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Extracellular ATP-Dependent Suppression of Proliferation and Induction of Differentiation of Human HL-60 Leukemia Cells by Distinct Mechanisms

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ABSTRACT. Extracellular ATP suppressed the growth of HL-60 leukemia cells and induced their differentiation as revealed by *N-formyl*-methionyl-leucyl-phenylalanine-induced β-glucuronidase release. ATP degraded to ADP, AMP, and adenosine, and the effect of ATP on cell growth was mimicked by these metabolites added to the cultures. The stable analog α,β-methylene ATP, however, had only a weak inhibitory effect on cell growth. Adenine nucleotide-induced growth suppression was reversed by uridine, suggesting the involvement of intracellular pyrimidine starvation secondary to adenosine accumulation. Consistent with this, ATP induced intracellular starvation of pyrimidine nucleotides, and this effect was also prevented by pretreatment of cells with uridine. The order of effectiveness of ATP-induced differentiation of HL-60 cells, unlike that for growth suppression, was ATP > ADP > AMP, and adenosine had no effect. Furthermore, uridine had no effect and the stable analog, α,β-methylene ATP also induced HL-60 cell differentiation, suggesting that differentiation was due to ATP per se. We tested the hypothesis that ATP-induced differentiation arises from activation of adenylyl cyclase by the novel $P2Y_{11}$ receptor using the cell-permeable inhibitor of protein kinase A, Rp-CPT-cAMPS (8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate, Rp isomer). Rp-CPT-cAMPS (1–100 μM) prevented ATP-induced differentiation of HL-60 cells as assessed by fMLP-induced β-glucuronidase release. However, Rp-CPT-cAMPS did not prevent ATP-induced growth suppression. Taken together, the data indicate that extracellular ATP suppresses HL-60 growth and induces their differentiation by distinct mechanisms. Growth suppression arises from adenosine generation and consequent pyrimidine starvation. Differentiation arises, at least in part, from a distinct mechanism involving the activation of cell surface P2 receptors coupled to cAMP generation and activation of protein kinase A. BIOCHEM PHARMACOL **60**;11: 1585–1591, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. P2 receptor; extracellular ATP; leukemia; growth suppression; differentiation; HL-60 cells

The finding that extracellular ATP suppresses the growth and induces the differentiation of human HL-60 promyelocytic leukemia cells [1] suggests that acute promyelocytic leukemia may respond to agonists of one or more P2 receptors. However, understanding the mechanisms responsible for ATP-induced growth suppression and differentiation represents an important first step in evaluating this concept. Extracellular ATP suppresses the growth of a variety of cells in culture, including human PC-3 prostate cancer cells [2], human CAPAN-1 pancreatic adenocarcinoma cells [3], human HT29 colon adenocarcinoma cells [3], and mouse 3T6 fibroblasts [4]. Various mechanisms have been proposed, including activation of P2 purinoceptors [2] and pyrimidine starvation induced by adenosine derived from the extracellular breakdown of adenine nucleotides [4]. Significantly, in this latter study, the effects of repetitive exposure to low-dose adenosine (mimicking the delayed release of adenosine from extracellular ATP) had a much greater growth-suppressing effect than a single exposure to high-dose adenosine [4].

Extracellular ATP induces marked growth suppression of HL-60 leukemia cells as well as granulocytic differentiation as indicated by chemotactic peptide-stimulated β-glucuronidase release [1], raising the possibility that the two phenomena share a common mechanism. HL-60 cells express a P2 receptor that is coupled to cAMP generation [1, 5] and are known to express the cloned $P2Y_{11}$ receptor which couples to adenylyl cyclase [6]. Recent work confirms that the adenylyl cyclase-coupled P2 receptor in HL-60 cells and

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the $P2Y_{11}$ receptor share a common pharmacology [7, 8]. This receptor exhibits the potency order ATP > ADP > AMP and adenosine is without effect.

