^{-5} M.

Drug administration

Adenosine (hemisulphate), NECA, **R**-PIA, S-PIA, acetylcholine chloride, noradrenaline bitartrate and 8-phenyltheophylline (8-PT) were obtained from Sigma. CGS 21680C was obtained from CIBA-Geigy Corporation, Summit, New Jersey, U.S.A. Adenosine, NECA, CGS 21680C and acetylcholine were dissolved in distilled water; **R**- and S-PIA were dissolved in a 1:1 mixture of dimethyl formamide (DMF) and methanol; noradrenaline was made up as a 10 mM stock solution in 0.1 mM ascorbic acid (to prevent oxidation); 8-PT was made up in a mixture of methanol and 1 M NaOH (80%:20%).

Adenosine and its analogues, in the dose range 10^{-10}–10^{-6} mol, were injected in turn as 0.1 ml boluses into the HA, via a rubber septum in the circuit. They were administered in a regimen so that injections of a given concentration of all compounds were made over a minimum period of time. Injections of the vehicle (water or DMF/methanol) were given at the end of each experiment in order to account for any injection artefact or haemodynamic response; water caused no change in pressure other than the injection artefact, while DMF/methanol, at a concentration equivalent to the highest dose of **R**- and S-PIA only, caused a small decrease in HA pressure which was subtracted from the relevant PIA responses.

The above protocol was employed in 2 separate groups of rabbits: in Group I (6 rabbits) the effects of adenosine, NECA, **R**-PIA, and S-PIA were compared; in Group II (4 rabbits) the effects of adenosine, NECA and CGS 21680C were compared.

The P_1-purinoceptor antagonist 8-PT was then added to the perfusate to a final concentration of 10^{-5} M and after 10–15 min, HA injections of adenosine (Group I) or NECA, CGS 21680C and adenosine (Group II) were repeated over the same concentration range as before.

In order to confirm retained vasodilator competence of the HA bed following 8-PT administration, 10^{-9} mol and 10^{-8} mol acetylcholine were injected into the HA in 5 of the 10 livers, both in the presence and in the absence of 8-PT in the perfusate.

Statistics and presentation of data

Responses were recorded as changes in perfusion pressure (mmHg). Student's paired and unpaired t tests were used, as appropriate, to test the significance of differences between responses, $P < 0.05$ being taken as significant. All results are quoted as mean ± s.e.mean.

The vasodilator potency of each agent was defined by the PD_2, the negative logarithm of the number of mol of drug required to elicit a half-maximal response.

Results

Perfusion indices

Group I (NECA, adenosine, **R**-PIA, S-PIA). Basal perfusion pressures in the HA and PV were 102 ± 11 mmHg and 12 ± 1 mmHg respectively. Pressures in the HA and PV increased to 148 ± 8 mmHg and 15 ± 2 mmHg respectively following the addition of noradrenaline to the perfusate. The total volume of bile collected was 12.7 ± 2.8 ml over the 145 ± 8 min perfusion period, approximating to an hourly mean output of 5.3 ml.

Group II (NECA, CGS 21680C, adenosine). Basal perfusion pressures in the HA and PV were 75 ± 22 mmHg and 8 ± 2 mmHg respectively. Pressures in the HA and PV increased to 148 ± 19 mmHg and 14 ± 3 mmHg respectively following the addition of noradrenaline to the perfusate. The total volume of bile collected was 9.8 ± 1.7 ml over the 155 ± 5 min perfusion period, equivalent to an hourly mean output of 3.8 ml.

