

Effects of purine and pyrimidine nucleotides on intracellular Ca^{2+} in human eosinophils: Activation of purinergic P2Y receptors

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Background: Extracellular adenosine 5'-triphosphate (ATP) increases human eosinophil intracellular Ca^{2+} concentration; the mechanism of action is not fully known. ATP, a physiologic regulator, acts through 2 purinergic receptor types: cation channels (P2X) and G protein-coupled receptors (P2Y).

Objective: This study is aimed at identifying the functional purinergic receptors in human eosinophils.

Methods: The relative potency of ATP, uridine (UTP), cytidine (CTP), and inosine (ITP) 5'-triphosphates (P2Y agonists); 2-methylthio-ATP (P2Y₁ agonist); and 2 P2X agonists, α,β -methylene-ATP and β,γ -methylene-ATP on intracellular Ca^{2+} concentration was examined in Ca^{2+} -sensitive Fura-2-labeled human eosinophils. For comparison, ATP effects were similarly studied in human neutrophils. P2X/P2Y mRNA expression in cells was examined by reverse transcription and PCR.

Results: The nucleotide potency order was $\text{UTP} \geq \text{ATP} > \text{ITP} \gg 2\text{-methylthio-ATP} > \alpha,\beta\text{-methylene-ATP} = \beta,\gamma\text{-methylene-ATP} = \text{CTP} = 0$ in eosinophils. Pertussis toxin (500 ng/mL) pretreatment abolished the effect of lower (10^{-6} mol/L) but not higher (10^{-5} mol/L) concentrations of ATP in eosinophils, whereas it attenuated the effects of 10^{-4} mol/L ATP in neutrophils. The phospholipase C inhibitor U73122 (2 $\mu\text{mol/L}$) partially inhibited the effect of ATP in eosinophils but totally blocked it in neutrophils. Both cells constitutively express mRNA for P2X₁, P2X₄, P2X₅, P2Y₁, and P2Y₂, but not P2X₇, with much weaker expressions of P2X₄ and P2X₅ in neutrophils. Eosinophils cultured with the T_H1 cytokine, IFN- γ , expressed mRNA for P2X₇, a receptor linked to apoptosis. **Conclusions:** These results suggest that the P2 purinergic receptor signal transduction pathways in eosinophils and neutrophils are different and are mediated by more than 1 subtype of functional P2Y receptors. (J Allergy Clin Immunol 2001;107:849-55.)

Key words: ATP, UTP, ITP, CTP, P2, purinergic receptor, eosinophils, neutrophils, intracellular calcium, pertussis toxin, U73122

Adenosine 5'-triphosphate (ATP) and its metabolites are released into the extracellular environment from several cell types under physiologic and pathophysiologic conditions.¹ Extracellular ATP acts on effector cells in vivo through cell surface receptors called P2 purinergic receptors linked to specific signal transduction pathways.^{1,2} P2 receptors are classified into 2 families: P2X and P2Y. P2X consists of ATP-gated cation channels, and P2Y consists of G protein-coupled receptors. Each family has multiple members (P2X_{1-n} and P2Y_{1-n}), which have been cloned and characterized on the basis of potency rank order of ATP and its analogs in inducing cell-specific effects.³

Activation and recruitment of eosinophils commonly occurs in asthma.⁴ It has been reported that extracellular ATP can increase the intracellular calcium ion concentration ($[\text{Ca}^{2+}]_i$) in cultured eosinophils derived from human cord blood,⁵ and this action of ATP results in a strong chemotactic response in blood eosinophils.⁶ However, the mechanism of action of ATP on human eosinophils, including culpable receptors and signal transduction pathways, awaits delineation. We have determined the function and expression of P2 purinergic receptors in human eosinophils obtained from donors with a history of mild allergy and/or asthma, using pharmacologic and molecular techniques. For comparison, similar studies were performed in neutrophils, another type of granulocyte, derived from same donors. Part of this work has been presented as an abstract.⁷

METHODS

Materials

The following materials were purchased: ATP; uridine 5'-triphosphate (UTP); inosine 5'-triphosphate (ITP); cytidine 5'-triphosphate (CTP); α,β -methylene-ATP ($\alpha,\beta\text{mATP}$); β,γ -methylene ATP ($\beta,\gamma\text{mATP}$); 2-methylthio-ATP (2mS-ATP); pertussis toxin (PTX); dexamethasone; Histopaque 1.077; FCS; HBSS (Sigma Chemical Co, St Louis, Mo); U73122 (BIOMOL, Plymouth Meeting, Pa); Fura-2-AM (Molecular Probes, Eugene, Ore); recombinant human IFN- γ ; IL-5; and GM-CSF (R&D Systems, Minneapolis, Minn). Other chemicals and buffer salts were purchased as analytical grade reagents from Fisher Scientific (Pittsburgh, Pa).