In the present study, we investigated the mechanisms of ATP-induced HL-60 growth suppression and differentiation to establish whether a common mechanism is responsible, e.g. via the activation of $P2Y_{11}$ receptors. We found that the growth suppression effect of ATP was mimicked by ADP, AMP, and adenosine and arose from intracellular pyrimidine starvation. Uridine prevented ATP-dependent growth suppression and pyrimidine starvation. However, the differentiation effect of ATP was not prevented by uridine. It was selective for adenine nucleotides, especially ATP and ADP, and arose from the activation of protein kinase A. Therefore, $P2Y_{11}$ is implicated in the process of ATP-induced differentiation of HL-60 cells, but is not directly involved in ATP-induced growth suppression.

MATERIALS AND METHODS

HL-60 Cell Culture

Human HL-60 leukemia cells (ATCC) were grown in suspension culture at 37° in HEPES-buffered RPMI-1640 (pH 7.4; Sigma) supplemented with 10% fetal bovine serum (CSL) and gentamicin (5 μg/mL; Sigma). Cell density was maintained within the range 1–10 × 10^5 cells mL^{-1}. Cell densities were determined using a Coulter counter. Doubling times of undifferentiated HL-60 cells were 24–30 hr.

fMLP Stimulated β-Glucuronidase Release*

HL-60 cells were pretreated with cytochalasin B (5 μg/mL; Sigma) and then exposed to the chemotactic peptide fMLP (1 μM; Sigma) as described previously, using 4-methylumbelliferyl-β-D-glucuronide (Calbiochem) as the fluorescent substrate [9, 10]. ATP-treated cells were exposed to a single dose of ATP (0.01–1 mM) and assayed for fMLP-induced β-glucuronidase release in physiological saline solution (145 mM NaCl, 5.0 mM KCl, 10 mM D-glucose, 1.0 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 10 mM H-HEPES, pH 7.4) after a further 2 days in culture. β-Glucuronidase release was expressed as a percentage with respect to total cellular enzyme activity determined after exposure of cells to 0.1% Triton X-100 in physiological saline solution. Small differences in the total cellular level of β-glucuronidase between different treatments were not sufficient to affect any of the experimental outcomes.

* *Abbreviations:* cAMP, adenosine 3′,5′-cyclic monophosphate; fMLP, *N-formyl*-methionyl-leucyl-phenylalanine; hENT1, human equilibrative nucleoside transporter 1; hENT2, human equilibrative nucleoside transporter 2; NBTI, nitro-benzyl-thio-inosine; and Rp-8CPT-cAMPS, 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate, Rp isomer.

Ion Pair HPLC for Analysis of ATP Breakdown

The breakdown of ATP was evaluated by ion-pair HPLC. ATP (100 μM) was added to cultured HL-60 cells, and samples of extracellular fluid were then collected at various times after the cells had been sedimented with a microfuge (10,000 *g*, 1 min). The samples were analysed immediately or stored at −20° until required. For ion-pair HPLC, an end-capped C-18 Goldpack HPLC column (5 μm, 250 mm × 4.6 mm, Activon) was attached to a Pharmacia-LKB HPLC system (Pharmacia). Tetrabutylammonium chloride (TBA-Cl, Sigma) was used as the ion-pair reagent. Adenosine, AMP, ADP, and ATP were sequentially eluted from the HPLC column at a flow rate of 1 mL min^{-1} using a gradient from Buffer A (80 mM KH_2PO_4, 5 mM TBA-Cl, pH 5.0) to Buffer B (50% buffer A and 50% acetonitrile). After pre-equilibration of the column with 24% B, the gradient profile was: 0–3 min, 24–50% B; 3–13 min, 50% B. The observed retention times for adenine nucleotide and adenosine standards (all Sigma) were: adenosine, 4.3 ± 0.01 min; AMP, 5.2 ± 0.01 min; ADP, 8.7 ± 0.01 min; and ATP, 10.8 ± 0.01 min (means ± SEM; N = 13).

Extraction of Nucleotides from Cells

Cultured HL-60 cells were sedimented and an equal volume of 0.8 M perchloric acid was added to the cell pellet, vortexed, left for 20 min, then centrifuged (1900 *g*, 15 min, 4°). The acid-soluble supernatant was neutralised by vortexing with an equal volume of trioctylamine:trichlorotrifluoroethane (2.2:7.8 v/v) then centrifuged (15 min, 4°) to separate the phases. The upper aqueous phase was collected and analysed for nucleoside triphosphates (NTPs) by HPLC.