Dose-response to adenosine and analogues

Group I (NECA, adenosine, R-PIA, S-PIA). Bolus injections of NECA, adenosine, **R**- and S-PIA produced dose-dependent vasodilator responses in the HA (Figure 1). Dose-response curves for the 4 agonists are illustrated in Figure 2a. The maximum response produced by NECA and adenosine was similar, but the PD_2 for each differed significantly (8.6 ± 0.2 and 7.6 ± 0.2 respectively; $P = 0.004$). The PD_2s for **R**- and S-PIA were estimated as 7.0 and 6.5 respectively; more accurate calculations could not be made because maximum responses were not achieved due to the limited solubility of these agents. The rank order of vasodilator potency was therefore: NECA > adenosine > **R**-PIA > S-PIA; their potency, expressed relative to the PD_2 of adenosine, was in the approximate ratio 10.0:1.0:0.25:0.08.

Group II (NECA, CGS 21680C, adenosine). Dose-response curves for NECA, CGS 21680C and adenosine are illustrated in Figure 3a; each drug produced a similar maximum response, though this was about 5 mmHg less than that in Group I. The PD_2 for each drug differed (8.5 ± 0.1, 8.1 ± 0.2 and 7.6 ± 0.1 for NECA, CGS 21680C and adenosine respectively). There was a statistically significant difference between the PD_2s of NECA and adenosine ($P = 0.002$) and of CGS 21680C and adenosine ($P = 0.05$), but not between those of NECA and CGS 21680C ($P = 0.08$). The rank order of agonist potency was thus: NECA > CGS 21680C > adenosine; their potency, expressed relative to the PD_2 of adenosine, was in the ratio 7.9 : 3.2 : 1.0.

Effect of 8-phenyltheophylline

Group I (NECA, adenosine, **R**-PIA, S-PIA). 8-PT attenuated responses to adenosine, as indicated by a shift to the right in

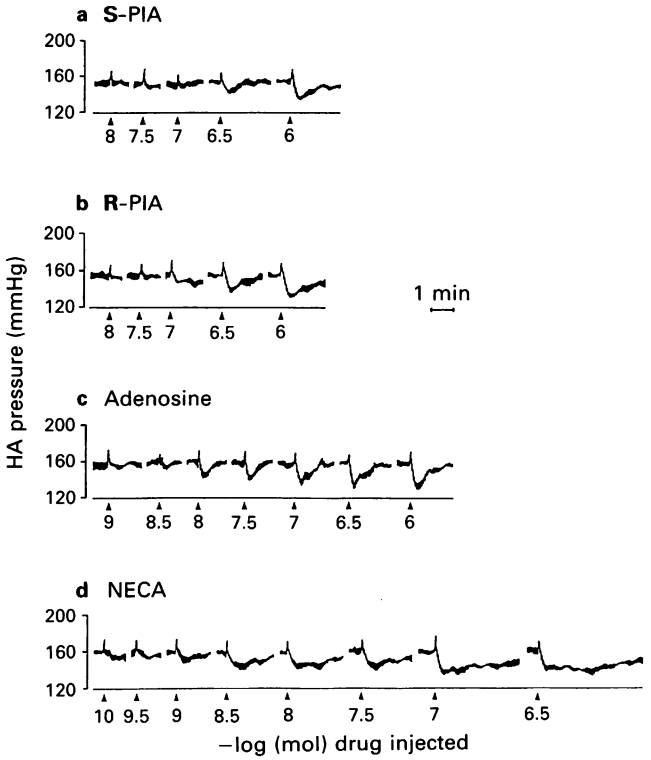

Figure 1 Vasodilator responses to (a) S-N^6-phenyl-isopropyl-adenosine (S-PIA), (b) R-PIA, (c) adenosine and (d) 5'-N-ethyl-carboxamido-adenosine (NECA) in the hepatic arterial (HA) vascular bed of an isolated, dual-perfused rabbit liver (0.1 ml of each agent at the doses indicated), showing the relative potency of the four agonists. The spike prior to response is an injection artefact.