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Supported by NIH grants AI-33941 (D.G.R.) and AI-20634 (E.S.S.) and the Margaret Wolf Memorial Fund.

Received for publication September 28, 2000; revised January 19, 2001; accepted for publication January 19, 2001.

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0091-6749/2001 \$35.00 + 0 1/84/114658

doi:10.1067/mai.2001.114658

Abbreviations used

ATP:	Adenosine 5'-triphosphate
[Ca ²⁺] _i :	Intracellular Ca ²⁺ concentration
CTP:	Cytidine 5'-triphosphate
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
ITP:	Inosine 5'-triphosphate
α,βmATP:	α,β-Methylene-ATP
β,γmATP:	β,γ-Methylene-ATP
2mS-ATP:	2-Methylthio-ATP
PLC:	Phospholipase C
PTX:	Pertussis toxin
RT-PCR:	Reverse transcription and PCR
UTP:	Uridine 5'-triphosphate

Cells

Venous blood of human volunteers with a history of mild allergy and/or asthma was collected (approved by the institutional review board) for the isolation of eosinophils and neutrophils. Eosinophils were purified from blood by Histopaque 1.077 density centrifugation and negative immunomagnetic separation method as previously described.⁸ Eosinophil purity was routinely 97% or greater. When both eosinophils and neutrophils were required, neutrophils (density 1.080 layer [$>98\%$]) were obtained from blood by discontinuous Percoll density gradient centrifugation as reported earlier.⁹ Pooled eosinophil-enriched layers (density 1.090 and 1.095) were then subjected to immunomagnetic removal of contaminating neutrophils as described above to obtain purified eosinophils of apparent homogeneity ($\sim 100\%$). For cytokine experiments, eosinophils suspended in RPMI 1640 medium ($\sim 2.5 \times 10^6$ cells/mL) containing 10% FCS and gentamicin (50 $\mu\text{g/mL}$) were cultured (5% CO₂, 37°C) in a non-tissue-culture-treated 24-well polystyrene plate (Corning Costar, Corning, NY), with or without any one of the following: dexamethasone (1 $\mu\text{mol/L}$), recombinant IFN- γ (5000 U/mL), IL-5 (10 ng/mL), or GM-CSF (10 ng/mL). After 18 hours of culture, cells were harvested and counted, and their viability was determined by trypan blue exclusion.

Human lung macrophages ($\geq 99.0\%$) were isolated as described earlier.¹⁰ Peripheral blood mononuclear (mostly lymphocytes) cells from the blood of a patient with chronic lymphocytic leukemia were isolated by Histopaque 1.077 density centrifugation.

Assays

Measurements of [Ca²⁺]_i in eosinophils and neutrophils were performed in HEPES buffer (133 mmol/L NaCl, 6 mmol/L KCl, 1 mmol/L MgSO₄, 1 mmol/L CaCl₂, 20 mmol/L HEPES, 1.2 mmol/L potassium phosphate, 5.5 mmol/L dextrose, and 0.1% [wt/vol] BSA, pH 7.4) as reported earlier.⁹

For reverse transcription and PCR (RT-PCR) analysis of P2 receptor mRNA expression, reverse transcription of total cellular RNA and amplification of cDNA was performed as previously reported.¹¹ In brief, total RNA from eosinophils or other cells was isolated by standard guanidinium isothiocyanate lysis, phenol/chloroform extraction, and isopropyl alcohol precipitation. cDNA from total RNA was prepared by reverse transcription with poly-dT (15-mer) as primer and Moloney murine leukemia virus reverse transcriptase. Negative control reaction for reverse transcription was also run in which no enzyme was added, and the resulting reaction mixture was used for PCR to check for genomic DNA contamination. The primers for the P2 receptors were as follows:

P2X₁, 5'-CACTTTGTGGAGAACGGGACAACACTAC-3'-sense,
5'-CCC-ATGTCCTCAGCGTATTGAACTTC-3'-antisense;
P2X₄, 5'-TG-CTCATCCGACGCCGAAAGT-3'-sense,
5'-AGTGGTTCGATCTGGAATCTCGG-3'-antisense;

P2X₅, 5'-TCCTGGCGTACCTGGTCGTATGG-3'-sense,
5'-CAGTCGCTGTCCTTGGAGCACG-3'-antisense;
P2X₇, 5'-TGAAGTCTCTGCCTGGTGCCCAT-3'-sense,
5'-CGGAAAATGGGACACTGTGGATTCTG-3'-antisense;
P2Y₁, 5'-CCGCCGTCTCCTCGTCGTTCAAAT-3'-sense,
5'-TTGAGGGGGTACACCACACCGCTGA-3'-antisense; and
P2Y₂, 5'-GCCCTTTGCCGTCATCCTTGTC-3'-sense,
5'-TCCAGCGAGCGGAAGGAGTAGTAG-3'-antisense.