HPLC Analysis of Cell Extracts

Samples were analysed for NTPs by gradient anion-exchange chromatography on a strong anion exchange column (Partisil 10 SAX, Phenomenex) equilibrated with buffer C (7.0 mM KH_2PO_4, pH 3.0). Elution of the extracts was achieved with a concave gradient from buffer C to buffer D (250 mM KH_2PO_4/500 mM KCl, pH 3.0) over 45 min followed by isocratic elution with buffer D for a further 45 min. NTPs were detected at 260 nm and quantified using Nelson Model 2600 Chromatography Software.

RESULTS

Effects of Extracellular Adenine Nucleotides on Growth of HL-60 Cells

Exposure of HL-60 cells to a single dose of extracellular ATP or one of its breakdown products ADP, AMP, or adenosine (initial concentration 10–1000 μM) suppressed the subsequent growth of HL-60 cells cultured in RPMI-1640 plus 10% fetal bovine serum. The effect was first apparent after two days and persisted for up to 7 days in

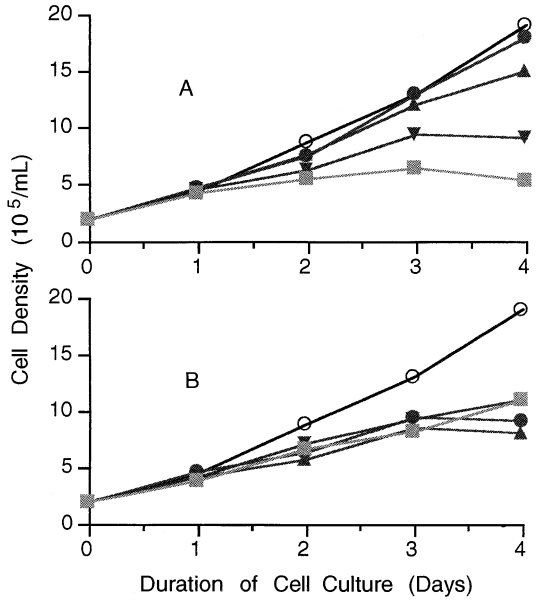

FIG. 1. The effect of adenine nucleotides and adenosine on HL-60 cell growth. (A). Time- and concentration-dependent inhibition of cell growth by extracellular ATP (○, zero; ●, 30 μM; ▲, 100 μM; ▼, 300 μM; ■, 1000 μM). (B). Time dependence of the inhibitory effect of ATP and its breakdown products (all 300 μM) on cell growth (○), control; ●, ATP; ▲, ADP; ▼, AMP; ■, adenosine). HL-60 cells were cultured under standard conditions in RPMI-1640 medium (fetal bovine serum 10%). The data show the means of four experiments. The standard error bars (≤20%) have been omitted for clarity.

culture (Fig. 1). We examined the concentration dependence of the effects and found that all four agents were approximately equipotent. Half-maximal inhibition of cell proliferation by ATP was observed at a concentration of about 250 μM (Fig. 2).

Stability of ATP Under Culture Conditions and Effect of α,β-Methylene ATP on HL-60 Cell Growth

ATP was rapidly broken down with the concomitant generation of ADP, AMP, and adenosine under standard culture conditions. At a cell density of 5×10^5 cells/mL, ATP (100 μM) was broken down with a half-life of about 1 hr and was undetectable after 6 hr (Fig. 3). Thus, the growth-suppressing effects of adenine nucleotides could arise from the generation of adenosine and its intracellular accumulation. Consistent with this proposal, the stable analog α,β-methylene ATP (0.1–1 mM) was markedly less effective than ATP as an inhibitor of HL-60 cell growth (Fig. 4).

