Contribution of P₁- (A_{2b} subtype) and P₂-purinoceptors to the control of vascular tone in the rat isolated mesenteric arterial bed

¹Annalisa Rubino, Vera Ralevic & Geoffrey Burnstock

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT

1 The direct vascular effects of adenosine and ATP were compared in the isolated and perfused mesenteric arterial bed of the rat. The actions of analogues of adenosine and ATP were also examined.

2 In preparations at basal tone, adenosine lacked vasoconstrictor actions, while ATP elicited dose-dependent vasoconstrictor responses. When the tone of preparations was raised by adding methoxamine to the perfusate, adenosine and its stable analogue, 2-chloroadenosine (2-CADO) elicited dose-dependent vasodilatation. The A₂ adenosine receptor agonist, 5'-N-ethylcarboxamidoadenosine (NECA) was active at lower doses than adenosine, while the A_{2a}-selective agonist, CGS 21680 and the selective A₁ agonist, N⁶-cyclopentyladenosine (CPA) failed to induce vasodilatation. ATP and its analogue, 2-methylthio ATP, elicited dose-dependent vasodilatation at doses 400 fold lower than adenosine.

3 Vasodilator responses to adenosine and 2-CADO were sensitive to antagonism by 1 μ M 8-sulphophenyltheophylline (8-SPT) and were unaffected by inhibition of nitric oxide synthase by N^ω-nitro-L-arginine methyl ester (L-NAME). In contrast, vasodilator responses to ATP were not sensitive to antagonism by 8-SPT and were almost abolished by L-NAME treatment.

4 These results indicate that in the rat mesenteric arterial bed, while both adenosine and ATP participate in the purinergic control of vascular tone, adenosine appears to be a weaker vasodilator than ATP and lacks vasoconstrictor action. A_{2b} adenosine receptors account for the adenosine-induced vasodilatation which is independent of the production of nitric oxide.

Keywords: Adenosine; ATP; mesenteric vascular bed; A_{2b} receptor

Introduction

Since the original observation by Drury & Szent-Gyorgyi (1929) on the cardiovascular effects of adenosine and adenine nucleotides a growing body of evidence has indicated that adenosine and ATP play different roles in the physiological control of cardiovascular function, acting via distinct receptor systems and mechanisms of transduction (see Burnstock, 1990). The characterization of distinct P₁- and P₂-purinoceptors which mediate the effects of adenosine and ATP respectively, was based on the rank order of potency of the agonists, sensitivity to methylxanthines and coupling to adenylate cyclase activity (Burnstock, 1978). Further subdivision of P₁-purinoceptors into A₁, A₂ (A_{2a} and A_{2b}) and A₃ subtypes is now established, the pharmacology of the A₄ subtype still being undefined (Collis & Hourani, 1993; Fredholm *et al.*, 1994; Linden, 1994). For ATP and other adenine nucleotide receptors the existence of two subtypes (P_{2x}- and P_{2y}-purinoceptors) was proposed by Burnstock & Kennedy (1985), based on the rank order of potency of several ATP analogues and selective antagonists and subsequently P_{2r}-, P_{2z}- and P_{2u}-purinoceptors were also identified (Fredholm *et al.*, 1994). Most recently, the voluminous literature describing the investigations of the pharmacological actions of purines in several tissues and the cloning of several subclasses of P₂-purinoceptors (Barnard *et al.*, 1994), has suggested the subdivision of P₂-purinoceptors into families of P_{2X} and P_{2Y}-purinoceptors to embrace all subdivisions of P₂-purinoceptors (Abbracchio & Burnstock, 1994).

