*Br. J. Pharmacol.* (1991), **102**, 645–650 

# The effects of adenosine triphosphate (ATP) and related purines on human isolated subcutaneous and omental resistance arteries

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**1** Human resistance arteries were obtained from specimens of omentum and subcutaneous fat removed at surgery. They were studied *in vitro* by use of a myograph technique to determine the effects of purines on the arteries.

**2** In preparations where tone had been raised with noradrenaline, low concentrations (1 nM–1 $\mu$M) of adenosine triphosphate (ATP) and 2-methylthioATP, but not $\alpha,\beta$-methyleneATP, produced concentration-dependent relaxation. There was a lack of relationship between the relaxation response to acetylcholine and that to ATP.

**3** In preparations under basal tone, high concentrations (1 $\mu$M–1 mM) of ATP, 2-methylthioATP and $\alpha,\beta$-methyleneATP produced concentration-dependent contractions.

**4** The rank order of potency of the purine nucleotide analogues for the relaxation response was 2-methylthioATP > ATP > $\alpha,\beta$-methyleneATP and for the contractile response it was $\alpha,\beta$-methyleneATP > ATP = 2-methylthioATP.

**5** Adenosine produced concentration-dependent relaxation in preparations under raised tone and was less potent than ATP but did not produce contraction in preparations at basal tone. Relaxation responses to adenosine, but not to ATP, were antagonized by 8-phenyltheophylline.

**6** These results indicate the presence of vasodilator $P_{2y}$- and $P_1$-purinoceptors and vasoconstrictor $P_{2x}$-purinoceptors on human resistance arteries isolated from omental and subcutaneous sites.

## Introduction

Purines have been shown to exert a variety of receptor-mediated effects in a number of tissues (Williams, 1987). The receptors through which these effects are manifest have been classified into $P_1$- and $P_2$-purinoceptors. These are distinguished on the basis of several criteria, including a different rank order agonist potency. At the $P_1$-purinoceptor, adenosine is more potent than adenosine 5'-triphosphate (ATP), whereas at the $P_2$-purinoceptor the reverse is true. Furthermore, the $P_1$-purinoceptor is susceptible to blockade by methylxanthines such as 8-phenyltheophylline whilst the $P_2$-purinoceptor is resistant to these antagonists (Burnstock, 1978). More recently it has become apparent that the $P_2$-purinoceptor population is not homogeneous. This has led to the the proposed subdivision of the $P_2$-purinoceptor into $P_{2x}$ and $P_{2y}$ subtypes which can be distinguished by the rank order of potency of ATP and its structural analogues. A rank order potency of $\alpha,\beta$-methyleneATP ($\alpha,\beta$-MeATP) > ATP = 2-methylthioATP (2-Me.S.ATP) is characteristic of the $P_{2x}$ purinoceptor subtype, whilst a rank order potency of 2-Me.S.ATP > ATP > $\alpha,\beta$-MeATP is characteristic of the $P_{2y}$ subtype (Burnstock & Kennedy, 1985).

In the vasculature, effects mediated through the $P_1$-purinoceptor and both $P_2$-purinoceptor subtypes have been demonstrated in a variety of preparations from several species (Alexander & Eyre, 1985; Burnstock & Kennedy, 1986; Houston *et al.*, 1987). However, in man, although the cardiovascular effects of adenosine are relatively well known (Sollevi, 1986; McCormack *et al.*, 1989), little is known about the effects of ATP or its analogues. Furthermore, even in animal tissues, most of the *in vitro* work on purinoceptors has been done on relatively large arteries that are unlikely to contribute significantly to peripheral vascular resistance (Mulvany, 1987). We have therefore determined the actions of the endogenous purines, adenosine and ATP, in human isolated resistance arteries and have begun to characterize the receptor subtypes involved using ATP analogues. A preliminary account of this work has previously been presented to the British Pharmacological Society (Martin *et al.*, 1988).