Relationship of Slow Adenosine Generation to ATP-Induced Growth Suppression

The foregoing data led us to investigate whether adenine nucleotides induce growth inhibition via the generation of adenosine followed by its cellular accumulation and pyrimidine starvation. Such a mechanism underlies the growth-suppressing effect of extracellular ATP on 3T6 fibroblasts [4]. We used HPLC on Partisil-SAX to quantify the intracellular levels of ATP, GTP, and UTP in perchloric acid extracts of control and ATP-treated HL-60 cells after 4 days in culture. Exposure of HL-60 cells to extracellular ATP (initial concentration 1 mM) led to a severe depletion of intracellular UTP from 50 ± 9.3 amol/cell (N = 6) to

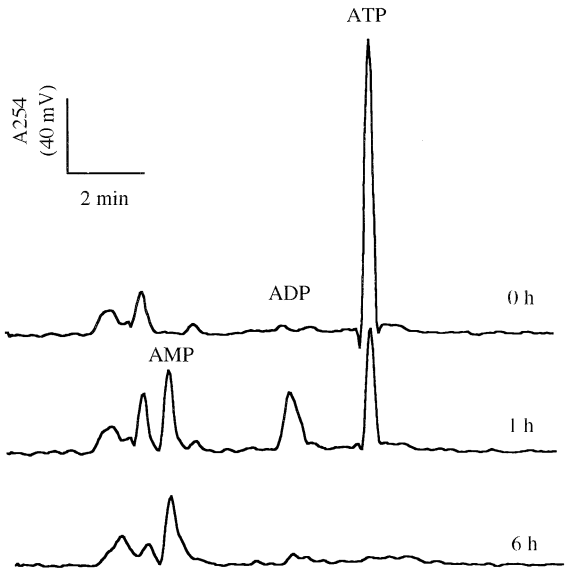

FIG. 3. Breakdown of ATP in HL-60 cell culture. HL-60 cells (10^5/mL) were cultured under standard conditions for various times in the presence of ATP (initial concentration 100 μM). The cells were then sedimented and the supernatants analysed for the presence of ATP, ADP, and AMP by ion-pair HPLC. Peak elution was monitored by absorbance at 254 nm and the data recorded using the program *Chart* for MacIntosh.

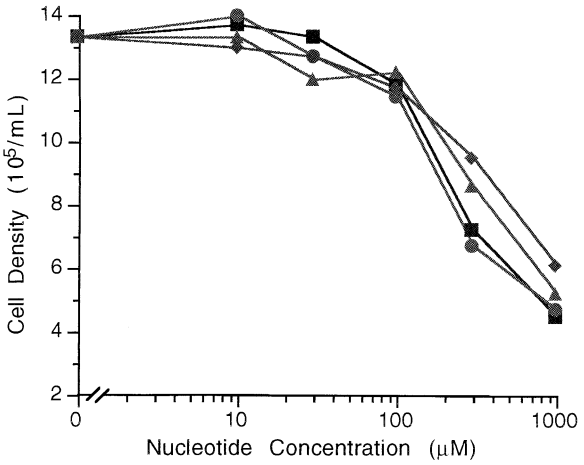

FIG. 2. Concentration dependence of adenosine- and adenine nucleotide-induced growth inhibition. HL-60 cells were cultured under standard conditions for 4 days. Nucleotides and adenosine were introduced on day zero into the cultures at the initial concentrations shown. The initial cell density was 2×10^5/mL (●, ATP; ■, ADP; ▲, AMP; ◆, adenosine). The data show the means of three experiments. The standard error bars (≤20%) have been omitted for clarity.

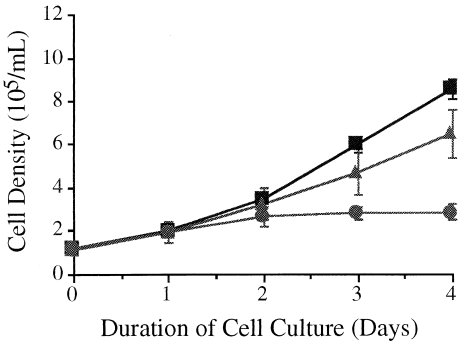

FIG. 4. Effect of the stable ATP analog α,β-methylene ATP on HL-60 cell growth suppression. HL-60 cells were cultured under standard conditions in the absence (■) or presence of 0.5 mM α,β-methylene ATP (▲) or 0.5 mM ATP (●). The data are means ± SEM (N = 4).