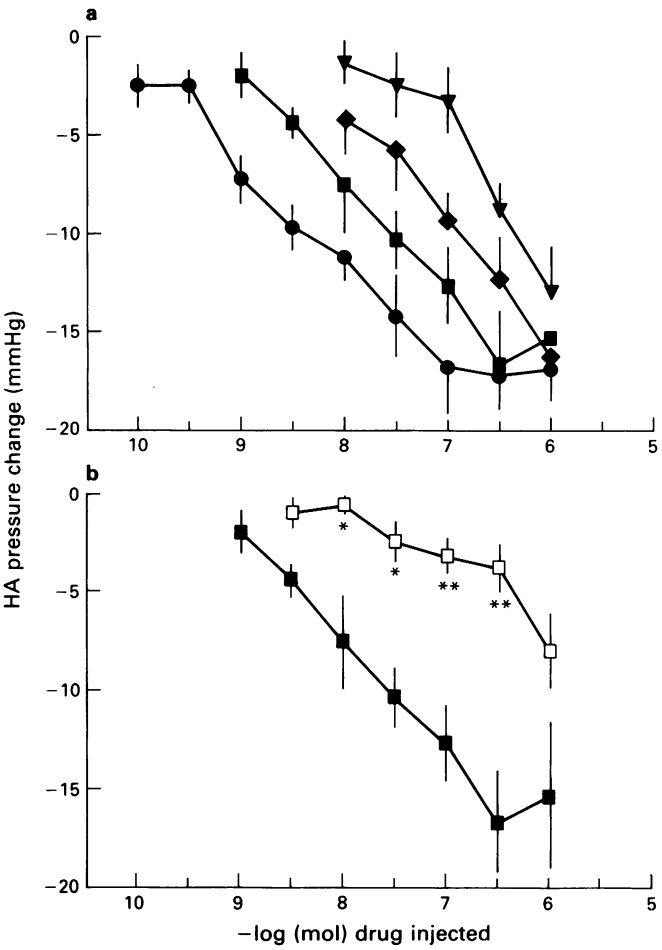

Figure 2 (a) Hepatic arterial (HA) vasodilator response to increasing doses of 5'-N-ethyl-carboxamido-adenosine (NECA) (●), adenosine (■), R-N^6-phenyl-isopropyl-adenosine (R-PIA) (◆) and S-PIA (▼) in the isolated, dual-perfused rabbit liver (Group I). (b) Hepatic arterial (HA) vasodilator response to increasing doses of adenosine before (■) and during (□) administration of 10^{-5} M 8-phenyltheophylline (8-PT) (Group I). Statistically significant differences between responses before and during 8-PT administration: * $P < 0.05$; ** $P < 0.01$.

its dose-response curve (Figure 2b). The PD_2 was 6.7 ± 0.2, which was significantly different from the normal value of 7.6 ± 0.2 ($P = 0.04$). The difference between responses to adenosine before and after the addition of 8-PT was statistically significant at the 4 doses from 10^{-8}–3×10^{-7} mol. The effect of 8-PT on responses to NECA, R- and S-PIA was not studied.

Group II (NECA, CGS 21680C, adenosine). 8-PT resulted in a substantial shift to the right of the dose-response curve of each of the three agents (Figure 3). The vasodilatation produced by each compound was significantly reduced at all doses used except the highest (10^{-6} mol; see Figure 3b).

Response to acetylcholine

Acetylcholine (10^{-9} and 10^{-8} mol) decreased HA pressure by 12.8 ± 1.2 and 17.7 ± 3.7 mmHg respectively before the addition of 8-PT to the perfusate. After the addition of 8-PT to the perfusate, the corresponding pressure decreases were almost identical (12.2 ± 1.3 and 17.6 ± 2.7 mmHg).

Discussion

The order of potency we have demonstrated for adenosine and its analogues to produce vasodilatation of the HA vascular bed is characteristic of P_1-purinoceptors of the A_2 sub-type (Collis, 1985; Burnstock & Kennedy, 1986; Williams, 1987). Moreover, the potency ratio of the 4 agents employed in Group I experiments is within the range expected from previously published studies on the A_2 receptor (Collis & Brown, 1983; Collis, 1985; 1989); for example, we have shown NECA to be 40 times more potent than R-PIA in dilating the HA, while R-PIA had only three times the potency of S-PIA. In addition, 8-PT resulted in a significant inhibition of the dilator response to adenosine, providing evidence for the existence of a population of P_1-purinoceptors in the HA vascular bed. We therefore conclude that A_2 receptors mediate adenosine-induced dilatation of the HA bed in the rabbit liver.