## Methods

Arteries were obtained from omentum and subcutaneous fat, removed at surgery from 113 patients (aged 17–89 years, median 56 years) and collected into modified Krebs buffer of composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, glucose 11.1, $NaH_2PO_4$ 1.0, $NaHCO_3$ 25, $CaCl_2$ 2.5 and $Na_2EDTA$ 0.03. Artery segments (internal diameter 98–708 $\mu$m, median 234 $\mu$m) were dissected free of surrounding tissue and mounted on two 40 $\mu$m wires in a myograph for the measurement of isometric tension (Mulvany & Halpern, 1977). The myograph chamber contained 10 ml of Krebs buffer maintained at 37°C and aerated with 95% $O_2$:5% $CO_2$. The arteries were allowed to equilibrate for at least 1 h and then set to a normalised resting internal circumference equal to 0.9 × L100, where L100 is the internal circumference producing a wall tension equivalent to that produced by a distending wall pressure of 100 mmHg, calculated from the Laplace relationship as described by Mulvany & Halpern (1977). Under these conditions, the arteries generated contractile responses to potassium-induced depolarization that were near to maximal (unpublished data).

Following a further 30 min equilibration, the arteries were exposed to a potassium depolarizing solution (KDS) composed of Krebs buffer in which the NaCl and KCl concentrations were reversed. Arteries that did not generate a tension equivalent to 90 mmHg (calculated by Laplace's relationship) were discarded. After washout and recovery, the arteries were exposed to noradrenaline (10 $\mu$M), repeat KDS and noradrenaline (1 $\mu$M) with washout and recovery between each. When stable tone had been induced by noradrenaline (1 $\mu$M) the functional integrity of the endothelium was assessed by the addition of acetylcholine (1 $\mu$M) (Thom *et al.*, 1987). The resultant relaxation was taken as indicative of the release of endothelium derived relaxing factor (EDRF) and the presence of a functional endothelium. In initial experiments, the arteries were preconstricted with noradrenaline (1 $\mu$M) and when stable

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tone had been induced, ATP (1 nM–1 mM) or adenosine (1 nM–1 mM) were added cumulatively. In subsequent experiments, the effects of shorter purine concentration-ranges were investigated with and without preconstriction. Concentration-response curves to ATP (1 nM–1 μM), 2-Me.S.ATP (100 pM–1 μM), α,β-MeATP (100 pM–10 nM), and adenosine (10 nM–10 μM) were generated following preconstriction with noradrenaline (1 μM) by cumulative addition of the purine. Concentration-response curves to ATP (1 μM–1 mM), 2-Me.S.ATP (10 nM–1 mM), α,β-MeATP (10 nM–1 mM) and adenosine (1 μM–1 mM) were generated without preconstriction by both cumulative addition of the purine and by the preparation of composite concentration-response curves. For the preparation of composite concentration-response curves, each artery segment was exposed to a single concentration of purine and the resultant responses of several segments exposed to different purine concentrations were grouped together. In this way, each artery segment was exposed to an exogenously applied purine once only and the problem of tachyphylaxis was avoided.

In some experiments, propranolol (4 μM), indomethacin (10 μM), phentolamine (10 μM) or 8-phenyltheophylline (10 μM) was added to the myograph chamber, 30 min before the first purine dose. Concentration-response data from individual artery segments were fitted to a logistic function by a computer programme (Barlow, 1983) and values for $EC_{50}$, namely the concentration of purine producing 50% of the maximum response to that purine, and maximum responses were derived. Results obtained following preconstriction are expressed as percentage reduction in preconstricted tone and those obtained without preconstriction as a percentage of the KDS response. $EC_{50}$ and maximum responses are expressed as mean ± s.e.mean.

### *Drugs*

Acetylcholine chloride (Sigma), adenosine (Sigma), adenosine 5'-triphosphate disodium salt (Sigma), indomethacin (Sigma), α,β-methyleneadenosine 5'-triphosphate lithium salt (Sigma), 2-methylthioadenosine 5'-triphosphate sodium salt (Research Biochemicals Inc.), noradrenaline bitartrate (Sigma), phentolamine mesylate (Ciba Geigy), 8-phenyltheophylline (Sigma) and (±)-propranolol hydrochloride (Sigma) were used. Indomethacin and 8-phenyltheophylline stock solutions were made up in 80% v/v methanol containing 0.2 mM NaOH and dilutions made in distilled water. All other drugs were dissolved and diluted in Krebs buffer.

## Results

Following preconstriction with noradrenaline, the addition of ATP typically produced concentration-dependent relaxation at low concentrations. When the concentration of ATP reached about 1 μM, the effect of ATP reversed and transient concentration-dependent contraction occurred. In contrast, adenosine produced concentration-dependent relaxation which was not reversed at high concentrations (Figure 1).

