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Pharmacological profile of a novel cyclic AMP-linked P2 receptor on undifferentiated HL-60 leukemia cells

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1 Extracellular ATP ($EC_{50} = 146 \pm 57$ μM) and various ATP analogues activated cyclic AMP production in undifferentiated HL-60 cells.

2 The order of agonist potency was: ATPγS (adenosine 5′-O-[3-thiotriphosphate]) $\geqslant$ BzATP (2′&3′O-(4-benzoylbenzoyl)-adenosine-5′-triphosphate) $\geqslant$ dATP > ATP. The following agonists (in order of effectiveness at 1 mM) were all less effective than ATP at concentrations up to 1 mM: β,γ methylene ATP $\geqslant$ 2-methylthioATP > ADP $\geqslant$ Ap4A (P^1, P^4-di(adenosine-5′) tetraphosphate) $\geqslant$ Adenosine > UTP. The poor response to UTP indicates that $P2Y_2$ receptors are not responsible for ATP-dependent activation of adenylyl cyclase.

3 Several thiophosphorylated analogs of ATP were more potent activators of cyclic AMP production than ATP. Of these, ATPγS ($EC_{50} = 30.4 \pm 6.9$ μM) was a full agonist. However, adenosine 5′-O-[1-thiotriphosphate] (ATPαS; $EC_{50} = 45 \pm 15$ μM) and adenosine 5′-O-[2-thiodiphosphate] (ADPβS; $EC_{50} = 33.3 \pm 5.0$ μM) were partial agonists.

4 ADPβS ($IC_{50} = 146 \pm 32$ μM) and adenosine 5′-O-thiomonophosphate (AMPS; $IC_{50} = 343 \pm 142$ μM) inhibited cyclic AMP production by a submaximal concentration of ATP (100 μM). Consistent with its partial agonist activity, ADPβS was estimated to maximally suppress ATP-induced cyclic AMP production by about 65%. AMPS has not been previously reported to inhibit P2 receptors.

5 The broad spectrum P2 receptor antagonist, suramin (500 μM), abolished ATP-stimulated cyclic AMP production by HL-60 cells but the adenosine receptor antagonists xanthine amine congener (XAC; 20 μM) and 8-sulpho-phenyltheophylline (8-SPT; 100 μM) were without effect.

6 Extracellular ATP also activated protein kinase A (PK-A) consistent with previous findings that PK-A activation is involved in ATP-induced differentiation of HL-60 cells (Jiang *et al*., 1997).

7 Taken together, the data indicate the presence of a novel cyclic AMP-linked P2 receptor on undifferentiated HL-60 cells.

Keywords: P2 receptor; HL-60 cell; cyclic AMP

Introduction

Receptors for extracellular ATP fall into two main classes, the P2X series of ionotropic receptors and the P2Y series of metabotropic receptors (for reviews: Fredholm *et al*., 1994; Boarder *et al*., 1995; Harden *et al*., 1995). The metabotropic receptors cloned to date all belong to the seven transmembrane domain family of G-protein coupled receptors and are, in general, Ca^{2+}-mobilizing because they are coupled to the activation of phospholipase C. They differ pharmacologically in their selectivity for ATP analogues. Some receptors, such as $P2Y_2$ (previously P_{2U}), $P2Y_4$ and $P2Y_6$ receptors, also exhibit selectivity for uridine nucleotides (Alexander & Peters, 1997).

Although the cloned ATP receptors are Ca^{2+}-mobilizing, some ATP receptors also activate cyclic AMP production and several different mechanisms by which ATP receptors activate cyclic AMP production have been identified. One mechanism operates *via* the production of arachidonic acid and its subsequent conversion to prostaglandins E_1 and E_2, which are recognized activators of receptors linked to adenylyl cyclase (Huang *et al*., 1991; Wang *et al*., 1992; Post *et al*., 1996). This mechanism operates in certain cells that express $P2Y_2$ receptors (e.g., Madin Darby kidney cells; Post *et al*., 1996) and is, therefore, activated by UTP. Another less-well defined mechanism operates *via* ATP receptors that are inhibited by P1 receptor antagonists such as xanthine amine congener (XAC) and 8-sulpho-phenyl-theophyline (8-SPT) (Griese *et al*., 1991; Cote *et al*., 1993; Matsuoka *et al*., 1995). Our work (Jiang *et al*., 1997) and that of Communi *et al*. (1997) indicate that some ATP receptors may be coupled directly to adenylyl cyclase independent of arachidonic acid generation and P1 receptors.