This conclusion was reinforced by the results from Group II, which showed a similar relative potency between adenosine and NECA as found in Group I and an inhibition by 8-PT of all three agonists used. In addition, the high-affinity A_2 agonist CGS 21680C stimulated HA vasodilatation with a potency three times greater than adenosine and one half (though not significantly different from) that of NECA. CGS 21680C is 140 fold more selective for A_2 receptors than for A_1 receptors, and has been reported to be equipotent with NECA in vasodilating the coronary artery (Hutchison *et al.*, 1989). The lesser maximal responses to adenosine and NECA in Group II compared to those in Group I cannot be explained by differences in perfusion characteristics or any other known factor; however, the maximal responses obtained from each group were internally consistent, and therefore the existence of a difference between the groups does not invalidate any of the conclusions drawn. Our use of acetylcholine before and after application of 8-PT demonstrated the retention of non-purinergic relaxation of HA smooth muscle in both groups.

We are not aware of any previous paper demonstrating the existence of A_2 purinoceptors in the HA vascular bed. However, Schütz *et al.* (1982) found evidence for A_2 receptors

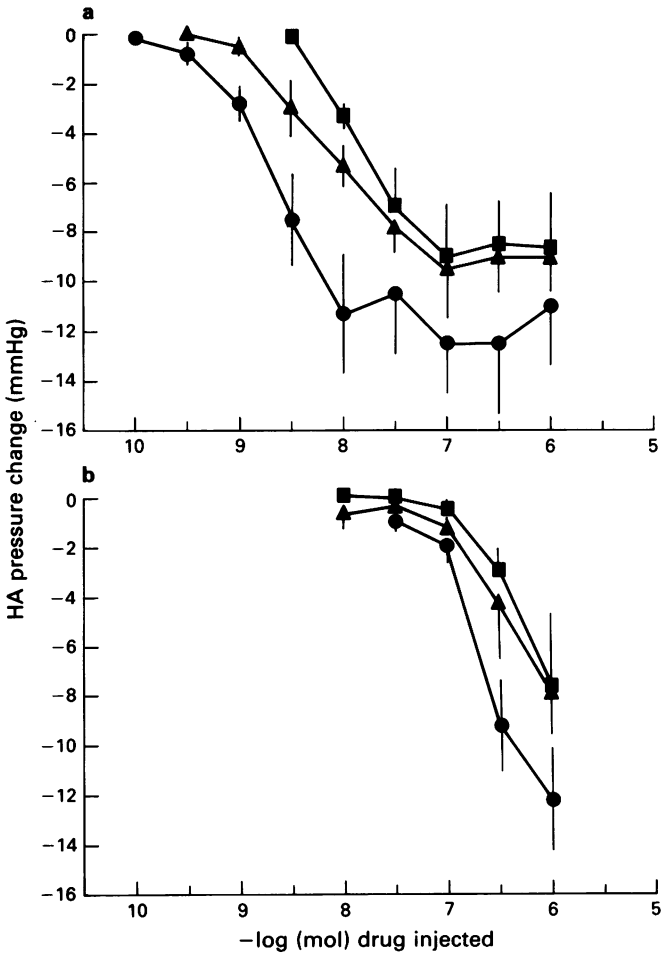

Figure 3 (a) Hepatic arterial (HA) vasodilator response to increasing doses of 5'-N-ethyl-carboxamido-adenosine (NECA) (●), CGS 21680C (▲) and adenosine (■) in the isolated, dual-perfused rabbit liver (Group II). (b) Hepatic arterial (HA) vasodilator response to increasing doses of NECA (●), CGS 21680C (▲) and adenosine (■) during administration of 10^{-5} M 8-phenyltheophylline (8-PT) (Group II). Statistically significant differences ($P < 0.05$) were observed between responses before and during 8-PT administration (cf. Figure 3a) at the following log doses of all three agonists: −8, −7.5, −7 and −6.5 mol.