After preconstriction, both subcutaneous and omental arteries relaxed similarly in response to low concentrations of ATP (1 nM–1 μM) with a maximum response of 83% of the preconstricted tone and $EC_{50}$ of 52 nM (Figure 2, inset). This response did not show tachyphylaxis. 2-Me.S.ATP (100 pM–1 μM) also produced relaxations in preconstricted arteries and was more potent than ATP, having an $EC_{50}$ of 12 nM (Figure 2). However, α,β-MeATP (100 pM–10 nM) failed to relax these arteries reproducibly. Arteries that relaxed well to ATP (100 nM) were not necessarily those that relaxed well to acetylcholine (1 μM) (Figure 3). This lack of relationship was evident in both omental and subcutaneous arteries. When added after noradrenaline, adenosine (10 nM–10 μM) also produced a concentration-dependent relaxation with a maximum response of 87% of the preconstricted tone and $EC_{50}$ of 6.2 μM. Adenosine relaxation showed a similar lack of relationship to acetylcholine relaxation as did ATP. Neither the ATP nor the adenosine relaxant response were significantly altered by indomethacin (10 μM) or propranolol (4 μM) whilst 8-phenyltheophylline (10 μM) competitively antagonized the adenosine response, but not the ATP response (Figure 4).

Both omental and subcutaneous arteries contracted similarly in response to high concentrations of ATP (1 μM–1 mM) (Figure 5, inset). This contraction required the presence of extracellular calcium, was poorly sustained and showed marked tachyphylaxis. Exposure of the arteries to ATP (1 mM) greatly reduced subsequent contractile responses to ATP and prior exposure to α,β-MeATP (3 μM) abolished contractile responses to subsequent exposure to ATP. Taking the transient maximum tension developed in response to ATP as a

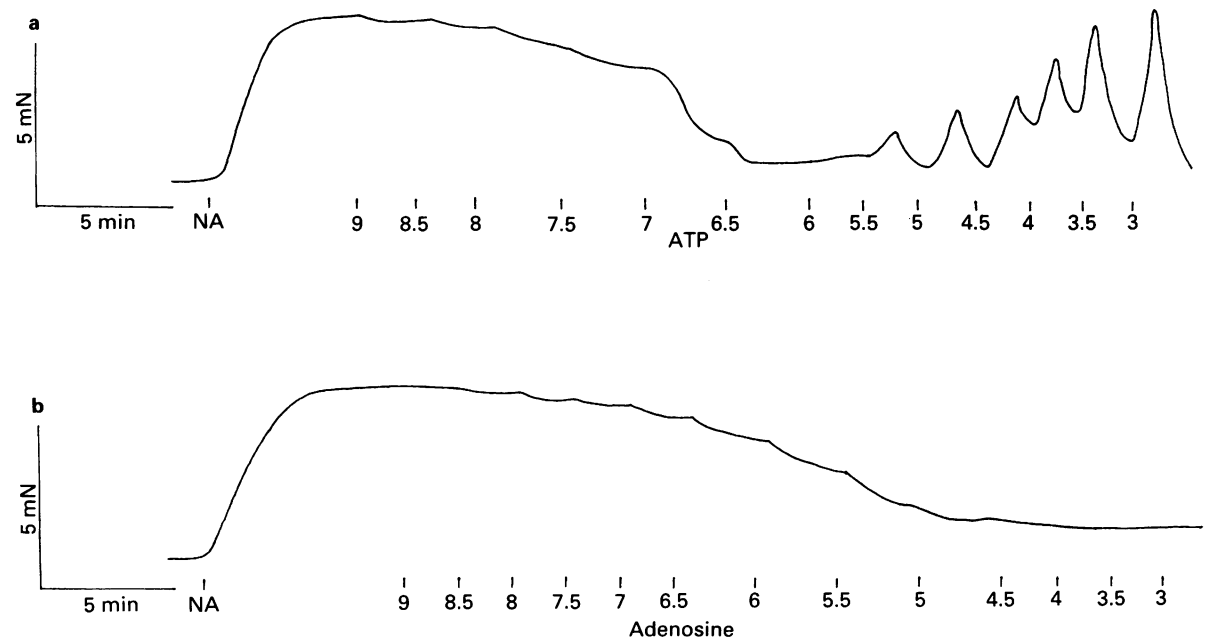


**Figure 1** Representative traces showing the effects of ATP and adenosine on human isolated resistance arteries. (a) Omental artery with an internal diameter of 229 μm. (b) Omental artery with an internal diameter of 255 μm. Noradrenaline (NA; 1 μM), ATP and adenosine were added at the points shown. Concentrations are shown as −log molar concentrations.