Recently, we and others have shown that extracellular ATP activates cyclic AMP production in undifferentiated HL-60 cells (Choi & Kim, 1997; Jiang *et al*., 1997). Furthermore, extracellular ATP, but not UTP, induces granulocytic differentiation of HL-60 cells in a cyclic AMP-dependent fashion (Jiang *et al*., 1997). The effects of ATP on differentiation and cyclic AMP production could not be mimicked by ADP, AMP or adenosine.

We have now completed a pharmacological characterization of a large number of ATP analogs on cyclic AMP production in HL-60 cells. This work confirms that the receptor is a P2 receptor. Analogs with increased potency and full efficacy include adenosine 5′-O-[3-thiotriphosphate] (ATPγS), 2′& 3′-O-(4-benzoylbenzoyl)-adenosine-triphosphate (Bz-ATP) and dATP. Adenosine 5′-O-[2-thiodiphosphate] (ADPβS) and adenosine 5′-O-[1-thiotriphosphate] (ATPαS) were high potency partial agonists. ADPβS, adenosine 5′-O-

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thiomonophosphate (AMPS) and suramin were identified as inhibitors. These results suggest the existence of a novel P2 receptor on HL-60 cells. Cyclic AMP production by this receptor is sufficient to activate protein kinase A (PK-A) which, in turn, induces the granulocytic differentiation of HL-60 cells (Jiang *et al*., 1997).

Methods

HL-60 cell culture

HL-60 leukemia cells were grown in suspension culture in HEPES-buffered RPMI-1640 (pH 7.4) supplemented with 10% FBS (fetal bovine serum) and gentamicin (5 μg ml^{-1}) at 37°C. Cell density was maintained within the range $1-10\times10^5$ cells ml^{-1}. Cell counts were performed routinely using a Coulter Counter. Doubling times of undifferentiated HL-60 cells were approximately 24–30 h.

Cyclic AMP assay

HL-60 cells were harvested in log phase by centrifugation and resuspended at a density of 10^6 cells/ml in either physiological saline solution (PSS) (mM): NaCl, 145; KCl, 5.0; HEPES, 10; glucose, 10; $CaCl_2$, 1.0; $MgCl_2$, 1.0; NaH_2PO_4, 1.0 (pH 7.4) or fresh RPMI-1640 medium supplemented with 10% FBS. 0.2 ml aliquots of cells were stimulated with nucleotides at various concentrations in the presence or absence of 3-isobutyl-l-methyl xanthine (IBMX) (0.5 mM) at 37°C. For the determination of total cyclic AMP production, reactions were stopped by the addition of 0.5 ml EDTA (1.0 mM) in 90% ethanol. For the determination of intracellular and extracellular cyclic AMP, the reactions were first centrifuged for 15 s in a bench-top microfuge and the supernatants transferred to new tubes. The reactions were then stopped as described above. The ethanol-containing supernatants were then evaporated by vacuum centrifugation and the remaining precipitates were re-dissolved in 0.2 ml cyclic AMP assay buffer (Na acetate, 50 mM; $CaCl_2$, 25 mM and bovine serum albumin, 0.1%, pH 6.2). The radioimmune assay for cyclic AMP was performed as described previously (Luttrell & Henniker, 1991).

Protein kinase-A assay

Undifferentiated HL-60 cells were harvested in log phase by centrifugation and resuspended in RPMI-1640 plus 10% FBS at a density of 2×10^5/ml. The cells were then incubated in the presence or absence of extracellular ATP (1 mM) at 37°C. At various times, reactions were stopped by centrifugation, the cell pellets washed immediately in ice cold Dulbecco's phosphate-buffered saline, and then resuspended on ice in 1 ml of an isotonic permeabilization buffer that contained (mM): NaCl, 150; $MgCl_2$, 1.5; Tris-HCl, 20 (pH 7.9 at 20–25°C); DTT (dithiotreitol), 1 mM; glycerol, 20%; Triton X-100, 1.0%; IBMX, 0.5 mM; leupeptin, 1 μg ml^{-1}; aprotinin, 4 μg ml^{-1} and PMSF (phenylmethanesulfonyl fluoride), 0.17 mM. After 10 min, the suspension was centrifuged at 39,000 *g* for 10 min and the resulting supernatant was assayed for PK-A either immediately or after storage at −80°C.

