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The effects of purine compounds on the isolated aorta of the frog *Rana temporaria*

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1 In the isolated aorta of the frog, *Rana temporaria*, adenosine concentration-dependently, endothelium-independently relaxed adrenaline pre-constricted vessels. None of the adenosine analogues including D-5'-(N-ethylcarboxamide) adenosine (NECA), **R**- and **S**-N^6-(2-phenylisopropyl) adenosine (**R**- and **S**-PIA) and 2-chloroadenosine (2-CA), or the more selective A_1, A_2 and A_3 agonists cyclopentyladenosine (CPA), CGS 21680 and N^6-(3-iodobenzyl) adenosine-5'-*N*-methylcarboxamide (IB-MECA) respectively, had any effect.

2 The non-selective adenosine antagonist, 8-*p*-sulphophenyl-theophylline (8-*p*SPT; 30 μM) failed to inhibit adenosine relaxations, as did N^G-nitro-L-arginine methyl ester (L-NAME; 0.1 mM) and indomethacin (30 μM).

3 Adenosine 5'-triphosphate (ATP), α,β-methylene ATP (α,β-MeATP), β,γ-methylene ATP (β,γ-MeATP), 2-methylthio ATP (2-MeSATP) and uridine 5'-triphosphate (UTP) all concentration-dependently contracted the frog aorta. ATP and α,β-MeATP were equipotent and more potent than UTP and β,γ-MeATP; 2-MeSATP had little activity.

4 The P_2-purinoceptor antagonist, suramin (0.1 mM) inhibited contractions to α,β-MeATP but not to ATP. Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 30 μM) also inhibited contractions to α,β-MeATP but not to ATP. Contractions to ATP were, however, inhibited by indomethacin (30 μM).

5 In conclusion, in the frog aorta there appears to be a novel subclass of P_1-purinoceptor mediating vasodilatation, although like the A_3 subclass it is not blocked by methylxanthines; a P_2-purinoceptor mediates vasoconstriction which resembles a P_{2X} subtype, based on the agonist potency of α,β-MeATP being more potent than 2-MeSATP (UTP has moderate activity) and PPADS is an effective antagonist. There is no evidence for the presence of a P_{2Y}-purinoceptor, mediating vasodilatation, in this preparation.

Keywords: Frog aorta; adenosine; ATP; purinoceptor

Introduction

Purine nucleotides and nucleosides have long been known to have effects on the cardiovascular system of mammals. Adenosine was shown to inhibit activity in mammalian hearts in 1929 by Drury & Szent Györgyi, and it was observed that ATP was more effective at causing heart block in the guinea-pig than adenosine (Drury, 1936). Further investigations into the effect of adenosine and ATP on the cardiovascular system followed (Gaddum & Holtz, 1933; Richards, 1934; Green & Stoner, 1950) resulting in detailed documentary evidence of the important role of both adenosine and ATP in the maintenance of mammalian vascular tone (see Olsson & Pearson, 1990) via the heart and systemic vascular systems (Su, 1981; 1985; Burnstock & Kennedy, 1986; Burnstock, 1987a,b; 1989; 1990b; Ralevic & Burnstock, 1991).

Once it was recognised that adenosine and ATP had different effects on mammalian systems and were therefore acting via separate receptors, a formal classification was proposed (Burnstock, 1978). Receptors selective for adenosine and adenosine monophosphate (AMP) were designated as P_1-purinoceptors and those selective for ATP and adenosine diphosphate (ADP) called P_2-purinoceptors. P_1-purinoceptors, susceptible to methylxanthine blockade, were divided into A_1- and A_2-receptors (Van Calker *et al.*, 1979; Londos *et al.*, 1980). More recently an A_3-receptor subclass has been cloned (Meyerhof *et al.*, 1991; Zhou *et al.*, 1992), N^6-(3-iodobenzyl)-adenosine-5'-*N*-methylcarboxamide (IB-MECA) is a selective agonist at this receptor (Jacobson *et al.*, 1993; Gallo-Rodriguez *et al.*, 1994) and D-5'-(N-ethylcarboxamide) adenosine (NECA) and N^6-(2-phenylisopropyl) adenosine (PIA) are equipotent, but it is resistant to methylxanthine blockade. P_2-purinoceptors were initially divided into P_{2X} and P_{2Y} subclasses (Burnstock & Kennedy, 1985); other subdivisions have since been recognised, P_{2Z}, P_{2T} and P_{2U} classes (Gordon, 1986; Hoyle & Burnstock, 1991a; Stone, 1991; Hoyle, 1992; O'Connor, 1992). Additional subdivisions have been proposed, P_{2R}, P_{2S} and P_3, but these have not been widely accepted (see Hoyle & Burnstock, 1991a, b; Stone, 1991). In the most recent proposal for purinoceptor subclassification by Abbracchio & Burnstock (1994), adopted in principle by the IUPHAR Nomenclature Committee (see Fredholm *et al.*, 1994), two families of purinoceptors are recognised, P2X (ligand-gated cation channels) and P2Y (G-protein mediated), each with their own subclasses, $P2X_{1-4}$ and $P2Y_{1-7}$.