These were designed on the basis of published cDNA sequences (Genbank, NIH, Bethesda, Md) and were purchased from Operon Inc (Alameda, Calif). For PCR, a 3-step protocol of 30 cycles (not found to be saturating) of denaturation (30 seconds at 94°C), annealing (30 seconds at 65°C, except at 60°C for P2Y₂), and polymerization (30 seconds at 72°C), followed by a 5-minute extension at 72°C was used. Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a constitutively expressed "housekeeping" gene, was also performed as a control. For negative controls in PCR, similar reactions were run without any cDNA added to the PCR reaction.

RNase protection assay was performed as reported earlier.¹² In brief, PCR products of P2X₇ and GAPDH genes were purified, ligated to TA cloning vectors (PCR-II; Invitrogen, Carlsbad, Calif) and cloned in *Escherichia coli*. Plasmids containing DNA templates for both P2X₇ and GAPDH were used to synthesize respective ³²P-labeled antisense RNA probes. Total cellular RNA (5–10 μg) was mixed with 2 μL Pellet Paint (Novagen, Madison, Wis), lyophilized, dissolved in hybridization buffer containing 80% formamide, mixed with ³²P-labeled probes for P2X₇ and GAPDH, and incubated at 45° to 50°C overnight; the next day, RNase-A was added to digest the unhybridized RNA, which was then treated with proteinase K in SDS-containing buffer to remove RNase. Duplex RNA was isolated by phenol-chloroform extraction, precipitated by ethanol, dissolved in gel-loading buffer, and analyzed by electrophoresis on 5% acrylamide-8M urea gels followed by autoradiography.

RESULTS**Effect of nucleotides on human eosinophils and neutrophils**

P2 purinergic receptors are known to be activated by purine (eg, ATP) and/or pyrimidine (eg, UTP, CTP) nucleotides.¹³ Therefore we compared the effects of ATP (a P2X and P2Y agonist) and UTP (a P2Y agonist)¹⁴ on [Ca²⁺]_i in Fura-2 loaded eosinophils. As shown in Fig 1, both ATP and UTP (10^{-7} – 10^{-4} mol/L) caused a significant rise in [Ca²⁺]_i as compared with the P2Y₁ agonist^{15–17} 2mS-ATP, which was only minimally effective ($\geq 3 \times 10^{-4}$ mol/L). Results are expressed as the increase from the baseline value of [Ca²⁺]_i (range 100–150 nmol/L). At the lower and upper parts of the dose-response curves, the potencies were similar; however, UTP was significantly more potent than ATP at around 10^{-6} mol/L ($P < .04$). The apparent median effective concentration values for UTP and ATP were approximately 6×10^{-7} mol/L and 10^{-6} mol/L, respectively. Among the other naturally occurring nucleotides, ITP (10^{-4} mol/L), reported to be a P2Y₂ agonist,¹⁸ elicited a weaker response than ATP, whereas CTP (10^{-4} mol/L) did not have any effect ($n = 2$, data not shown).

α,βmATP and β,γmATP are potent agonists at P2X₁ and P2X₃ receptors but have little effect on P2Y receptors.¹⁹ Neither compound ($\leq 10^{-4}$ mol/L) affected [Ca²⁺]_i in eosinophils ($n = 2$, data not shown). P2X₁ and P2X₃ recep-

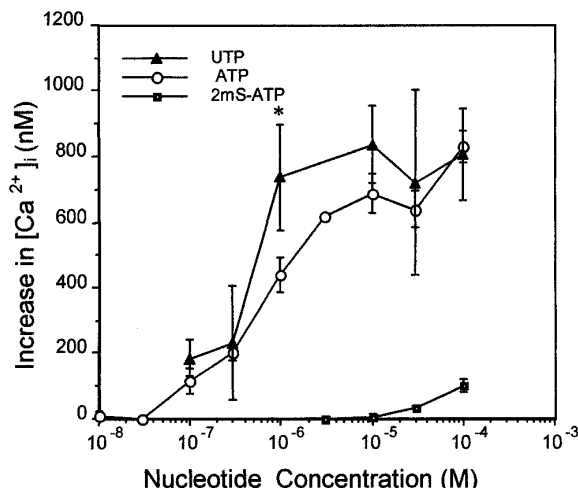


FIG 1. Dose-response effect of ATP ($n = 2-9$), UTP ($n = 2-5$) and 2mS-ATP ($n = 3$) on human eosinophils. The difference between the effects of ATP and UTP is statistically significant only at 10^{-6} mol/L (* $P < .05$).