9.7 ± 3.2 amol/cell (N = 5; Student's unpaired *t*-test: $P = 0.01$) after 4 days of culture, but there was no significant effect on purine nucleotides. The inhibitory effect of extracellular ATP (1 mM, 4 days) on intracellular UTP levels was reversed by inclusion of 0.1 mM uridine in the culture medium; the intracellular UTP level in ATP-treated cells co-incubated with uridine was 114 ± 56 amol/cell (N = 3; $P = 0.04$ compared with ATP alone).

Uridine Reversal of ATP-Induced Growth Suppression

The finding that uridine reversed ATP-induced pyrimidine starvation prompted us to investigate whether uridine might also reverse ATP-induced growth suppression of HL-60 cells. Inclusion of uridine (1–1000 μM) in the culture medium reversed ATP-induced growth suppression in a time- (Fig. 5A) and concentration (Fig. 5B)-dependent manner. At 4 days, the EC_{50} was approximately 5 μM. Uridine (up to 1 mM) also reversed the growth-suppressing effect of adenosine (0.1–0.5 mM), but not at 1 mM adenosine. Uridine had a small growth-promoting effect on control cells (Fig. 5). These data indicate that HL-60 cells are partially starved of pyrimidines under control conditions, but that adenine nucleotides, by acting as a source of adenosine for cellular accumulation, induce severe pyrimidine starvation leading to growth arrest.

Effects of Inhibitors of Adenosine Transport

HL-60 cells are known to express a minimum of three nucleoside transporters including hENT1 (previously *ES*), hENT2 (previously *EI*), and Na^+-dependent nucleoside transporters. Inhibitors of the hENT1 (NBTI, 1 μM; dipyridamole 10 μM) and hENT2 (dipyridamole, 10 μM) transporters did not prevent ATP-induced growth suppression (data not shown). These data suggest a role for a different nucleoside transporter in adenine nucleotide-induced growth suppression. The Na^+-dependent nucleoside transporter, for which inhibitors are currently not available, is a candidate.

Effects of Extracellular ATP and Its Analogs on HL-60 Cell Differentiation

Exposure of HL-60 cells to extracellular ATP (initial concentration 10–1000 μM) induced differentiation of HL-60 cells as revealed by fMLP-induced β-glucuronidase release (Fig. 6) after 2 days in culture. Uridine, which reversed ATP-induced growth suppression of HL-60 cells, had no effect on ATP-induced differentiation (Fig. 6A). ATP-treated (1 mM, 2 days) HL-60 cells released 15.6 ± 1.7% (N = 5) of their β-glucuronidase content in response to 1 μM fMLP. ATP-treated cells cultured in the presence of 1 mM uridine released 15.4 ± 0.8% (N = 8) of their β-glucuronidase content in response to 1 μM fMLP. Uridine had no effect on fMLP-induced β-glucuronidase release in untreated cells, which was 3.5 ± 0.6% (N = 6) in the absence of uridine and 2.7 ± 0.3% (N = 4) in the presence of 1 mM uridine (Fig. 6A).

As described above, the stable analog of ATP, α,β-methylene ATP, had little or no effect on cell growth. However, α,β-methylene ATP (1 mM) induced differentiation of HL-60 cells with an efficacy similar to ATP as assessed by fMLP-induced β-glucuronidase release. Whereas ATP-treated (1 mM, 2 days) cells released 15.4 ± 1.8% (N = 5) β-glucuronidase in response to 1 μM fMLP, α,β-methylene ATP-treated (1 mM, 2 days) cells released 11.8 ± 1.2% (N = 6).