The SignaTECT PK-A assay system was used according to the manufacturer's instructions with a minor modification. Incubations (5 min at 30°C) were performed by the addition of 2 μl of the sample supernatant to 9 μl of a solution that contained 0.1 mM ATP, 12.5 nM γ-[^{32}P]-ATP (ca. 4000 Ci mmol^{-1}), 0.1 mM biotinylated PK-A substrate, 0.1 mM IBMX, 1 × SignaTECT PK-A assay buffer ± 5 μM cyclic AMP. Reactions were stopped by the addition of 5 μl of 7.5 M guanidine HCl and 3 μl aliquots were then spotted onto streptavidin-linked capture membranes which were then washed and dried according to the manufacturer's instructions. Phosphorylation of the biotin-labelled peptide substrate by γ-[^{32}P]-ATP was determined by scintillation counting of dried capture membranes in 2 ml of Ecolume scintillation fluid. The PK-A activation ratio was calculated by dividing the PK-A activity obtained in the absence of exogenous cyclic AMP by the PK-A activity obtained in the presence of exogenous cyclic AMP.

Data analysis

The data are reported as means ± s.e.m. Student's unpaired and paired *t*-tests were used to test the statistical significance of the differences observed between experimental means. Solid lines on the concentration-response curves were fitted with the Hill equation using non-linear regression (DeltaGraph Professional software). EC_{50} values (means ± s.e.m.) were determined using MacCurveFit 1.0 software. IC_{50} values for inhibitory analogs of ATP were obtained in a similar way using the equation:

$$y = y_{max} - c(y_{max} - y_{min})/(i + c) \quad (1)$$

where y is cyclic AMP production (pmol/10^6 cells), y_{max} is the maximum cyclic AMP production, y_{min} is the minimum cyclic AMP production (non-zero for a partial agonist), c is the concentration of inhibitor (μM), i is the IC_{50} concentration.

Materials

HEPES-buffered RPMI-1640 and Dulbecco's phosphate buffered saline was from Sigma (Sigma, St Louis, MO, U.S.A.). Fetal bovine serum was from CSL (Melbourne, Victoria, Australia). Nucleotides were from Sigma with the exception of ATPγS and ADPβS (Boehringer Mannheim, Castle Hill, NSW, Australia), ATPαS (Calbiochem CA, U.S.A.) and 2-methylthio-ATP (RBI, Natick, MA, U.S.A.). 8-SPT, XAC, suramin and PPADS were from RBI. IBMX was from Sigma. The SignaTECT PK-A assay system was from Promega (Sydney, Australia). Ecolume scintillation fluid was from ICN (Seven Hills, NSW, Australia). All other reagents were analytical grade or equivalent.

Results

Effects of ATP on cyclic AMP production and intracellular cyclic AMP levels

Extracellular ATP (10–1000 μM) stimulated total cyclic AMP production by undifferentiated HL-60 cells in a time-dependent fashion (Figure 1). This effect occurred independent of whether the cells were exposed to ATP in physiological saline solution or in serum-containing RPMI-1640 medium (not shown). In cells exposed to 1 mM ATP in serum-containing RPMI-1640, cyclic AMP production rose promptly to reach a maximum after 10–20 min and thereafter decayed slightly (Figure 1). Exposure of HL-60 cells to 0.5 mM IBMX for 10 min resulted in a small increase in basal cyclic AMP levels from 1.6 ± 0.4 pmol/10^6 cells (n = 3) to 9.0 ± 1.3 pmol/10^6 cells (n = 3) and, in addition, IBMX enhanced ATP-induced cyclic AMP production (Figure 1), presumably by inhibiting

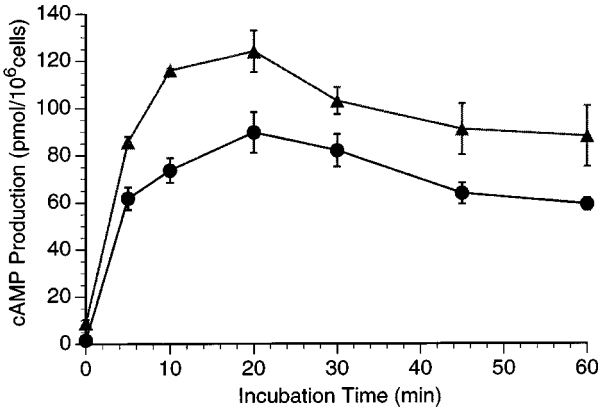

Figure 1 Time-course for the effect of extracellular ATP on total cyclic AMP production by undifferentiated HL-60 cells. HL-60 cells (10^6 cells ml^{-1}) were exposed to ATP (1 mM) and then incubated in 0.2 ml of RPMI-1640 medium plus FBS 10% in the absence (●) or presence (▲) of IBMX (0.5 mM) for various times at 37°C. Data are expressed as means ± s.e.mean. Basal cyclic AMP levels were 1.6 ± 0.4 pmol/10^6 cells ($n = 3$). Exposure of cells to IBMX (0.5 mM) for 10 min elevated basal cyclic AMP levels to 9.0 ± 1.3 pmol/10^6 cells ($n = 3$).