Purinergic control of vascular function is achieved as a result of direct actions of adenosine and ATP on vascular smooth muscle or by actions via the endothelium and indirectly, via modulation of perivascular neurotransmission. In most organs the primary role of adenosine is to couple blood

perfusion of tissues to cellular energy state and available evidence suggests that adenosine plays an important role as a powerful direct muscle vasodilator in several vascular beds, such as the coronary and cerebral vasculature (Kalaria & Harik, 1988; Olsson & Pearson, 1990; Vials & Burnstock, 1993). Vasoconstrictor responses to adenosine have been shown in the pulmonary, placental and renal circulation (Lippton *et al.*, 1989; Olsson & Pearson, 1990). Vascular responses to ATP may also consist of vasodilatation or vasoconstriction, depending on the vascular tone and on the purinoceptor subtype activation (Ralevic & Burnstock, 1992). The endothelium via production of endothelium-derived relaxing factors, such as nitric oxide (NO), is involved in the vasodilator responses to several agents and the vasodilator activity of purines has been shown to be either endothelium-dependent or independent (Furchgott, 1984; Ralevic & Burnstock, 1992). In the rat isolated mesenteric vascular bed both P_{2x}- and P_{2y}-purinoceptor subtypes have been characterized, mediating vasoconstrictor and endothelium-dependent vasodilator responses to ATP, respectively (Ralevic & Burnstock, 1988). Furthermore the inhibitory modulation of adenosine on sympathetic and sensory-motor neurotransmission has been shown in the same vascular model (Jackson, 1987; Rubino *et al.*, 1993). However, no information is available on the direct effects of adenosine in the resistance vessels of the mesenteric vascular bed and on the receptor subtype(s) mediating such actions.

The present investigation was therefore designed to examine the direct actions of adenosine on the rat mesenteric arterial bed and to compare them with the effects of ATP. In addition we have investigated the vasodilator potency of analogues of adenosine and ATP. The vascular effect of the purines was further characterized by using the P₁-purinoceptor antagonist 8-(*p*-sulphophenyl)theophylline (8-SPT) and an inhibitor of endothelial NO formation, N^ω-nitro-L-arginine methyl ester (L-NAME).

¹ Author for correspondence.

Methods

Experiments were carried out in the mesenteric arterial bed, prepared for perfusion as previously described (Rubino *et al.*, 1993). Briefly, male Wistar rats (300–350 g) were killed by asphyxiation with carbon dioxide, the abdomen was opened and the superior mesenteric artery exposed and cannulated. The superior mesenteric vein was severed, the gut dissected away and perfusion (5 ml min^{-1}) started with Krebs solution containing (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, NaHCO_3 16.3, MgSO_4 0.61, CaCl_2 2.52 and glucose 7.8, gassed with 95% O_2 , 5% CO_2 and maintained at 37°C . The preparations were allowed to equilibrate for 30 min before experimentation. Because flow through the mesenteric arterial bed was kept constant (5 ml min^{-1}) throughout the experiment, changes in perfusion pressure (mmHg) were indicative of changes in mesenteric vascular resistance. These were measured with a pressure transducer (model P23XL, Viggo Spectramed, Oxnard, CA, U.S.A.) on a side arm of the perfusion cannula and were recorded on a polygraph (model 79D, Grass Instrument Co., Quincy, Mass, U.S.A.). Concentration-response curves to adenosine and ATP were constructed by perfusing the preparations with increasing concentrations of the two agonists. However, due to the high cost of the experiments, dose-response curves to their analogues were obtained by applying the drugs as bolus injections of $50 \mu\text{l}$ via an injection port proximal to the preparation. When the effects of 8-SPT and L-NAME were tested, injections of adenosine, 2-chloroadenosine (2-CADO) and ATP were repeated on the same preparation in the absence and in the presence of the drugs.

Statistical analysis

Results are presented in the text and figures as means $\pm$ standard error of the mean (s.e.mean), followed by number of observations in parentheses (n). Student's t test for paired data was used to test for statistical significance and a value of $P < 0.05$ was considered statistically significant.

Drugs

Adenosine (hemisulphate), 5'-N-ethylcarboxamidoadenosine (NECA), 2-chloroadenosine (2-CADO), adenosine 5'-triphosphate (ATP), methoxamine hydrochloride and N^o-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma.

8-(*p*-Sulphophenyl)theophylline (8-SPT), CGS 21680, cyclopentyladenosine (CPA) and 2-methylthio ATP (2-meSATP) were from RBI. Stock solutions (10^{-2} M) of all drugs except CGS 21680 which was dissolved in 50% dimethylsulphoxide (DMSO), were in distilled water.