Both P_1- and P_2-purinoceptors have important actions on the cardiovascular system. Generally, adenosine dilates mammalian blood vessels by acting on A_2-receptors located on the smooth muscle (Berne *et al.*, 1983; Burnstock & Kennedy, 1986; Collis, 1989) whereas activation of A_1-receptors often mediates prejunctional inhibition (Verhaeghe *et al.*, 1977). Stimulation of P_{2X}-purinoceptors mediates vasoconstriction via an action on receptors located on the smooth muscle (Burnstock & Kennedy, 1986; Burnstock, 1990a, c) and activation of endothelial P_{2Y}-purinoceptors results in vasodilatation, via the release of endothelium-derived relaxing factor (EDRF) now known to be nitric oxide (NO) (De Mey & Vanhoutte, 1981; Houston *et al.*, 1987; Pearson & Gordon, 1989) and prostacy-

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clin (Moncada & Vane, 1979), although P_{2Y}-purinoceptors have been identified on the vascular smooth muscle of some vessels (Mathieson & Burnstock, 1985; Brizzolara & Burnstock, 1991).

While the effects of both adenosine and ATP have been extensively studied on mammalian systems, there is, in contrast, only limited information available on the role of purines in the control of the cardiovascular system of lower vertebrates. A presynaptic A_1-receptor has been identified, stimulation of which inhibits sympathetic nerve activity to arterioles of frog cutaneous muscle (Fuglsang *et al.*, 1989). There are several studies examining the effect of purine compounds on the amphibian heart. P_1-purinoceptors have been demonstrated in frog heart (Burnstock & Maghji, 1981; Lazou & Beis, 1987). P_2-purinoceptors have been identified in axolotl, frog and toad atria (Meghji & Burnstock, 1983a, b; Hoyle & Burnstock, 1986). A non-adrenergic, non-cholinergic (NANC) excitatory transmission has been demonstrated in the frog heart (Donald, 1985) which is mediated by a P_2-purinoceptor (Burnstock & Meghji, 1981), ATP being a cotransmitter with adrenaline (Hoyle & Burnstock, 1986; Bramich *et al.*, 1990). Studies have revealed P_1-purinoceptors on the coronary artery of trout and skate (Small *et al.*, 1990; Farrell & Davie, 1991) and trout gill vasculature (Colin & Leray, 1979; Colin *et al.*, 1979), dogfish atria and aorta (Meghji & Burnstock, 1984; Evans, 1992). ATP has been shown to dilate the portal vein of the lizard (Ojewole, 1983). The present investigation was carried out to study the effects of adenosine and ATP in the isolated aorta of the frog and to identify purinoceptor subtypes using agonist potency orders and selective antagonists.

Methods

Frogs (*Rana temporaria*) of either sex, supplied by Blades Biological, Cowden, Edenbridge, U.K., were stunned and decapitated. The frog was pithed and the brain destroyed with a metal seeker. The aortic branches were rapidly removed and placed in a physiological saline solution. Ring segments of approximately 4–5 mm, one from each arch, were dissected free and mounted in 10 ml organ baths containing gassed (95% O_2/5% CO_2) saline of the following composition (mM): NaCl 111.1, KCl 1.88, NaH_2PO_4 0.08, $NaHCO_3$ 2.35, $CaCl_2$ 1.08 and glucose 1.11. All experiments were carried out at room temperature, 23 ± 1.0°C.

Segments of aorta were mounted horizontally in the organ bath by inserting two tungsten wires through the lumen of the vessel. One wire was used to anchor the vessel to a rigid support, the other attached to a Grass force-displacement transducer FT03C. Mechanical activity was displayed on a Grass ink-writing oscillograph. An initial load of 0.75–1 g was applied and the vessels were allowed to equilibrate for 1 h.

With each vessel, a concentration-response curve for adrenaline was constructed in order to obtain an EC_{50} value. This concentration of adrenaline was used to constrict the vessel to investigate vasodilator responses. The integrity of the endothelium was tested at the beginning of the experiment by the vasodilator response to acetylcholine (ACh; data not shown).

Vasodilator responses to adenosine and analogues NECA, **R** and **S**-PIA, 2-chloroadenosine (2-CA), cyclopentyladenosine (CPA), CGS 21680 and IB-MECA were examined on adrenaline (EC_{50}) pre-constricted vessels. The concentration-response curve to adenosine was repeated in the presence of the P_1-purinoceptor antagonist 8-*p*-sulphophenyl-theophylline (8-*p*SPT, 30 μM), the NO-synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mM) and the prostanoid inhibitor, indomethacin (30 μM).

Vasoconstrictor concentration-response curves to ATP, α,β-MeATP, β,γ-MeATP, UTP and 2-methylthio ATP (2-MeSATP) were constructed at basal tone. The concentration-response curves to ATP and α,β-MeATP were repeated in the presence of the P_2-purinoceptor antagonists, suramin (0.1 mM) and pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 30 μM) and the prostanoid inhibitor, indomethacin (30 mM). All antagonists were allowed to equilibrate for 20 min before concentration-response curves were repeated.

Drugs used

Adrenaline bitartrate, adenosine, NECA, **R**-PIA, **S**,PIA, 2-CA, ATP, α,β-MeATP, β,γ-MeATP, UTP, ACh, indomethacin, L-NAME, dimethyl sulphoxide (DMSO) and ascorbic acid were supplied by Sigma Chemical Co; 2-MeSATP, CPA, CGS 21680 (2-[-p-(2-carboxyethyl) phenethylamino]-5'-N-ethylcarboxamido adenosine) and 8-*p*SPT by Research Biochemicals Inc.; IB-MECA was the kind gift of Prof. K. Jacobson (NIH, U.S.A.), suramin was the kind gift of I.C.I. and PPADS a kind gift from Prof. Lambrecht (Frankfurt, Germany). Adrenaline was dissolved in 100 μM ascorbic acid; IB-MECA in DMSO to give a stock solution, the first dilution in 50% DMSO/50% distilled water, and subsequent dilutions in distilled water; indomethacin in 0.2 M $NaCO_3$ solution and NECA, **R**- and **S**-PIA, CPA and CGS 21680 in DMSO to produce a stock solution, with subsequent dilutions in distilled water. All other drugs were dissolved in distilled water.