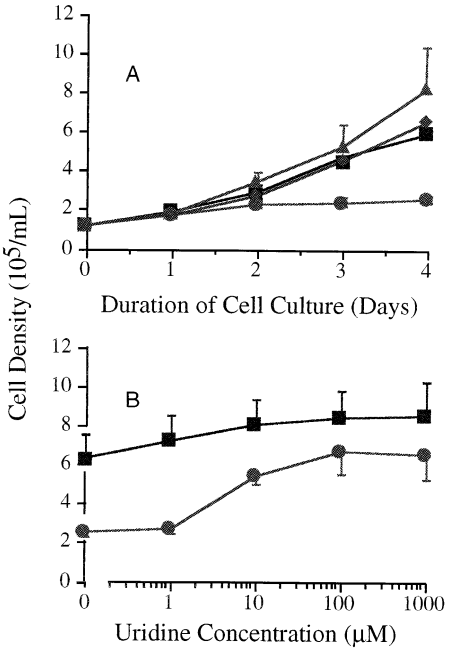

FIG. 5. Effect of uridine on ATP-induced growth suppression of HL-60 cells. HL-60 cells were cultured under standard conditions in the absence or presence of ATP (initial concentration 1 mM). (A) Time-dependent reversal of ATP-induced growth suppression by 1.0 mM uridine (■, control cells; ●, ATP-treated cells; ▲, control cells plus uridine; ◆, ATP-treated cells plus uridine). (B) Concentration dependence of the effects of uridine on control and ATP-treated cells after culture for four days (■, control cells; ●, ATP-treated cells). The data are means ± SEM (N = 4).

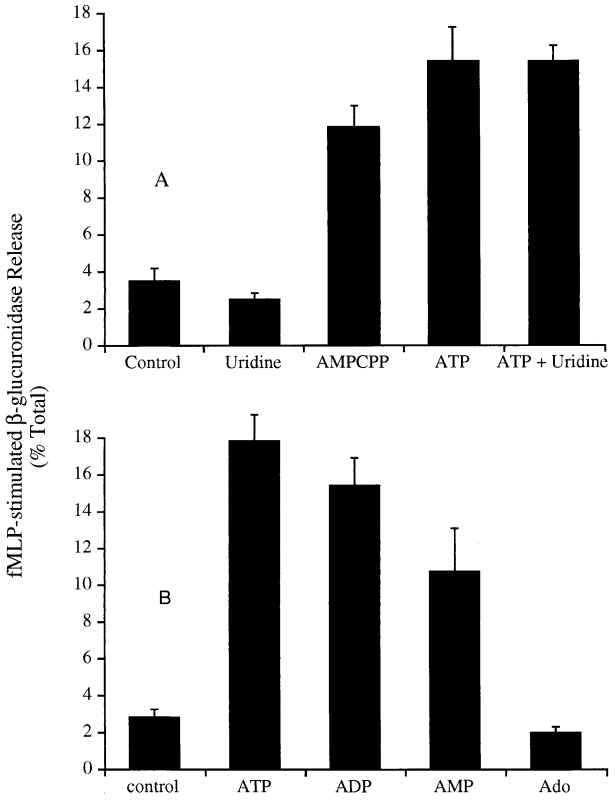

FIG. 6. Effect of pre-exposure of HL-60 cells to adenine nucleotides on fMLP-stimulated β-glucuronidase release in the presence and absence of uridine. In (A), HL-60 cells were cultured under standard conditions in the absence or presence of uridine (0.1 mM), AMPCPP (α,β-methylene ATP; 1 mM), ATP (1 mM), and ATP plus uridine. In (B), HL-60 cells were cultured in uridine (0.1 mM)-containing medium under standard conditions in the absence or presence of ATP, ADP, AMP, or adenosine (Ado) (all at an initial concentration of 1 mM). After 2 days in both (A) and (B), the cells were harvested and washed and then preincubated with cytochalasin B prior to exposure to 1 μM fMLP at 37° for 10 min in physiological saline solution. β-Glucuronidase released into the medium was assayed using 4-methylumbelliferyl-β-D-glucuronide as substrate and as a percentage of the β-glucuronidase released by 0.1% Triton X-100. The data are means ± SEM (N = 4–6).

The comparative effects of ATP, ADP, AMP, and adenosine on HL-60 cell differentiation were determined in the presence of uridine (0.1 mM) to eliminate their effects on growth suppression. HL-60 cells exposed to ATP, ADP, and AMP (1 mM, 2 days) all exhibited fMLP-induced β-glucuronidase release (Fig. 6B). The order of effectiveness was ATP > ADP > AMP. Adenosine was without effect when compared to control. Similarly, the $P2Y_2$ receptor activator UTP (up to 1 mM) had no effect on fMLP-stimulated β-glucuronidase release (not shown).