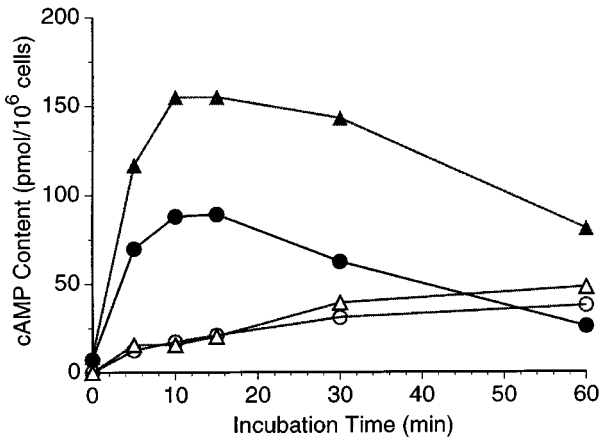

Figure 2 Time-course for the effect of extracellular ATP on intracellular and extracellular cyclic AMP levels by undifferentiated HL-60 cells. HL-60 cells (10^6 cells ml^{-1}) were exposed to ATP (1 mM) and then incubated in 0.2 ml of RPMI-1640 medium plus FBS 10% in the absence (●, ○) or presence (▲, △) of IBMX (0.5 mM) for various times at 37°C. The cell suspension was centrifuged and intracellular (●, ▲) and extracellular (○, △) cyclic AMP levels were then determined. A representative experiment (total of three similar experiments) is shown.

cyclic AMP phosphodiesterase. ATP-induced cyclic AMP production occurred intracellularly as expected for receptor-dependent activation of adenylyl cyclase (Figure 2). However, ATP also induced a progressive increase in the extracellular cyclic AMP level presumably as a result of cyclic AMP release into the medium (Figure 2).

Extracellular ATP stimulated PK-A activity

Extracellular ATP also stimulated PK-A activity and the time-course for the activation of PK-A (Figure 3) was similar to that observed for the ATP-induced elevation of intracellular cyclic AMP levels (Figure 2). Peak PK-A activity was observed after about 10 min exposure to extracellular ATP and thereafter recovered to baseline levels (Figure 3). dbcAMP (N^6,2′-O-dibutyryl-adenosine-3′,5′-cyclic monophosphate: 0.5 mM) also activated PK-A. However, consistent with the idea that dbcAMP induces a sustained elevation in intracullar cyclic AMP levels, PK-A remained fully active throughout 60 min of exposure to dbcAMP (Figure 3).

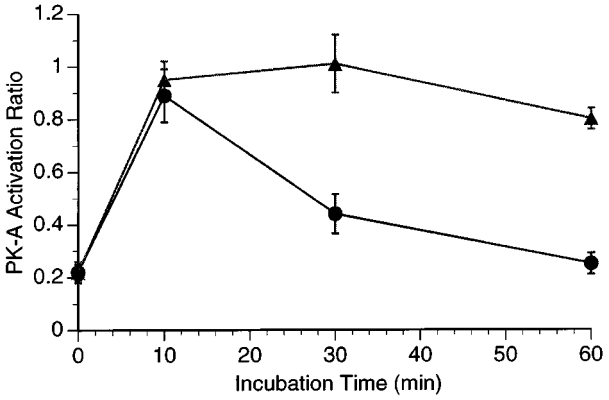

Figure 3 Time-course for the effect of extracellular ATP on PK-A activity. HL-60 cells (2×10^5/ml) were incubated in RPMI-1640 medium plus FBS 10% in the presence of 1 mM ATP (●) or 0.5 mM dbcAMP (▲) for various times at 37°C. The cells were then washed, permeabilized with Triton X-100 and assayed for the presence of PK-A activity. PK-A activity is expressed as the activation ratio (i.e., PK-A activity in the absence of exogenous cyclic AMP divided by PK-A activity in the presence of 5 μM cyclic AMP). Data are expressed as means ± s.e.mean.