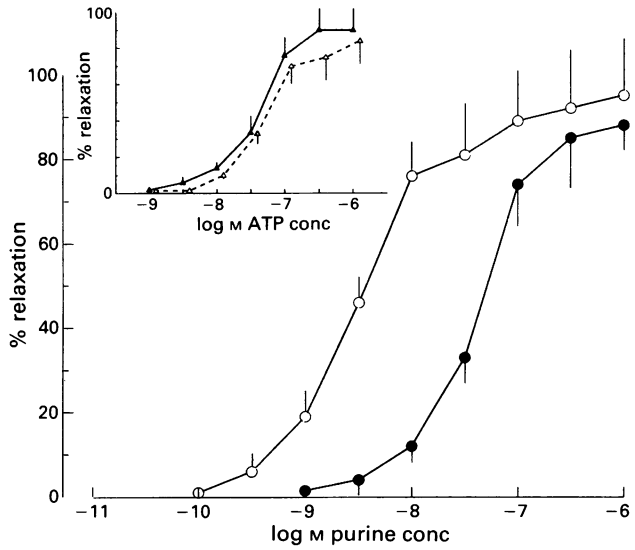


**Figure 2** Concentration-response curves (CRC) for ATP (●) ($n = 20$) and 2-methylthioATP (○) ($n = 8$) in pooled human resistance arteries. Inset: CRC for ATP in omental arteries (▲) ($n = 17$) and subcutaneous arteries (△, broken line) ($n = 12$). (Points represent the mean % relaxation of NA-induced tone with s.e.mean shown by vertical lines).

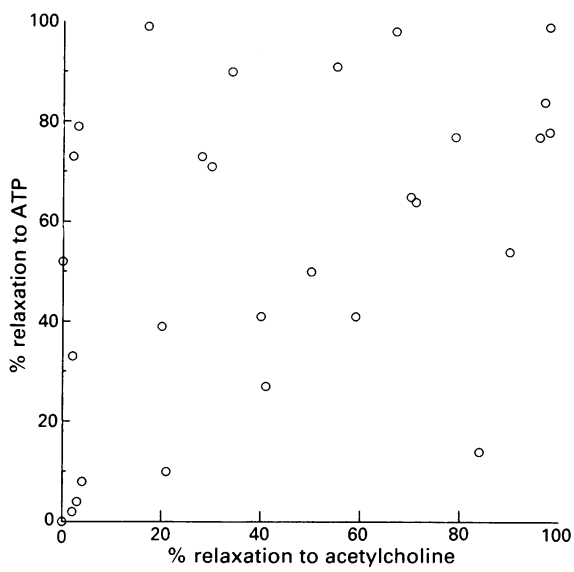


**Figure 3** Percentage relaxation of pooled human resistance arteries to ATP (100 nM) against % relaxation of the same arteries to acetylcholine (1 $\mu$M) ($n = 29$). Points represent the % relaxation of NA-induced tone. Determinations for acetylcholine were followed immediately by washout, re-constriction and determination of ATP responses.

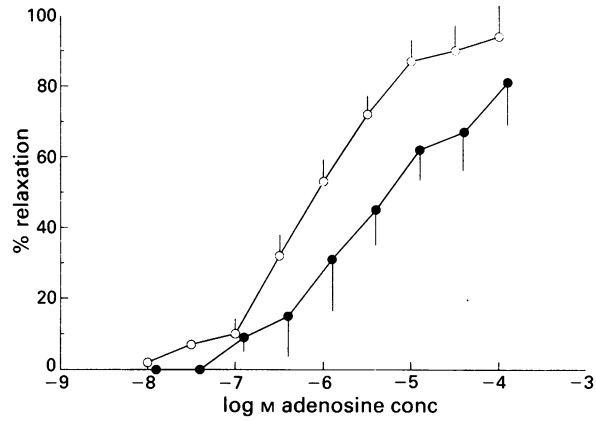


**Figure 4** Concentration-response curves for adenosine in the absence (○) ($n = 10$) and presence of 10 $\mu$M 8-phenyltheophylline (●) ($n = 6$) in pooled human resistance arteries. Points represent the mean % relaxation of NA-induced tone with s.e.mean shown by vertical lines. ANOVA, $P < 0.05$.