Results

Dose-response to adenosine, ATP and their analogues

Basal perfusion pressure in the isolated mesenteric vascular bed was 40.6 ± 2.9 ($n=12$) mmHg. In preparations at basal tone, adenosine used at doses of up to 10^{-4} mol failed to induce vasoconstriction (see Figure 1). Similarly, the adenosine analogues, 2-CADO, NECA, CPA and CGS 21680 lacked vasoconstrictor activity. In contrast, ATP (3×10^{-7} – 10^{-5} mol) elicited dose-dependent vasoconstrictor responses (Figure 1) achieving 42.2 ± 6.0 ($n=4$) mmHg constriction at the highest dose tested.

The addition of methoxamine (3 – $10 \mu\text{M}$) to the perfusate raised the vascular tone by 80.2 ± 3.7 ($n=12$) mmHg and this tone was maintained throughout the experiment. In preparations in which the tone had been raised by methoxamine, continuous perfusion with ATP (10^{-8} – 10^{-5} M) elicited concentration-dependent vasodilatation (Figure 2). At higher concentrations (10^{-6} – $3 \times 10^{-4} \text{ M}$) adenosine mimicked the vasodilator action of ATP. Similarly, bolus injection of ATP and its analogue 2-meSATP elicited dose-dependent vasodilatation at doses ranging from 10^{-11} to $3 \times 10^{-8} \text{ mol}$, while adenosine was active at higher doses (Figures 1 and 2). At the highest dose tested (10^{-6} mol), adenosine relaxed by 49.5 ± 5.1 ($n=7$) % the methoxamine-sustained tone of the preparations. The vasodilator responses to 2-CADO were superimposable on those to adenosine, the maximal vasodilatation achieved being $52.6 \pm 4.2\%$ ($n=4$) of the raised tone. The A_2 -selective agonist, NECA, appeared to be more potent, inducing a vasodilator response of $44.0 \pm 3.2\%$ ($n=4$) when injected as a bolus of 10^{-6} mol (Figure 2). No responses were detected following injection of CGS 21680 and CPA up to 10^{-6} mol (Figure 2).

Figure 1 Representative tracings showing vasoconstrictor (upper tracings, preparation at basal tone) and vasodilator (lower tracings, preparation with tone raised by $10 \mu\text{M}$ methoxamine) responses of the rat mesenteric vascular bed to increasing doses of adenosine and ATP. Vascular responses are shown as increase in the perfusion pressure (mmHg). Doses were applied as $50 \mu\text{l}$ bolus injection and are labelled in the figure as ($-\log \text{ mol}$).

Effect of 8-sulphophenyltheophylline

In the presence of 8-SPT ($1 \mu\text{M}$) the tone of the preparations was not significantly different from controls, being $73.9 \pm 5.2 \text{ mmHg}$ ($n=8$) above the basal value in the presence of methoxamine. 8-SPT attenuated the vasodilator responses to adenosine and its stable analogue 2-CADO, as indicated by the shift to the right in their dose-response curves (Figure 3). The vasodilatation induced by these agents was significantly reduced at all the doses, including the highest (Figure 3). In contrast, the dose-effect curve for ATP in the presence of 8-SPT was superimposable on that obtained in the absence of the antagonist (Figure 3).

Effect of L-NAME

Perfusion with L-NAME resulted in an increased sensitivity of the preparations to the vasoconstrictor action of methoxamine. Vasodilator responses were therefore evaluated in the presence of a reduced concentration of methoxamine ($7 \mu\text{M}$) which allowed the tone to be raised by $80.2 \pm 4.0 \text{ mmHg}$ ($n=4$), as in control conditions (see above). L-NAME ($30 \mu\text{M}$)

only partially but not significantly reduced the vasodilatation elicited by adenosine and 2-CADO (Figure 4). In contrast, L-NAME ($30 \mu\text{M}$) produced a drastic reduction of vasodilator responses to all doses of ATP tested (Figure 4).