Effects of ATP analogs

A variety of ATP analogues, including the stable analog β,γ-methylene ATP, also stimulated cyclic AMP production (Figures 4, 5; Table 1). After 5 min incubation, the order of potency was ATPγS ≥ BzATP ≥ dATP > ATP. The following analogues (in order of effectiveness at 1 mM) were all less effective than ATP at concentrations up to 1 mM: β,γ methylene ATP ≥ 2-methylthioATP > ADP ≥ Ap4A ≥ Adenosine > UTP (Figure 4, Table 1). Ap3A, GTP, CTP and AMP, at concentrations up to, and including 1 mM, were without significant effect (cyclic AMP production ≤ 5 pmol/10^6 cells after 5 min stimulation).

Agonist effects of thiophosphorylated analogs of ATP

Because ATPγS was a more potent activator of cyclic AMP generation than ATP, we tested the effects of several other thiophosphorylated analogs, ATPαS, ADPβS and AMPS. ATPαS and ADPβS, like ATPγS, exhibited higher potency than ATP (Figure 5; Table 1). However, both compounds were partial agonists and maximally activated cyclic AMP production to about 50% and 20% respectively of the maximal effect of ATP. AMPS (10 – 1000 μM) also stimulated cyclic AMP production but was a very weak agonist. At the highest concentration tested (1 mM) AMPS promoted cyclic AMP production to about 10% of the maximal effect induced by ATP.

Antagonist activity of ADPβS and AMPS

We screened ADPβS and AMPS as potential antagonists of ATP-induced cyclic AMP production. Undifferentiated HL-60 cells were exposed to ATP (100 μM) in the presence or absence

of various concentrations of ADPβS or AMPS, without pre-incubation because of their agonist effects. Both agents inhibited ATP-induced cyclic AMP production (Figure 6). For ADPβS, apparent $IC_{50} = 146 \pm 32$ μM and for AMPS, apparent $IC_{50} = 343 \pm 142$ μM. Because of its partial agonist activity, however, the maximum inhibition induced by ADPβS was about 65% of the control value as determined by non-linear regression fit of Equation 1 to the data in Figure 6. The predicted maximum inhibitory effect of AMPS, however, was about 90% determined in similar fashion.

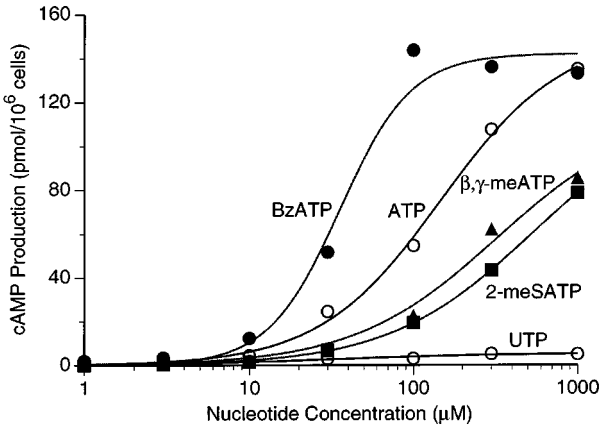

Figure 4 Concentration dependence for the effects of ATP and selected ATP analogs on cyclic AMP production. HL-60 cells (10^6 cells/ml^{-1}) were exposed to various concentrations of ATP and selected ATP analogs (agents with known selectivities for various P2 receptors) in 0.2 ml of RPMI-1640 medium plus FBS 10% for 5 min at 37°C in the presence of IBMX (0.5 mM). The data were obtained from three to six experiments. For clarity the means, but not the s.e.means (all less than 25% of their corresponding mean values), are shown. The lines were fitted to the data by non-linear regression using the Hill equation. EC_{50} values are recorded in Table 1. BzATP, 2′&3′-O-(4-benzoylbenzoyl)-adenosine-5′-triphosphate; β,γ-meATP, (β,γ-methylene ATP; 2-meSATP,2-methylthioATP.