Statistical analysis

Vasodilator responses are expressed as mean % relaxation of the adrenaline (EC_{50} concentration) contraction ± standard error (s.e.) (*n*) and vasoconstrictor responses are expressed as a mean maximum tension developed in g or as a % of the maximum response in the absence of antagonist ± s.e. (*n*). Significance was tested by analysis of variance followed by Tukey's test, or Student's paired *t* test. A probability of $P < 0.05$ was taken as significant.

Results

Vasodilator response to adenosine

Adrenaline, known to be the principle transmitter in sympathetic nerves in amphibians (see Burnstock, 1969), concentration-dependently constricted isolated preparations of frog aorta. The EC_{50} value for each preparation was calculated and this concentration was used to constrict the vessel to study vasodilator responses. The mean pD_2 ($-\log EC_{50}$ value) for adrenaline was 6.07 ± 0.10 ($n = 18$).

Adenosine (1 μM–3 mM) concentration-dependently, endothelium-independently relaxed adrenaline pre-constricted vessels. As adenosine did not consistently cause a maximum vasodilatation, a pD_2 value could not be calculated.

The adenosine analogues NECA, **R**- and **S**-PIA and 2-CA and the more selective A_1, A_2 and A_3 agonists CPA, CGS 21680 and IB-MECA respectively, were all without effect, up to a concentration of 30 μM ($n = 5$).

The non-selective adenosine antagonist 8-*p*SPT (30 μM) had no effect on the dilator activity of adenosine. Since adenosine failed to reach a maximum response in either the absence or presence of 8-*p*SPT, pD_2 values could not be compared; however, Student's paired *t* test revealed no statistical difference in the concentration-response curve for adenosine in the absence and presence of 8-*p*SPT ($P > 0.05$) (Figure 1).

Concentration-response curves for adenosine were repeated in the presence of L-NAME (0.1 mM; $n = 6$) and indomethacin (30 μM; $n = 6$). There was no significant difference in concentration-response curves for adenosine in the absence or presence of these agents ($P > 0.05$).

Vasoconstrictor responses to ATP and analogues

At basal tone ATP, α,β-MeATP, β,γ-MeATP and UTP all concentration-dependently constricted the isolated aorta, 2-

MeSATP being virtually without activity. None of the analogues reached a maximum response, therefore the purine compounds were compared by expressing contractions as mean tensions developed in mg. Analysis of variance followed by a Tukey's test revealed an order of potency for the agonists to be: α,β-MeATP = ATP > UTP = β,γ-MeATP > > 2-MeSATP (Figure 2).

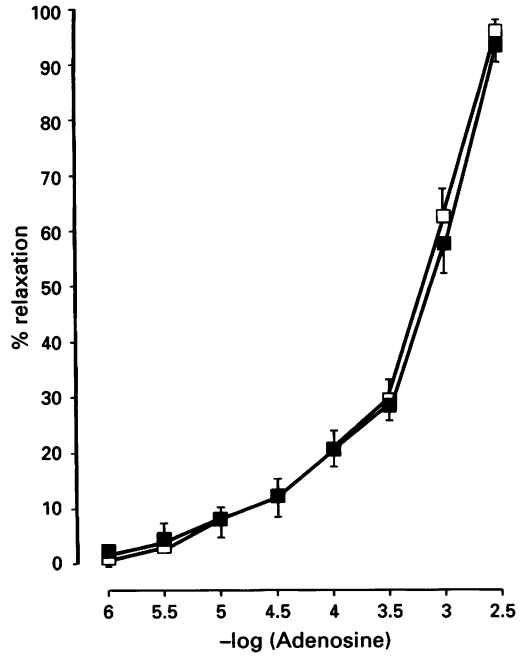

Figure 1 Cumulative concentration-response curve for adenosine on adrenaline (EC_{50} concentration) preconstricted aorta of the frog *Rana temporaria* in the absence (■, $n=6$) and presence (□, $n=6$) of 8-*p*SPT (30 μM). Symbols represent mean % relaxation ± s.e. (unless masked by the symbol).

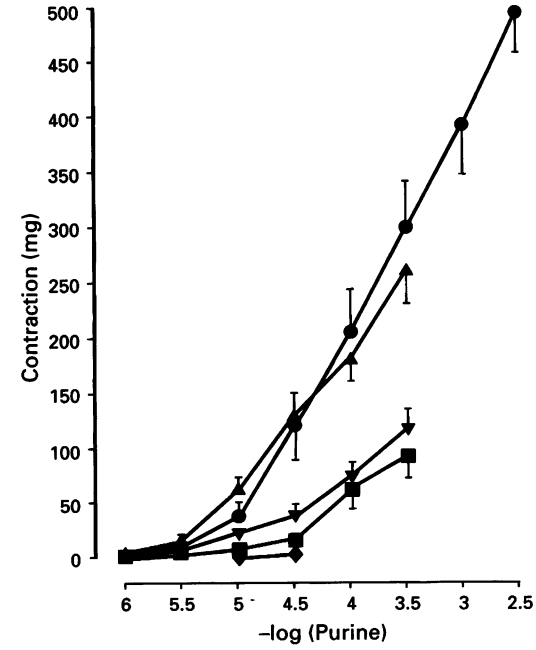

Figure 2 Concentration-response curves for ATP (●, $n=21$) and its stable analogues α,β-MeATP (▲, $n=26$), β,γ-MeATP (■, $n=7$) and 2-MeSATP (◆, $n=16$) and the pyrimidine UTP (▼, $n=8$) on the isolaed aorta of the frog *Rana temporaria*. All symbols represent mean contraction in mg ± s.e. (unless masked by the symbol).