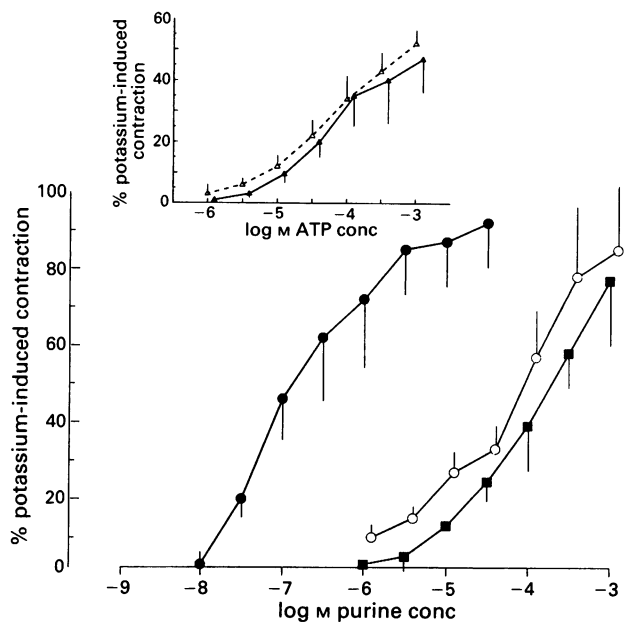


**Figure 5** Concentration-response curves (CRC) to high concentrations of ATP (■) ($n = 21$, 3 each dose), $\alpha,\beta$-methyleneATP (●) ($n = 24$, 3–5 each dose) and 2-methylthioATP (○) ($n = 21$, 3 each dose) in pooled human resistance arteries determined by a composite method. Points are percentages of the KDS-induced contraction determined in the same artery and are shown as means with s.e.mean shown by vertical lines. Inset: CRC to high concentrations of ATP in omental arteries (△, broken line) ($n = 14$) and subcutaneous arteries (▲) ($n = 11$). Points represent % of the KDS-induced contraction determined in the same artery and are shown as means ± s.e.mean.

measure of the maximum response, this was 55% of the KDS response. The maximum response was increased to 82% when the composite method was used but the $EC_{50}$ was unaltered, being 72 $\mu$M and 74 $\mu$M by the cumulative and composite methods respectively (Figure 6).

$\alpha,\beta$-MeATP (10 nM–1 mM) also produced contractions and was more potent ($EC_{50}$ 0.12 $\mu$M) than ATP, whilst 2-Me.S.ATP (10 nM–1 mM) was approximately equipotent ($EC_{50}$ 60 $\mu$M) with ATP in this respect (Figure 5). The contractile responses to ATP were unaffected by indomethacin (10 $\mu$M), 8-phenyltheophylline (10 $\mu$M) or phentolamine (10 $\mu$M). When added alone, adenosine (1 $\mu$M–1 mM) did not produce contractions.

## Discussion

The greater potency of 2-Me.S.ATP in comparison with $\alpha,\beta$-MeATP regarding relaxation of noradrenaline preconstricted human resistance arteries, suggests an action at the $P_{2y}$-purinoceptor. Unlike the response to ATP, the relaxation response to adenosine was antagonized by the $P_1$-purinoceptor antagonist, 8-phenyltheophylline (Griffith *et al.*, 1981), suggesting an action of adenosine at the $P_1$-purinoreceptor. Low concentrations of ATP (less than 1 $\mu$M) produced relaxant responses but higher concentrations of adenosine were required to produce a comparable effect.

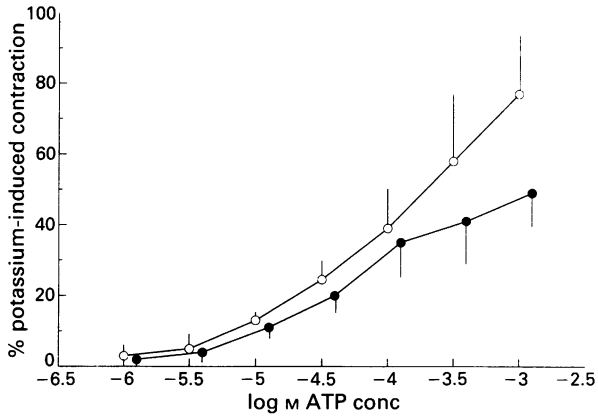


**Figure 6** Concentration-response curves to high concentrations of ATP in pooled human resistance arteries determined by a cumulative method (●) ($n = 25$) and a composite method (○) ($n = 21$, 3 each dose). Points represent % of the KDS-induced contraction determined in the same artery, and are shown as means with s.e.mean shown by vertical lines.