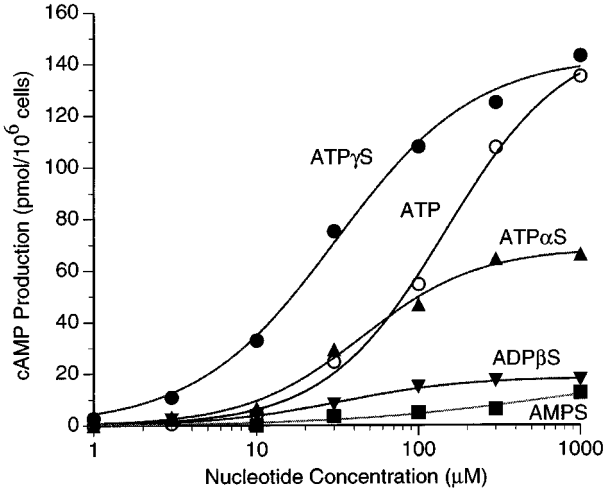

Figure 5 Concentration dependence for the effects of thiophosphorylated ATP analogues on cyclic AMP production. HL-60 cells (10^6 cells ml^{-1}) were exposed to various concentrations of different thiophosphorylated ATP analogues in 0.2 ml of RPMI-1640 medium plus FBS 10% for 5 min at 37°C in the presence of IBMX (0.5 mM). ATP data from Figure 4 are shown for comparison. The data were obtained in three to six experiments. For clarity the means, but not the s.e.means, are shown. The lines were fitted to the data by non-linear regression using the Hill equation. EC_{50} values are recorded in Table 1. AMPS, adenosine 5′-O-thiomonophosphate; ADPβS, adenosine 5′-O-[2-thiodiphosphate]; ATPαS, adenosine 5′-O-[1-thiotriphosphate]; ATPγS, adenosine 5′-O-[3-thiotriphosphate].

Table 1 Pharmacological analysis of ATP analogues as agonists of cyclic AMP production in HL-60 cells

ATP analog	*EC_{50}* (μM)*	*Maximum cyclic AMP production* (pmol/10^6 cells)
ATPγS	30.4 ± 6.9	139 ± 8.5*
ADPβS	33.3 ± 5.0	17.8 ± 0.8*,‡
BzATP	33.5 ± 12.5	134 ± 10.3*
dATP	34.5 ± 6.4	106 ± 6.3*
ATPαS	44.6 ± 14.5	66.3 ± 6.6*,‡
ATP	146 ± 57	145 ± 21*
Adenosine 5′-tetraphosphate		112 ± 11 (3)†
β,γ-methylene ATP		83.0 ± 8.1 (3)†
2-methylthio ATP		76.3 ± 13 (4)†
8-Br ATP		57 ± 22 (3)†
ADP		29.4 ± 5.2 (3)†
Ap4A		24.8 ± 6.1 (3)†
Adenosine		20.7 ± 5.8 (3)†
Ap5A		14.0 ± 2.0 (3)†
AMPS		13.1 ± 1.3 (3)†,‡
UTP		5.9 ± 0.4 (3)†

Undifferentiated HL-60 cells were incubated in RPMI-1640 medium (FBS 10%) for 5 min at 37°C in the presence of IBMX (0.5 mM). Reactions were stopped and the cyclic AMP produced estimated as described in Methods. The following ATP analogs were without significant effect on cyclic AMP production (≤ 5 pmol/10^6 cells after a 5 min exposure at a concentration of 1 mM): AMP, Ap3A, GTP, CTP. Values were obtained by fitting the Hill equation to dose-dependence curves including those shown in Figures 4 and 5. *Values (mean ± s.e.mean) were determined by non-linear regression analysis (MacCurveFit 1.0 for Macintosh) using the Hill equation. †Values reported as means ± s.e.mean (number of experiments) were determined following exposure of cells to ATP analogs at a concentration of 1 mM. ‡Partial agonist; AMPS, like ADPβS and ATPαS, may be a partial agonist.

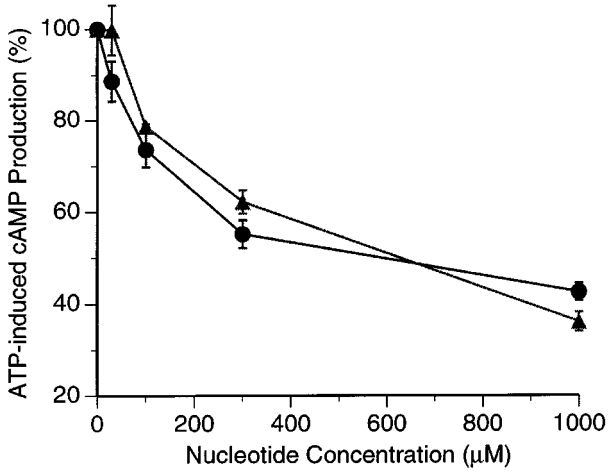

Figure 6 Concentration dependence for the inhibitory effects of ADPβS and AMPS on ATP-induced cyclic AMP production. HL-60 cells (10^6 cells ml^{-1}) were exposed to ATP (100 μM) and various concentrations of ADPβS (●) or AMPS (▲) in 0.2 ml of RPMI-1640 medium plus FBS 10% for 10 min at 37°C in the presence of IBMX (0.5 mM). Data are expressed as means ± s.e.mean.