Repeated administration of α,β-MeATP (30 μM; $n=3$) did not desensitize the vessel either to an increased concentration of α,β-MeATP or to ATP administration and in both cases a further contraction was observed. In adrenaline pre-constricted vessels, neither ATP (up to 3 mM) nor 2-MeSATP (up to 30 μM) caused vasodilatation; ATP generally raised the tone of the vessel still further, while 2-MeSATP had no effect.

Effect of antagonists on constrictor responses

Suramin (0.1 mM; $n=6$) significantly ($P<0.05$) inhibited constrictor responses to α,β-MeATP (Figure 3a). As maximum responses were not reached, pD_2 values could not be calculated. Suramin (0.1 mM; $n=9$) had no inhibitory effect on ATP (Figure 3b).

PPADS (30 μM) significantly ($P<0.05$) inhibited constrictor responses to α,β-MeATP ($n=6$; Figure 4a), but not ATP ($n=5$; Figure 4b).

Indomethacin (30 μM) significantly ($P<0.05$) inhibited the response to ATP ($n=7$; Figure 5), but not α,β-MeATP ($n=9$). In the presence of indomethacin (30 μM) neither suramin (0.1 mM; $n=7$) nor PPADS (30 μM; $n=7$) caused any further inhibition of the responses to ATP.

Discussion

The vasodilator activity of adenosine in the frog aorta is consistent with its vasodilator action in both mammals (see Burnstock, 1990b) and fish vessels, including the trout and skate coronary arteries (Small *et al.*, 1990; Farrell & Davie, 1991) and dogfish aorta (Meghji & Burnstock, 1984). In mammals the subclass of P_1-purinoceptor mediating vasodilatation of most vessels is the A_2-receptor (Burnstock, 1990b). Adenosine dilates the frog aorta in a concentration-dependent, endothelium-independent manner; however none of the adenosine analogues, which are used to classify the subtype of P_1-purinoceptor in mammalian systems, had any vasodilator activity, these include NECA, **R**- and **S**-PIA and 2-CA. In addition, the selective A_1, A_2 and A_3 analogues CPA, CGS 21680 and IB-MECA respectively (Lohse *et al.*, 1988; Hutchison *et al.*, 1989; Jacobson *et al.*, 1993; Gallo-Rodriguez *et al.*, 1994) were without effect upto a concentration of 30 μM. These more selective analogues are significantly more potent than adeno-

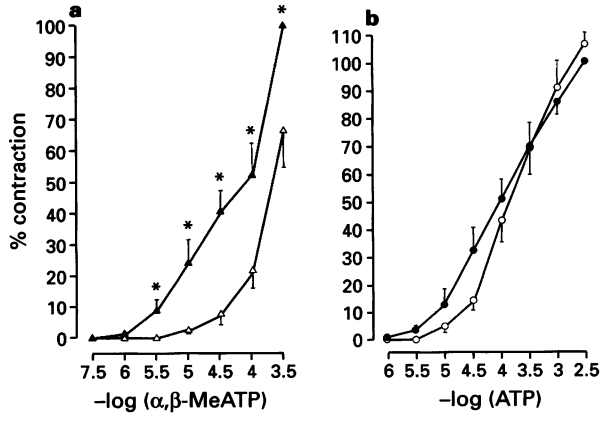

Figure 3 Concentration-response curves for α,β-MeATP and ATP in the isolated aorta of the frog *Rana temporaria*. (a) Concentration-response curve for α,β-MeATP in the absence (▲, $n=6$) and presence (△, $n=6$) of suramin (0.1 mM). Symbols represent mean % contraction ± s.e. (unless masked by the symbol). *Indicates statistical significance ($P<0.05$). (b) Concentration-response curve for ATP in the absence (●, $n=9$) and presence (○, $n=9$) of suramin (0.1 mM). Symbols represent mean % contraction ± s.e. (unless masked by the symbol).

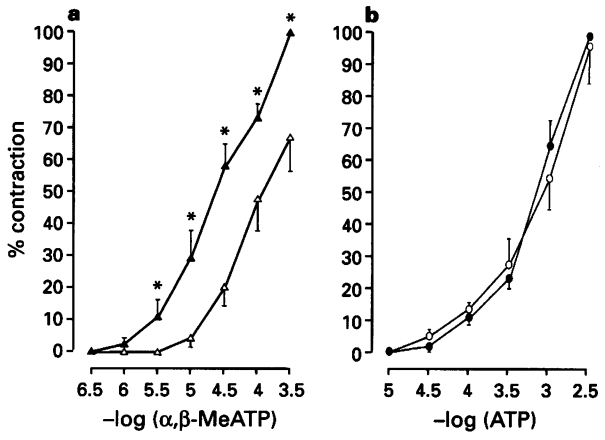

Figure 4 Concentration-response curve for α,β-MeATP and ATP on the isolated aorta of the frog *Rana temporaria*. (a) Concentration-response curve for α,β-MeATP in the absence (▲, $n=6$) and presence (△, $n=6$) of PPADS (30 μM). Symbols represent mean % contraction ± s.e. (unless masked by the symbol). *Indicates statistical significance ($P<0.05$). (b) Concentration-response curve for ATP in the absence (●, $n=6$) and presence (○, $n=6$) of PPADS (30 mM). Symbols represent mean % contraction ± s.e. (unless masked by the symbol).