This greater potency of ATP, together with the insensitivity to methylxanthine blockade, suggests that in these arteries *in vitro*, relaxant responses to ATP do not occur solely following the hydrolysis of ATP to adenosine. This might have been expected due to the nucleotidase enzymes present in vascular endothelial cells (Pearson & Gordon, 1985). This alone does not necessarily exclude the possibility that the effects of ATP are partially mediated via the $P_1$-purinoceptor as in the guinea-pig atrium (Moody *et al.*, 1984) and rat coronary vasculature (Hopwood & Burnstock, 1987). Nevertheless, the potent effect of the slowly degradable analogue, 2-Me.S.ATP, provides strong evidence for a substantial $P_{2y}$-purinoceptor-mediated component to the relaxant response and the lack of effect of 8-phenyltheophylline suggests that any $P_1$-purinoceptor-mediated component must be small.

In a variety of arteries, including the rat mesenteric bed (Ralevic & Burnstock, 1988), dog coronary artery (Houston *et al.*, 1987), pig aorta (Martin *et al.*, 1985) and rat femoral artery (Kennedy *et al.*, 1985), $P_2$-purinoceptors mediating relaxation to ATP and its analogues, with orders of agonist potency similar to that determined in this study, have been located on the vascular endothelium and are presumed to produce relaxation via the release of endothelium-derived relaxing factor (EDRF) (Furchgott, 1983). Cautious extrapolation of these animal data may suggest that the $P_{2y}$-purinoceptor-mediated responses of the human arteries used in this study are also mediated via the release of EDRF. However, there was no evidence that arteries with a functional endothelium relaxed any better to ATP than those lacking a functional endothelium. ATP may be inducing relaxation in an endothelium-independent manner (Mathieson & Burnstock, 1985; Burnstock, 1987). No deliberate attempt was made to remove the endothelium, but some damage was inevitable during the dissection and mounting of the arteries. Previous attempts in our hands to disrupt the endothelium deliberately by passing a third 40 $\mu$m wire through the lumen of mounted arteries have given variable results. Reversible loss has been shown to occur in mouse cerebral microvessels exposed to endothelial damage by a laser (Rosenblum, 1988). White & Angus (1987) have also expressed difficulty in reliably removing the endothelium from small calibre arteries, but Osol *et al.* (1989) have described a successful method.

Adenosine also produced relaxation, following preconstriction with noradrenaline, by an essentially endothelium-independent mechanism, as determined by the same criteria used for the ATP-induced relaxations. This observation is consistent with the proposed smooth muscle location of the post-junctional $P_1$-purinoceptor determined in a variety of vascular preparations (Mathieson & Burnstock, 1985; Su, 1985a; McCormack *et al.*, 1989). When added to arteries at basal tone, ATP had no effect at low concentrations, but produced contraction at high concentrations (greater than 1 $\mu$M). These were characteristically poorly sustained and, when cumulative concentration-response curves were constructed, necessitated the addition of incremental doses of agonist at arbitrary 2 min intervals. Once a full concentration-response curve to ATP had been prepared and the myograph chamber washed-out, further addition of ATP either failed to elicit a response or produced only a greatly diminished response. This tachyphylaxis was even more pronounced following exposure to $\alpha,\beta$-MeATP and, together with the order of agonist potencies and insensitivity to 8-phenyltheophylline, suggests an action at the $P_{2x}$-purinoceptor (Burnstock & Kennedy, 1985).

The tachyphylaxis of the contractile response to ATP may invalidate the cumulative concentration-response curves. Composite concentration-response curves were therefore prepared in order to avoid this problem. The maximum response to ATP obtained by the composite method was increased compared with that obtained by the cumulative method. This suggests that tachyphylaxis to the ATP contractile response can occur within 12 min (6 × 2 min dose intervals). It is consequently of note that the $EC_{50}$ values obtained by the two methods were similar. In contrast to ATP, adenosine consistently failed to produce contraction. This is apparent in the majority of arteries described (Burnstock, 1978), except some renal arteries (Williams, 1987). Ramagopal *et al.* (1988), using human epicardial coronary arteries, reported only relaxant responses to adenosine. On the other hand adenosine has been shown to contract strips of the guinea-pig pulmonary artery, and to enhance the contractile response of this tissue to transmural electrical stimulation and applied noradrenaline probably by a postjunctional mechanism (Wiklund *et al.*, 1987).