The effects of recognized P2 and P1 receptor antagonists

The broad-spectrum P2 inhibitor suramin (500 μM) abolished ATP-induced cyclic AMP generation (not shown). PPADS (pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonate; 100 μM), a recognized inhibitor of $P2X_1$ and $P2Y_1$ receptors, however, was without effect. Furthermore, the P1 receptor antagonists XAC (20 μM) and 8-SPT (100 μM), which have been shown to inhibit ATP-induced cyclic AMP production in some cell types (Griese *et al*., 1991; Cote *et al*., 1993; Matsuoka *et al*., 1995), were also without effect.

Discussion

We and others have recently shown that extracellular ATP activates cyclic AMP production by undifferentiated HL-60 cells *via* a P2 receptor (Choi & Kim, 1997; Jiang *et al*., 1997). The data presented here provide additional evidence to support the idea that a novel P2 receptor subtype is involved. Activation of this receptor leads to cyclic AMP production (Figure 1) including elevation of intracellular cyclic AMP levels (Figure 2). Cyclic AMP release into the extracellular fluid is partly responsible for the recovery of intracellular cyclic AMP levels to baseline. Breakdown of ATP leading to deactivation of its surface receptors is also likely to play a role in the recovery of intracellular cyclic AMP to baseline levels. We have previously demonstrated that the half-life of ATP (100 μM) under the conditions of these experiments is about 30 min (Jiang *et al*., 1997).

The activated receptor also elevates PK-A activity (Figure 3) with a similar time-course to the ATP-induced elevation of intracellular cyclic AMP levels. Unlike ATP, however, dbcAMP (0.5 mM) fully activated PK-A for greater than 60 min (Figure 3) presumably because extracellular dbcAMP readily permeates cell membranes thereby acting as a continuous supply of intracellular cyclic AMP.

The receptor involved in ATP-dependent cyclic AMP production is clearly distinct from the $P2Y_2$ (P_{2U}) receptor on HL-60 cells that activates PI-PLC and mobilizes Ca^{2+} ions from intracellular stores (Dubyak *et al*., 1988; Stutchfield & Cockroft, 1990) because UTP was only a very weak activator of cyclic AMP production (Figure 4 and Table 1). Furthermore, the potency order for the effects of ATP analogues on cyclic AMP generation is not consistent with the behaviour of any of the P2 receptors cloned to date. In particular, three ATP analogs (ATPγS, BzATP and dATP) were found to have enhanced potency when compared to ATP (Table 1). The enhanced potency of these analogs is not due to their greater stability with respect to the action of ecto-nucleotidases since under the incubation conditions used in the concentration-response experiments only 5–10% of ATP (100 μM) was broken down (Jiang *et al*., 1997).

The finding that BzATP is a potent activator of cyclic AMP production (Figure 4; Table 1) raises the possibility that a $P2X_7$ (P_{2Z}) receptor is involved (el-Moatassim & Dubyak, 1992; Rassendren *et al*., 1997). However, $P2X_7$ receptors are not expressed by HL-60 cells as revealed by the failure of ATP to induce plasma membrane permeabilization. This conclusion is supported by the observation that BzATP (100 μM) induced a transient elevation of $[Ca^{2+}]_i$ in fura-2 loaded HL-60 cells similar to that induced by activators of the $P2Y_2$ (P_{2U}) receptor, but did not provoke fura-2 release (Jiang and Conigrave, unpublished). Furthermore, ATPγS, which is only a weak activator of $P2X_7$ receptors (Wiley *et al*., 1990; el-Moatassim & Dubyak, 1992) was a more potent activator of cyclic AMP production (Table 1; Figure 5). Taken together, the data indicate that BzATP activates a distinct cyclic AMP-linked ATP receptor on HL-60 cells and has selectivity for this receptor in addition to its selectivity for $P2X_7$ receptors.