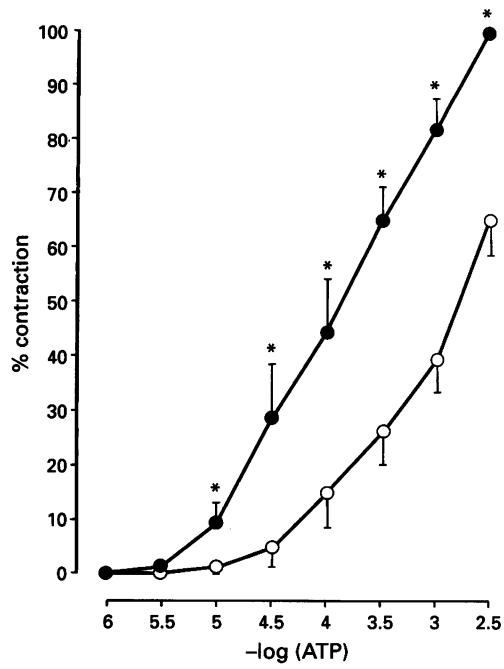

Figure 5 Concentration-response curve for ATP on the isolated aorta of the frog *Rana temporaria* in the absence (●, $n=7$) and presence (○, $n=7$) of indomethacin (30 μM). Symbols represent mean % contraction ± s.e. (unless masked by the symbol). *Indicates statistical significance ($P<0.05$).

sine itself, for instance an IC_{50} value of 4.3 μM for 2-CA has been reported for the guinea-pig aorta (Alexander *et al.*, 1994) whereas an EC_{50} of 115 nM for CGS 21680 has been reported in the rat aorta (Conti *et al.*, 1993). CPA modulates ACh release in the mouse hemidiaphragm with an IC_{50} of 0.08 μM (Nagano *et al.*, 1992). The novel A_3 agonist, IB-MECA has a K_i value of 1.1 nM in rat brain membranes (Gallo-Rodriguez *et al.*, 1994). Further, the non-selective P_1-purinoceptor antagonist, 8-*p*SPT, did not affect the dilator activity of adenosine. Thus, the adenosine receptor mediating vasodilatation in the frog aorta appears to be novel, although it does resemble the A_3-receptor in that methylxanthines are not effective blockers. Adenosine is not initiating relaxation via the production of NO or a prostanoid, since neither L-NAME, which inhibits the activity of NO-synthase (Rees *et al.*, 1989; 1990), nor indomethacin which inhibits prostanoid production (Vane, 1971), had any effect on the dilator response to adenosine. Both NO and prostanoids have been found to mediate the dilator response to adenosine in some mammalian preparations (Ciabattoni & Wennmalm, 1985; Vials & Burnstock, 1993a).

In addition to activating receptors that are located on the cell surface (A_1-, A_2- and A_3-receptors), adenosine is thought to interact with an intracellular 'P-site' (Londos & Wolff, 1977). Adenosine and 2-CA are found to activate this site which is believed to be linked to adenylyl cyclase (Londos *et al.*, 1980). NECA and PIA are inactive at this site, which is also insensitive to xanthine antagonism. It is unlikely that adenosine is mediating vasodilatation via activation of an intracellular P-site, similar to that found in the guinea-pig aorta (Collis & Brown, 1983), since 2-CA was inactive.

The activity of ATP analogues on the frog aorta can be characterized according to mammalian P_2-purinoceptor classification. ATP, α,β-MeATP, β,γ-MeATP and UTP all concentration-dependently constricted the frog aorta, ATP and α,β-MeATP being equipotent and more potent than UTP, β,γ-MeATP or 2-MeSATP. The activity of α,β-MeATP together with the more limited activity of 2-MeSATP suggest that the receptor is a member of the P_{2X}-purinoceptor family. A P_2-purinoceptor mediating vasoconstriction has also been described in the systemic vasculature of the trout (Wood, 1977). That the receptor is of the P_{2X} subtype is supported further, since the contractile response to α,β-MeATP is inhibited by the selective P_{2X}-purinoceptor antagonist, PPADS (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1993; 1994). This receptor differs from mammalian P_{2X}-purinoceptors since it is insensitive to α,β-MeATP desensitization. It is interesting that ATP itself is not inhibited by either the nonselective P_2-purinoceptor antagonist suramin (Dunn & Blakeley, 1988; Hoyle *et al.*, 1990) or PPADS. A separate property of suramin is its ability to inhibit Ca^{2+}/Mg^{2+}-dependent ectoATPases (Hourani & Chown, 1989; Ziganshin *et al.*, 1995); this fact may account for the lack of antagonism against ATP observed in this preparation. However, contractions to ATP were inhibited by indomethacin, demonstrating that much of its action is via the production of a prostanoid. UTP also initiates contractions, although less potently than either ATP or α,β-MeATP. A separate class of purinoceptors (named the P_{2U}-purinoceptor) has been proposed where UTP ≥ ATP (von Kügelgen *et al.*, 1987; Saiag *et al.*, 1990; 1992; Ralevic & Burnstock, 1991; O'Connor, 1992). Lack of selective antagonists makes identification of a separate P_{2U} subclass difficult. Responses to UTP are not affected by indomethacin (von Kügelgen *et al.*, 1987; Ralevic & Burnstock, 1991; Vials & Burnstock, 1993b).