Discussion

These data indicate that in the rat mesenteric arterial bed both P_1 - and P_2 -purinoceptors contribute to the regulation of vascular tone. Adenosine elicits vasodilatation via the A_{2B} receptor subtype and lacks vasoconstrictor effects, in contrast to ATP which is a powerful vasoconstrictor and a more potent vasodilator than adenosine.

When the preparations were perfused with increasing concentrations of adenosine and ATP, ATP was shown to be a more potent vasodilator than adenosine and this finding was confirmed when vascular responses to bolus injections of the two agonists and their analogues were evaluated. The vasodilator activity of ATP was mimicked by its analogue 2-meSATP used at the same doses, while the effect of adenosine was mimicked by 2-CADO and NECA. Dose-response curves for adenosine and 2-CADO were superimposable, while NECA elicited vasodilatation at lower doses. CPA, a selective agonist of A_1 adenosine receptors (Williams *et al.*, 1986), failed to in-

Figure 2 Dose-responses curves showing vasodilator responses of the rat mesenteric vascular bed to ATP ($\blacktriangle$), 2-meSATP ($\blacksquare$), adenosine ($\diamond$), 2-CADO ($\star$), NECA ($\blacktriangledown$), CPA ($\circ$) and CGS 21680 ($\bullet$). Vasodilator responses are shown as percent vasodilatation of the methoxamine sustained tone taken as 100% and are the mean of 4–7 preparations. (a) Shows vascular responses following perfusion of the preparations with increasing concentrations of ATP and adenosine; (b) shows vascular responses following bolus injection of increasing doses of agonists. Symbols show means $\pm$ s.e. mean. For abbreviations, see text.

Figure 3 Dose-responses curves showing vasodilator responses of the rat mesenteric vascular bed to ATP ($\square$, $\blacksquare$), adenosine ($\triangle$, $\blacktriangle$) and 2-CADO ($\circ$, $\bullet$) in the absence (solid symbols) and in the presence (open symbols) of $1 \mu\text{M}$ 8-sulphophenyltheophylline. Symbols indicate means of 4 preparations $\pm$ s.e. mean.

Figure 4 Vascular responses to adenosine (ADO) and 2-chloroadenosine (2-CADO) (a) and to ATP (b) in the absence (open columns) and in the presence (hatched columns) of N^{ω} -nitro-L-arginine methyl ester $30 \mu\text{M}$. Columns indicate means $\pm$ s.e. mean of 4–6 preparations.

duce vasodilatation. Similarly, CGS 21680, which has been suggested as an A_{2a} receptor-selective agonist (Jarvis *et al.*, 1989; Lupica *et al.*, 1990) was devoid of vasodilator activity.

The vasodilator activity of adenosine and 2-CADO was sensitive to the antagonistic action of methylxanthine as indicated by the shift to the right of their respective dose-response curves in the presence of 8-SPT. In contrast, the vasodilator activity of ATP was unaffected by 8-SPT. The different sensitivity of adenosine- and ATP-mediated vasodilatation to antagonism by methylxanthine is in favour of the hypothesis that distinct receptor populations, namely P_1 - and P_2 -purinoceptors, mediate the vasodilator action of adenosine and ATP, respectively. Furthermore, the order of potency of the adenosine analogues and the lack of activity of CGS 21680 suggest that in the rat mesenteric arterial bed, P_1 -purinoceptors of the A_{2b} subtype are responsible for the adenosine-mediated vasodilatation. The A_{2b} adenosine receptor has been characterized in other vascular preparations, such as guinea-pig aorta, where NECA was 400 fold more potent than CGS 21680 (Gurden *et al.*, 1993). The order of potency for NECA and CGS 21680 was reversed in dog and guinea-pig coronary artery, where the A_{2a} receptor subtype has been described (Gurden *et al.*, 1993; Vials & Burnstock, 1993). In the rabbit mesenteric artery the adenosine analogues, CGS 21680 and NECA, were equipotent as vasodilator agonists (Balwierczak *et al.*, 1991). It is noteworthy that the cloned A_{2b} receptor has been shown to be highly expressed in the rat intestine and bladder (Collis & Hourani, 1993), consistent with the functional data of this study.