Effects of Rp-8CPT-cAMPS on ATP-Induced Growth Suppression and Differentiation

We previously demonstrated a novel P2 receptor on HL-60 cells that is linked to activation of adenylyl cyclase [1, 5], and this receptor has been identified with the recently cloned $P2Y_{11}$ receptor [6]. The receptor is activated by adenine nucleotides with the potency order ATP ($EC_{50} \approx$ 100 μM) > ADP > AMP and is also activated by the stable analog α,β-methylene ATP ($EC_{50} \approx$ 300 μM). Activation of this receptor on HL-60 cells leads to the production of cAMP, a recognised differentiating agent for HL-60 cells. We used the cell-permeable PK-A inhibitor, Rp-8CPT-cAMPS, to test whether protein kinase A (PK-A) was involved in ATP-induced growth suppression or differentiation. Whereas Rp-8CPT-cAMPS (1–100 μM) had no effect on ATP-induced growth suppression over the succeeding 2–4 days, it suppressed ATP-induced β-glucuronidase release in a concentration-dependent fashion (Fig. 7). The IC_{50} for this effect was 2.3 ± 0.7 μM (SEM; N = 3).

DISCUSSION

In this study, we found that extracellular ATP suppressed the proliferation and stimulated the differentiation of HL-60 cells by distinct mechanisms. The growth-suppressing effect of extracellular ATP arose from pyrimidine starvation and was reversed by inclusion of uridine in the culture medium. Differentiation, however, arose from a distinct mechanism which was not blocked by uridine but was, instead, suppressed by the cell-permeable protein kinase A inhibitor, Rp-CPT-cAMPS.

Growth-inhibitory effects of adenine nucleotides have been described in a variety of cell types [2–4]. The proposed mechanisms, however, differ markedly. In some cases, it has been speculated that surface P2 receptors were responsible

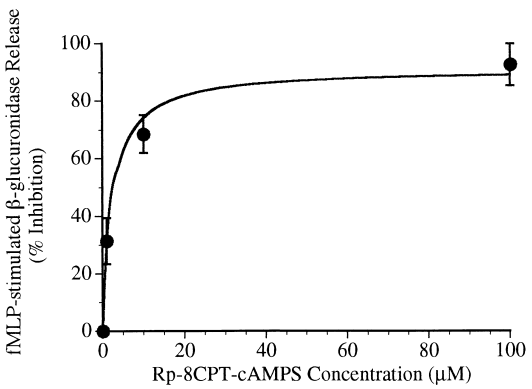

FIG. 7. Concentration-dependent inhibition of ATP-induced differentiation of HL-60 cells by Rp-8CPT-cAMPS. HL-60 cells were cultured at an initial cell density of 3×10^5/mL in the presence of ATP (initial concentration 1.0 mM) either in the absence or presence of the cell-permeable inhibitor of protein kinase A, Rp-8CPT-cAMPS (1–100 μM). Where included, cells were exposed to the PK-A inhibitor for 20 min prior to the addition of ATP. After 2 days, the cells were harvested, treated with cytochalasin B, and then assayed for fMLP-stimulated release of β-glucuronidase into the medium. fMLP-stimulated β-glucuronidase release was 12 ± 2% in ATP-treated cells in the absence of Rp-8CPT-cAMPS. The data are means ± SEM (N = 3).