In conclusion, this study has shown that adenosine induces vasodilatation of the frog aorta by a novel P_1-purinoceptor that has characteristics of the A_3-receptor, the dilatation being independent of the production of NO or a prostanoid. The presence of a purinoceptor of the P_{2X} family has been demonstrated in the frog aorta: α,β-MeATP responses are antagonized by the action of suramin and PPADS, much of the effect of ATP is via the production of a prostanoid, although the receptor is novel in that it is not susceptible to desensitization by α,β-MeATP. There is no evidence for the presence of a P_{2Y}-purinoceptor mediating vasodilatation in this preparation.

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References

ABBRACCHIO, M.P. & BURNSTOCK, G. (1994). Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol. Ther.*, **64**, 445–475.

ALEXANDER, S.P.H., LOSINSKI, A., KENDALL, D.A. & HILL, S.J. (1994). A comparison of A_2 adenosine receptor-induced cyclic AMP generation in cerebral cortex and relaxation of pre-contracted aorta. *Br. J. Pharmacol.*, **111**, 185–190.

BERNE, R.M., KNABB, R.M., ELY, S.W. & RUBIO, R. (1983). Adenosine in the local regulation of blood flow: a brief overview. *FASEB J.*, **42**, 3136–3142.

BRAMICH, N.J., EDWARDS, F.R. & HIRST, G.D.S. (1990). Sympathetic nerve stimulation and applied transmitters on the sinus venosus of the toad. *J. Physiol.*, **429**, 349–375.

BRIZZOLARA, A.L. & BURNSTOCK, G. (1991). Endothelium-dependent and endothelium-independent vasodilatation of the hepatic artery of the rabbit. *Br. J. Pharmacol.*, **103**, 1206–1212.

BURNSTOCK, G. (1969). Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacol. Rev.*, **21**, 247–324.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones*: a *Multidisciplinary Approach*. ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

BURNSTOCK, G. (1987a). Local control of blood pressure by purines. *Blood Vessels*, **24**, 156–160.

BURNSTOCK, G. (1987b). Present status of purinergic neurotransmission - implication for vascular control. In *Neuronal Messengers in Vascular Function*. ed. Nobin, A., Owman, C. & Arneklo-Nobin, B. pp. 327–340. Amsterdam: Elsevier Science Publishers.

BURNSTOCK, G. (1989). Vascular control by purines with emphasis on the coronary system. *Eur. Heart J.*, **10**, 15–21.

BURNSTOCK, G. (1990a). Noradrenaline and ATP as cotransmitters in sympathetic nerves. *Neurochem. Int.*, **17**, 357–368.

BURNSTOCK, G. (1990b). Dual control of local blood flow by purines. In *Biological effects of Extracellular ATP*. ed. Dubyak, G.R. & Fedan, J.S. pp. 31–44. New York: Ann. N.Y. Acad. Sci. Vol 603.

BURNSTOCK, G. (1990c). Classification and characterisation of purinoceptors. In *Purines in Cellular Signalling - Targets for New Drugs*. ed. Jacobson, K.A., Daly, J.W. & Manganiello, V. pp. 241–253. New York: Springer Verlag.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

BURNSTOCK, G. & KENNEDY, C. (1986). Purinergic receptors in the cardiovascular system. *Prog. Pharmacol.*, **6**, 111–132.

BURNSTOCK, G. & MEGHJI. P. (1981). Distribution of P_1- and P_2-purinoceptors in the guinea-pig and frog heart. *Br. J. Pharmacol.*, **73**, 879–885.

CIABATTONI, G. & WENNMALM, A. (1985). Adenosine-induced coronary release of prostacyclin at normal and low pH in isolated heart of rabbit. *Br. J. Pharmacol.*, **85**, 557–563.

COLIN, D.A., KIRSCH, R. & LERAY, C. (1979). Haemodynamic effects of adenosine on gills of the trout (*Salmo gairdneri*). *J. Comp. Physiol.*, **130**, 325–330.

COLIN, D.A. & LERAY, C. (1979). Interaction of adenosine and its phosphorylated derivatives with putative purinergic receptors in the gill vascular bed of rainbow trout. *Pflüger's Arch.*, **383**, 35–40.

COLLIS, M.G. (1989). The vasodilator role of adenosine. *Pharmacol. Ther.*, **41**, 143–162.

COLLIS, M.G. & BROWN, C.M. (1983). Adenosine relaxes the aorta by interacting with an A_2 receptor and an intracellular site. *Eur. J. Pharmacol.*, **96**, 61–69.

CONTI, A., MONOPOLI, A., GAMBA, M., BOREA, P.A. & ONGINI, E. (1993). Effects of selective A_1 and A_2 adenosine receptor agonists on cardiovascular tissues. *Naunyn-Schmied. Arch. Pharmacol.*, **348**, 108–112.

DE MEY, J.G. & VANHOUTTE, P.M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.*, **316**, 347–355.

DONALD, F.M.S. (1985). A non-adrenergic non-cholinergic (NANC) excitatory response in frog atria. *Br. J. Pharmacol.*, **85**, 211P.

DRURY, A.N. (1936). The physiological activity of nucleic acid and its derivatives. *Physiol. Rev.*, **16**, 292–325.

DRURY, A.N. & SZENT-GYÖRGYI, A. (1929). The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J. Physiol.*, **68**, 213–237.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.*, **93**, 243–245.

EVANS, D.H. (1992). Evidence for the presence of A_1 and A_2 adenosine receptors in the ventral aorta of the dogfish shark, *Squalus acanthias*. *J. Comp. Physiol.*, **B, 162**, 179–183.

FARRELL, A.P. & DAVIE, P.S. (1991). Coronary vascular reactivity in the skate, *Raja nasuta*. *Comp. Biochem. Physiol.*, **99C**, 555–560.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.*, **46**, 143–156.