In most vascular preparations vasodilator responses to adenosine appear to be endothelium-independent and the production of NO following adenosine receptor activation seems to play a minor role (Furchgott, 1984). However, A_2 adenosine receptors have been localized on endothelial cells in culture (Des Rosiers & Nees, 1987) and NO production has been suggested in the guinea-pig coronary circulation following activation of A_{2a} receptors (Vials & Burnstock, 1993). In this study the vasodilator effects of maximal doses of adenosine and 2-CADO were not significantly affected by a concentration of L-NAME that fully inhibited ATP-mediated vasodilatation thus suggesting that in the rat mesenteric circulation, adenosine-induced vasodilatation can be achieved independently from the production of NO. However, the contribution of NO to vasodilatation via A_{2b} receptors can be suggested in the light of the fact that L-NAME could slightly reduce, though not significantly, the vasodilator effect of adenosine and CADO. In contrast, ATP-mediated vasodilatation was largely dependent on NO formation, as shown previously in this preparation (Ralevic & Burnstock, 1988; 1992). These observations are in line with previous reports showing that in the rat mesenteric artery vasodilatation to ATP and adenosine is endothelium-dependent and -independent, respectively (Vourinen *et al.*, 1992).

In several vessels ATP has effects that are more potent than those of adenosine, for example in the constriction of the pulmonary circulation (Lippton *et al.*, 1989). Moreover, in the

coronary circulation of every mammalian species ATP is a powerful vasodilator, often more potent than adenosine (Olsson & Pearson, 1990; Corr & Burnstock, 1991). In this study ATP elicited vasoconstrictor responses while adenosine and its stable analogues failed to induce vasoconstriction. When vasodilator responses were evaluated, ATP was able to elicit vasodilatation at concentrations 400 fold lower than adenosine and its analogues, thus confirming data obtained in the isolated mesenteric artery where ATP is a more potent vasodilator than adenosine (Vourinen *et al.*, 1992). However, as first described by McGregor (1965) and confirmed by more recent investigations (Longhurst *et al.*, 1986), the isolated mesenteric arterial bed rather than vascular rings isolated from the main mesenteric artery, is representative of vascular responsiveness at the level of resistance vessels and the whole-bed preparation can therefore be considered as a valuable *in vitro* model of splanchnic circulation. In the light of these considerations, this study rather than previous observations (Vourinen *et al.*, 1992) suggests that ATP is a more powerful vasoactive agent than adenosine in the intestinal vasculature.

Adenosine and ATP may change vascular tone in either of two ways, directly through receptors on the vascular wall or indirectly as modulators of perivascular innervation. The inhibitory effect of adenosine on sympathetic neurotransmission has been well documented *in vitro* and *in vivo* in the rat mesenteric circulation (Lukacsko & Blumberg, 1982; Jackson, 1987). Moreover, previous observations in this laboratory demonstrated that in the rat mesenteric arterial bed, adenosine and its analogues modulate sensory-motor neurotransmission via prejunctional A_1 receptors at concentrations devoid of any direct action on vascular tone (Rubino *et al.*, 1993). From the observations of this study and from data described in the literature it can be speculated that adenosine and ATP both participate in the regulation of the mesenteric circulation via activation of distinct receptor systems and via different mechanisms which are independent or dependent on NO production for adenosine and ATP, respectively. However, ATP appears to be more powerful than adenosine in causing direct vasoactive actions, whilst adenosine exerts indirect effects as a modulator of perivascular sympathetic and sensory-motor neurotransmission. These observations are in contrast with the hypothesis of Berne (1963; 1980) in favour of adenosine, a breakdown product of ATP, as the main endogenous vasodilator and further support the view that ATP itself is a powerful regulator of vascular tone, producing both vasodilatation and vasoconstriction (Burnstock, 1993).

In conclusion, this study shows ATP as a more powerful vasoactive agent than adenosine in the regulation of the mesenteric circulation via direct vascular effects and demonstrates for the first time that in the rat mesenteric arterial bed, adenosine elicits vasodilatation via A_{2b} receptors, independently of NO formation.

This work was supported by the British Heart Foundation.

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(Received February 3, 1995
Accepted March 1, 1995)