The finding that ATPγS was also a more potent activator of cyclic AMP production led us to determine whether other thiophosphorylated analogues of ATP might have enhanced potency with respect to ATP. ATPαS and ADPβS were also found to have higher potency than ATP but were partial agonists (Figure 5). Furthermore, ADPβS ($IC_{50} = 146 \pm 32$ μM) and AMPS ($IC_{50} = 339 \pm 144$ μM) inhibited ATP (100 μM)-induced cyclic AMP production (Figure 6). Although ADPβS has been previously reported to inhibit a P2X receptor in PC-12 cells (Sela *et al*., 1991), AMPS has not been previously reported to inhibit the activity of P2 receptors. AMPS was a very weak agonist and may also be a partial agonist of the cyclic AMP-linked P2 receptor on HL-60 cells (Figure 5). Because of its weaker agonist activity, it may be of greater value than ADPβS as a selective inhibitor of the novel cyclic AMP-linked P2 receptor described in this study.

ADPβS has not been previously reported to act as a partial agonist as demonstrated here. ADPβS has been previously reported as an activator of $P2Y_1$ receptors which are coupled to phospholipase C but not adenylyl cyclase. ADPβS also activates distinct P2Y receptors on C6 glioma cells which are coupled to the inhibition of adenylyl cyclase (Boyer *et al*., 1995; Schachter *et al*., 1997). Because of its partial agonist behaviour, it is unlikely that ADPβS inhibits ATP-induced cyclic AMP production via the activation of a distinct P2 receptor on HL-60 cells. Furthermore, the potency of ADPβS for the inhibition of isoproterenol-stimulated cyclic AMP production in C6 glioma cells was high (EC_{50} about 100 nM; Boyer *et al*., 1995) whereas its potency for the activation of cyclic AMP production in HL-60 cells and its potency for the inhibition of ATP (100 μM)-induced cyclic AMP production were both much lower (EC_{50} about 30 μM, and IC_{50} about 150 μM, respectively). It is possible that some of the reported biological effects of ADPβS, particularly at high μM concentrations, might arise from partial activation or inhibition of cyclic AMP-linked ATP receptors like those on HL-60 cells rather than from the activation of Ca^{2+}-mobilizing $P2Y_1$ receptors or C6 glioma-like P2 receptors that are linked to the inhibition of cyclic AMP production.

We also examined the effects of various other potential antagonists of ATP-induced cyclic AMP production. The non-selective P2 receptor antagonist, suramin (0.5 mM) abolished ATP-induced cyclic AMP production. However, antagonists of P1 receptors (XAC, 20 μM and 8-SPT, 100 μM) which have been reported to inhibit ATP-induced cyclic AMP production in some cell types (Griese *et al*., 1991; Cote *et al*., 1993; Matsuoka *et al*., 1995) were without effect. PPADS (100 μM) an inhibitor of $P2X_1$ and $P2Y_1$ receptors (Ziganshin *et al*., 1993; Boyer *et al*., 1994) was also without effect thus excluding a role for these receptor sub-types in the cylic AMP signalling pathway.

Some features of this receptor resemble the recently cloned $P2Y_{11}$ receptor (Communi *et al*., 1997). Activation of the $P2Y_{11}$ receptor expressed in 1321N1 cells, by ATP induces cyclic AMP production with a similar partial potency order (ATP > 2-MeSATP > ADP) and EC_{50} value for ATP (about 100 μM). Interestingly, the $P2Y_{11}$ receptor also couples to phospholipase C and is expressed in retinoic acid or DMSO-differentiated HL-60 cells. It remains to be determined whether the novel receptor we have identified in undifferentiated HL-60 cells is related, or identical, to the cloned $P2Y_{11}$ receptor.

We have shown previously that the cell permeable inhibitor of PK-A, Rp-8Br-cAMPS, inhibits ATP-induced HL-60 cell differentiation (Jiang *et al*., 1997). Consistent with the idea that ATP induces HL-60 cell differentiation via cyclic AMP production and the activation of PK-A, cell permeable analogues of cyclic AMP such as dibutyryl cyclic AMP (Chaplinski & Niedel, 1982) and 8-Cl-cAMP (Rohlff *et al*., 1993) are also recognized inducers of HL-60 cell differentiation. The data reported here indicate that PK-A is activated by extracellular ATP with a similar time-course to that observed for the elevation of cellular cyclic AMP levels (Figures 2 and 3). The downstream signalling pathway that links ATP receptor activation to granulocyte differentiation has not yet been determined but is likely to involve members of the CREB/ATF-1 family of transcription factors (Montminy, 1997).

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