FUGLSANG, A., THERKILDSEN, P. & CRONE, C. (1989). Presynaptic modulation of sympathetic nerve transmission - an element in vasomotor control. *Int. J. Microcirc. Clin. Exp.*, **8**, 71–84.

GADDUM, J.H. & HOLTZ, P. (1933). The localization of the action of drugs on the pulmonary vessels of dogs and cats. *J. Pharmacol.*, **77**, 139–158.

GALLO-RODRIGUEZ, C ., JI, X., MELMAN, N., SIEGMAN, B.D., SANDERS, L.H., ORLINA, J., FISCHER, B., PU, Q., OLAH, M.E., VAN GALEN, J.M., STILES, G.L. & JACOBSON, K.A. (1994). Structure-activity relationships of N^6-benzyladenosine-5'-uronamides as A_3-selective adenosine agonists. *J. Med. Chem.*, **37**, 636–646.

GORDON, J.L. (1986). Extracellular ATP: effects, sources and fate. *Biochem. J.*, **233**, 309–319.

GREEN, H.N. & STONER, H.B. (1950). The effect of purine derivatives on the cardiovascular system. In *Biological Actions of the Adenine Nucleotides*. pp. 65–103. London: H.K. Lewis and Co.

HOURANI, S.M.O. & CHOWN, J.A. (1989). The effects of some possible inhibitors of ectonucleotidases on the breakdown and pharmacological effects of ATP in the guinea-pig urinary bladder. *Gen. Pharmacol.*, **20**, 413–416.

HOUSTON, D.A., BURNSTOCK, G. & VANHOUTTE, P.M. (1987). Different P_2-purinergic receptor subtypes of endothelium and smooth muscle in canine blood vessels. *J. Pharmacol. Exp. Ther.*, **241**, 501–506.

HOYLE, C.H.V. (1992). Transmission: Purines. In *Autonomic Neuroeffector Mechanisms*. ed. Burnstock, G. & Hoyle, C.H.V. pp. 367–407. Chur: Harwood Academic Publishers.

HOYLE, C.H.V. & BURNSTOCK, G. (1986). Evidence that ATP is a neurotransmitter in the frog heart. *Eur. J. Pharmacol.*, **124**, 285–289.

HOYLE, C.H.V. & BURNSTOCK, G. (1991a). ATP receptors and their physiological roles. In *Adenosine in the Nervous System*. ed. Stone, T.W. pp. 43–76. London: Academic Press.

HOYLE, C.H.V. & BURNSTOCK, G. (1991b). Purinergic receptors. In *Receptor Data for Biological Experiments*. ed. Doods, H.N. & Van Meel, J.C.A. pp. 54–61. Chichester: Ellis Horwood.

HOYLE, C.H.V., KNIGHT, G.E. & BURNSTOCK, G. (1990). Suramin antagonizes responses to P_2-purinoceptor antagonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia-coli. *Br. J. Pharmacol.*, **99**, 617–621.

HUTCHISON, A.J., WEBB, O.L., OEI, H.H., GHAI, G.R., ZIMMERMAN, M.B. & WILLIAMS, M. (1989). CGS 21680C, an A_2 selective adenosine receptor agonist with preferential hypotensive activity. *J. Pharmacol. Exp. Ther.*, **251**, 47–55.

JACOBSON, K.A., NIKODIJEVIC, O, SHI, D., GALLO-RODRIGUEZ, C., OLAH, M.E. STILES, G.L. & DALY, J.W. (1993). A role for central A_3-adenosine receptors. Mediation of behavioural depressant effects. *FEBS Lett.*, **336**, 57–60.

LAMBRECHT, G., FRIEBE, T., GRIMM, U., WINDSCHEIF, U., BUNGARDT, E., HILDEBRANDT, C., BÄUMERT, H.G., SPATZ-KÜMBEL, G. & MUTSCHLER, E. (1992). PPADS, a novel functionally selective antagonist of P_2-purinoceptor-mediated responses. *Eur. J. Pharmacol.*, **217**, 217–219.

LAZOU, A. & BEIS, I. (1987). Effects of adenosine perfusion on the metabolism and contractile activity of *Rana ridibunda* heart. *Comp. Biochem. Physiol.*, **86C**, 415–419.

LOHSE, M.J., KLOTZ, K.N., SCHWABE, U., CRISTALLI, G., VITTORI, S. & GRIFANTINI, M. (1988). 2-Chloro N^6-cyclopentyladenosine: a highly selective agonist at A_1 adenosine receptors. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 687–689.

LONDOS, C., COOPER, D.M.F. & WOLFF, J. (1980). Subclasses of external adenosine receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2551–2554.

LONDOS, C. & WOLFF, J. (1977). Two distinct adenosine-sensitive sites on adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 5482–5486.

MATHIESON, J.J.I. & BURNSTOCK, G. (1985). Purine-mediated relaxation and constriction of isolated rabbit mesenteric artery are not endothelium-dependent. *Eur. J. Pharmacol.*, **118**, 221–229.

MEGHJI, P. & BURNSTOCK, G. (1983a). The effects of adenyl compounds on the heart of the axolotl (*Ambystoma mexicanum*). *Comp. Biochem. Physiol.*, **76C**, 319–326.

MEGHJI, P. & BURNSTOCK, G. (1983b). An unusual excitatory action of adenosine on the ventricular muscle of the south African clawed toad (*Xenopus laevis*). *Eur. J. Pharmacol.*, **89**, 251–258.

MEGHJI, P. & BURNSTOCK, G. (1984). The effect of adenyl compounds on the heart of the dogfish, *Scyliorhinus canicula*. *Comp. Biochem. Physiol.*, **77C**, 295–300.