on rat liver membranes, and Buxton *et al.* (1987) showed that the PV of the perfused rat liver contains A_2 receptors, which, interestingly, they found to mediate vasoconstriction due to adenosine. A_2 receptors have been demonstrated in several other sites in the body (Burnstock & Kennedy, 1986); in the cardiovascular system their presence has been demonstrated in the smooth muscle of the coronary and cerebral circulations as well as in the aorta (Kusachi *et al.*, 1983; Edvinsson & Fredholm, 1983; Collis & Brown, 1983). Further work from our own laboratory using the present experimental model has established the existence of P_2-purinoceptor sub-types in the HA (Ralevic *et al.*, 1991), while Brizzolara & Burnstock (1991) have found both P_1 and P_2 receptors to be present in the common hepatic artery of the rabbit.

It is important to acknowledge that agonist potency alone cannot provide definitive evidence for the differentiation of A_1 and A_2 receptor populations in any vascular bed (Collis, 1985; Paton, 1988). Potency of an agonist is determined by its affinity for the receptor (its ability to bind to the receptor site) and by the efficacy with which it evokes the response (its ability to activate the receptor) as well as by tissue variables such as the number of receptors and the efficiency of coupling of the stimulus to the response (Collis, 1985; 1989). In addition, systems which remove and degrade adenosine may influence the potency of the analogues used to classify receptor subtypes (Collis, 1985). However, the advantage of using agents such as NECA and PIA is that these are relatively resistant to uptake and degradation, and although this resistance was not proven in the current study the considerably longer duration of the response to NECA than to adenosine supports this idea (see Figure 1). NECA was substantially more potent at eliciting vasodilatation than R- or S-PIA, consistent with an action at the A_2 receptor. Furthermore, the absence of stereoselectivity displayed for PIA, and the results with the selective A_2 agonist CGS 21680C are also strongly indicative of the presence of A_2 receptors in the rabbit HA vasculature. Recently, several selective A_2 antagonists have been developed (Bruns & Coughenour, 1987; Ghai *et al.*, 1987; Bruns *et al.*, 1988), which should prove valuable in the identification of A_2-purinoceptors as a complement to studies using agonist potency orders.

Adenosine can cause vasodilatation indirectly by inhibiting the release of noradrenaline from adrenergic nerve terminals, through activation of pre-junctional P_1-purinoceptors (Burnstock & Kennedy, 1986). Although these are normally of the A_1 sub-type, in the rat PV they appear to be of the A_2 sub-type (Kennedy & Burnstock, 1984). The vasodilatation evoked by adenosine in the current *in vitro* preparation is unlikely to have taken place by such a pre-junctional mechanism, because of the lack of sympathetic tone. *In vivo*, however, pre-junctional P_1-purinoceptor regulation of HA blood flow is more likely to take place; in addition, ecto-enzymatic breakdown of ATP co-released with noradrenaline at the nerve terminal (Brizzolara & Burnstock, 1990) can provide a local source of adenosine for such a mechanism.

Recent studies have suggested an important role for adenosine in the physiological control of HA blood flow (Lautt, 1985; 1988), notably in the vasodilator response of the HA to PV flow interruption (the HA 'buffer response') (Lautt *et al.*, 1985; Lautt & Legare, 1985; Mathie & Alexander, 1990). Our current evidence supporting the existence of A_2-purinoceptors in the HA bed indicates a specific mechanism by which adenosine-induced vasodilatation of the HA may take place, and reinforces the probable importance of purinergic vasoactive mechanisms in the hepatic circulation.

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