Purines are stored in endothelial cells, platelets and sympathetic nerve endings and can be released in response to physiological stimuli as well as ischaemia and injury (Pearson & Gordon, 1985; Born & Krater, 1985; Jennings & Steenbergen, 1985; Gordon, 1986). Despite rapid degradation of ATP to adenosine and re-uptake of adenosine into the cells, plasma purine levels, particularly levels of ATP, are likely to exceed those required to initiate $P_{2y}$-purinoceptor-mediated vasodilatation (Su, 1985b). ATP is released, with noradrenaline, from perivascular sympathetic nerves in a variety of tissues (Kugelgen & Starke, 1985; Kennedy *et al.*, 1986; Muramatsu & Kigoshi, 1987; Machaly *et al.*, 1988) and local concentrations may reach levels sufficient to initiate $P_{2x}$-purinoceptor-mediated effects. The rate of adenosine outflow from canine subcutaneous adipose tissue increases following nerve stimulation (Fredholm & Sollevi, 1981) and, in addition, adenosine and the $P_1$-purinoceptor antagonist, theophylline, affect responses to nerve stimulation in this tissue (Sollevi & Fredholm, 1983).

This study has identified vasodilator $P_{2y}$- and $P_1$-purinoceptors and vasoconstrictor $P_{2x}$-purinoceptors in human isolated arteries small enough to contribute significantly to peripheral vascular resistance (Mulvany, 1987). These receptors have similarly been demonstrated in a variety of animal studies (Burnstock & Kennedy, 1986; Williams, 1987; Hourani *et al.*, 1988). These data suggest that purines may play a significant role in human vasoregulation. Other studies of the effects of purines on resistance calibre arteries have almost entirely been restricted to those using perfused animal tissue preparations (Taylor *et al.*, 1989). As in the present study, Ralevic & Burnstock (1988) were unable to produce a vasodilator response to $\alpha,\beta$-MeATP in the perfused rat mesentery and, due to the weak vasodilator action of adenosine in this preparation, concluded that the breakdown of ATP to adenosine does not contribute significantly to the vasodilator action of ATP in this preparation. In a study using isolated myograph mounted arteries, White & Angus (1987) demonstrated relaxant effects of ATP and adenosine in

small as well as large dog coronary arteries. Liu *et al.* (1989) have shown preliminary evidence for the presence of $P_{2y}$- and $P_{2x}$-purinoceptors in human small pulmonary arteries. Hardebo *et al.* (1987) have demonstrated the presence of both $P_1$ and $P_2$-purinoceptors in human pial arteries of about 300–600 $\mu$m in diameter, using arteries from 11 patients. These authors observed a $P_1$-purinoceptor mediated relaxant response to adenosine with an $EC_{50}$ of 1.8 $\mu$M and maximum response of 73%, values similar to those obtained in the present study. They also identified a $P_{2y}$-like relaxant response, but no contractile response, to ATP. These data complement the present study in providing evidence for the widespread distribution of several purinoceptor subtypes in the human resistance vasculature.

In conclusion, the results of this study demonstrate the presence of both $P_{2x}$-purinoceptors mediating contraction and $P_{2y}$-purinoceptors mediating relaxation of human resistance arteries. The greater potency of $\alpha,\beta$-MeATP compared to ATP and 2-Me.S.ATP for the contraction response suggests an action at the $P_{2x}$-purinoceptor and the greater potency of 2-Me.S.ATP compared to ATP and $\alpha,\beta$-MeATP for the relaxant response suggests an action at the $P_{2y}$-purinoceptor. Relaxation produced by adenosine, but not ATP, was antagonized by 8-phenyltheophylline suggestive of a role for $P_1$-purinoceptors in these arteries. There is also evidence that the relaxant responses to ATP and adenosine are, at least in part, independent of endothelium in human resistance arteries.

We would like to thank surgical colleagues at St. Mary's Hospital for assistance with these studies. This work was supported by the British Heart Foundation.

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*(Received July 19, 1990*
*Revised October 30, 1990*
*Accepted November 8, 1990)*