(e.g. [2]). This hypothesis does not sit well, however, with recent data indicating a mitogenic role for P2 receptors [11–13]. In other cases, the intermediate generation of adenosine has been thought responsible, acting perhaps via surface P1 receptors [14]. In 3T6 fibroblasts, a persuasive case was made for ATP breakdown to adenosine followed by its intracellular accumulation and pyrimidine starvation [4]. This latter mechanism was found to be responsible for adenine nucleotide-induced growth suppression in HL-60 cells. In addition to its growth-inhibitory effect, extracellular ATP induced pyrimidine starvation. Incubation of ATP-treated cells with the pyrimidine nucleoside uridine (1–1000 μM) reversed ATP-induced pyrimidine starvation (Fig. 5). Uridine also reversed the growth-inhibitory effects of ATP and adenosine except at the highest adenosine concentrations tested (≥1 mM). Uridine has recently been reported to reverse adenosine-induced growth inhibition of HL-60 cells [15]. The failure of uridine to reverse adenosine-induced growth suppression at the highest adenosine concentrations is consistent with the observation that high concentrations of adenosine (≥1 mM) induce apoptosis in HL-60 cells [16], perhaps via activation of adenosine A3 receptors [17]. Unlike the situation in 3T6 cells, in which ATP-induced growth suppression was reversed by the hENT1 nucleoside transport inhibitor NBTI, the growth-suppressing effects of adenine nucleotides in HL-60 cells were not reversed by NBTI (1 μM) or the hENT1 and hENT2 nucleoside transport inhibitor, dipyridamole (10 μM). These data imply that HL-60 cells express an additional nucleoside transporter that is responsible for adenosine accumulation and the subsequent pyrimidine starvation and growth suppression. In addition to the facilitative hENT1 and hENT2 transporters, HL-60 cells are known to express a Na^+-dependent nucleoside transporter which is dependent upon the plasma membrane Na^+-gradient—it is present in undifferentiated cells and up-regulated upon differentiation [18, 19]. This transporter, for which selective inhibitors are currently not available, is a potential mediator of the extracellular ATP-dependent effects on growth and cellular pyrimidine levels.

Extracellular ATP-induced HL-60 cell differentiation was detected by the appearance of fMLP-induced β-glucuronidase release. The effect of adenine nucleotides (initial concentrations all 1 mM) on fMLP-stimulated β-glucuronidase release exhibited the efficacy order ATP > ADP > AMP. Adenosine, which was an equipotent inhibitor of cell growth, was, however, without effect. Consistent with the idea that the effect is selective for ATP, the stable analog of ATP, α,β-methylene ATP, also activated HL-60 cell differentiation. The $P2Y_2$ receptor activator, UTP, however, was ineffective. Furthermore, uridine (1–1000 μM), which reversed the growth-inhibitory effect of adenine nucleotides on HL-60 cells, had no effect on ATP-induced differentiation. Strikingly, ATP-induced differentiation was abolished by the cell-permeable PK-A inhibitor Rp-8CPT-cAMPS (1–100 μM). Rp-8CPT-cAMPS had no effect, however, on ATP-induced growth suppression. The data are consistent with the hypothesis that ATP-induced differentiation arises from the activation of a surface receptor coupled to the accumulation of cAMP and the consequent activation of protein kinase A. We, along with others [1, 20], previously identified a P2 receptor on HL-60 cells that is coupled to adenylyl cyclase activation. The pharmacological profile of this receptor (ATP > ADP ≈ α,β-methylene ATP > AMP) [5] together with its resistance to the cyclooxygenase inhibitor indomethacin, which inhibits $P2Y_2$-stimulated cAMP accumulation in MDCK cells [21], indicated the expression of a novel P2 receptor coupled to the activation of adenylyl cyclase. A seven transmembrane receptor that is a good candidate for these effects was recently cloned and shown to couple to adenylyl cyclase when expressed in CHO-K1 and 1321N1 astrocytoma cells [6]. This receptor, currently known as $P2Y_{11}$, is expressed by HL-60 cells [6], and recent work on the pharmacology of $P2Y_{11}$ receptors expressed in a variety of cell types confirms the identity of the cAMP-linked P2 receptor in HL-60 cells as $P2Y_{11}$ ([7, 8], The data in the current study indicate that this receptor plays a critical role in ATP-induced differentiation of HL-60 cells by activating adenylyl cyclase, leading to cAMP accumulation and the activation of protein kinase A. The role that the $P2Y_{11}$ receptor might play in white cell maturation is currently unknown but its message is rapidly up-regulated in response to key granulocytic differentiating agents including granulocyte-colony stimulating factor [22].

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