MEYERHOF, W., MÜLLER-BRECHLIN, R. & RICHTER, D. (1991). Molecular cloning of a novel putative G-protein coupled receptor expressed during rat spermogenesis. *FEBS Lett.*, **284**, 155–160.

MONCADA, S. & VANE, J.R. (1979). Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol. Rev.*, **30**, 293–331.

NAGANO, O., FÖLDES, F.F., NAKATSUKA, H., REICH, D., OHTA, Y., SPERLAGH, B. & VIZI, E.S. (1992). Presynaptic A_1-purinoceptor-mediated inhibitory effects of adenosine and its stable analogues on the mouse hemidiaphragm preparation. *Naunyn-Schmied. Arch. Pharmacol.*, **346**, 197–202.

O'CONNOR, S.E. (1992). Recent developments in the classification and functional significance of receptors for ATP and UTP, evidence for nucleotide receptors. *Life Sci.*, **50**, 1657–1664.

OJEWOLE, J.A.O. (1983). Analysis of the responses of rainbow lizard (*Agama agama* Linn.) isolated portal vein to electrical stimulation and to drugs. *Methods Find. Expt. Clin. Pharmacol.*, **5**, 673–684.

OLSSON, R.A. & PEARSON, J.D. (1990). Cardiovascular purinoceptors. *Physiol. Rev.*, **70**, 761–845.

PEARSON, J.D. & GORDON, J.L. (1989). P_2 Purinoceptors in the blood vessel wall. *Biochem. Pharmacol.*, **38**, 4157–4163.

RALEVIC, V. & BURNSTOCK, G. (1991). Roles of P_2-purinoceptors in the cardiovascular system. *Circulation*, **84**, 1–14.

REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

RICHARDS, F.A. (1934). The effect of adenylic acid and adenosine on the human heart and blood vessels. *J. Physiol.*, **81**, 10P.

SAIAG, B., MILON, D., ALLAIN, H., RAULT, B. & VAN DEN DRIESSCHE, J. (1990). Constriction of the smooth muscle of rat tail and femoral arteries and dog saphenous vein is induced by uridine triphosphate via 'pyrimidinoceptors', and by adenosine triphosphate via P_{2X}-purinoceptors. *Blood Vessels*, **27**, 352–364.

SAIAG, B., MILON, D., SHACOORI, V., ALLAIN, H., RAULT, B. & VAN DEN DRIESSCHE, J. (1992). Newly evidenced pyrimidinoceptors and the P_{2X}-purinoceptors are present on the vascular smooth muscle and respectively mediate the UTP- and ATP-induced contractions of the dog maxillary internal vein. *Res. Commun. Chem. Path. Pharmacol.*, **76**, 89–95.

SMALL, S.A., MACDONALD, C. & FARRELL, A.P. (1990). Vascular reactivity of the coronary artery in rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol.*, **258**, R1402–R1410.

STONE, T.W. (1991). Receptors for adenosine and adenine nucleotides. *Gen. Pharmacol.*, **22**, 25–31.

SU, C. (1981). Purinergic Receptors in Blood Vessels. In *Purinergic Receptors*. ed. Burnstock, G. pp. 95–117. London: Chapman & Hall.

SU, C. (1985). Extracellular functions of nucleotides in heart and blood vessels. *Annu. Rev. Physiol.*, **47**, 665–676.

VAN CALKER, D., MÜLLER, M. & HAMPRECHT, B. (1979). Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.*, **33**, 999–1005.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature*, **231**, 232–235.

VERHAEGHE, R.H., VANHOUTTE, P.M. & SHEPHERD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circ. Res.*, **40**, 208–215.

VIALS, A.J. & BURNSTOCK, G. (1993a). A_2-purinoceptor-mediated relaxation in the guinea-pig coronary vasculature: a role for nitric oxide. *Br. J. Pharmacol.*, **109**, 424–429.

VIALS, A.J. & BURNSTOCK, G. (1993b). Effects of pyrimidines on the guinea-pig coronary vasculature. *Br. J. Pharmacol.*, **110**, 1091–1097.

VON KÜGELGEN, I., HÄUSSINGER, D. & STARKE, K. (1987). Evidence for a vasoconstriction-mediating receptor for UTP, distinct from the P_2 purinoceptor, in rabbit ear artery. *Naunyn Schmied. Arch. Pharmacol.*, **336**, 556–560.

WOOD, C.M. (1977). Cholinergic mechanisms and the response to ATP in the systemic vasculature of the rainbow trout. *J. Comp. Physiol. B*, **122**, 325–345.

ZHOU, Q.Y., LI, C.Y., OLAH, M.E., JOHNSON, R.A., STILES, G.L. & CIVELLI, O. (1992). Molecular cloning and characterization of an adenosine receptor - the A_3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 7432–7436.

ZIGANSHIN, A.U., ZIGANSHINA, L.E., KING, B.F. & BURNSTOCK, G. (1995). Characteristics of ecto-ATPase of *Xenopus* oocytes and inhibitory actions of suramin on ATP breakdown. *Pflügers Arch.*, **429**, 412–418.

ZIGANSHIN, A.U., HOYLE, C.H.V., BO, X., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G. & BURNSTOCK, G. (1993). PPADS selectivly antagonizes P_{2X}-purinoceptor-mediated responses in the rabbit urinary bladder. *Br. J. Pharmacol.*, **110**, 1491–1496.

ZIGANSHIN, A.U., HOYLE, C.H.V., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G. & BURNSTOCK, G. (1994). Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.*, **111**, 923–929.

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