tors are known to get desensitized quickly by extracellular ATP, even by small, "nonpharmacologic" quantities of ATP constitutively released from cells.¹⁹ To eliminate this potential desensitization of P2X₁ and P2X₃ receptors, we repeated these experiments in the presence and absence of apyrase (which degrades extracellular ATP). Even with apyrase pretreatment, there was no increase in $[Ca^{2+}]_i$ caused by α, β mATP (data not shown), strongly suggesting the lack of P2X₁ and P2X₃ involvement.

The ATP-induced increase in $[Ca^{2+}]_i$ can occur as a result of the release of Ca^{2+} from intracellular stores, influx of external Ca^{2+} through transmembrane ionic channels, or both. To clarify which pathway or pathways may be involved in its action, we investigated the effect of ATP on eosinophils suspended in HEPES buffer containing 1 mmol/L $MgCl_2$ and 0.5 mmol/L EGTA but no Ca^{2+} . Removal of extracellular Ca^{2+} significantly attenuated, but did not eliminate, the ATP-induced increase in $[Ca^{2+}]_i$ (Fig 2). It appears that there is a dose-dependent ATP-induced (beginning at 10^{-8} mol/L) release of Ca^{2+} ions from intracellular stores, which plateaus in the absence of extracellular Ca^{2+} . In the presence of extracellular Ca^{2+} , the effect of ATP is larger; this difference in ATP response is statistically significant ($P < .05$) at concentrations of 10^{-5} mol/L or higher.

On the basis of the above differential effects on $[Ca^{2+}]_i$, we further investigated the potential P2 receptor signal transduction pathways in human eosinophils by determining the effects of PTX²⁰ and a phospholipase C (PLC) inhibitor U73122²¹ on the release of Ca^{2+} from internal stores (10^{-6} mol/L ATP) and on Ca^{2+} influx ($\geq 10^{-5}$ mol/L ATP). Fig 3, A, shows that PTX (500 ng/mL) pretreatment almost abolished the increase in $[Ca^{2+}]_i$ caused by 10^{-6} mol/L ATP and markedly attenuated (by 70%-80%) the effect of 10^{-5} mol/L ATP. The concentration-dependent

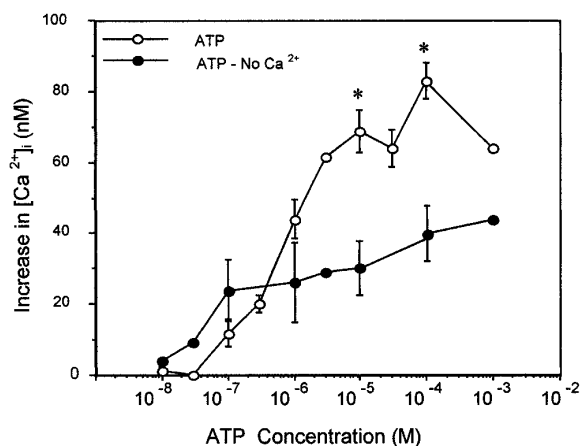


FIG 2. ATP-stimulated increase in cytosolic calcium ion in eosinophils in the presence (open circles) and absence (closed circles) of extracellular Ca^{2+} ($n = 2-5$). *Statistically significant ($P < .05$).

differences between the effects of 10^{-5} and 10^{-6} mol/L ATP were statistically significant ($P = .013$ and $P = .048$ in the presence and absence of extracellular Ca^{2+} , respectively). PTX-treated eosinophils were viable, as determined by trypan blue exclusion. Fig 3, B, shows that preincubation of eosinophils with the PLC inhibitor U73122 (2 μ mol/L) only partially inhibited the ATP-stimulated (10^{-4} and 10^{-6} mol/L) increase in $[Ca^{2+}]_i$. In both cases removal of extracellular Ca^{2+} reduced the level of $[Ca^{2+}]_i$ but did not abolish the effect of ATP. In contrast, both PTX and U73122 almost abolished the effect of ATP even at higher concentrations (up to 10^{-4} mol/L) in neutrophils (Fig 4), suggesting that different P2 purinergic receptors/signal transduction pathways mediate ATP's effect in eosinophils and neutrophils.

P2 receptor mRNA expression by RT-PCR

To further investigate ATP's signal transduction, we examined the constitutive expression of mRNA of P2 receptors in eosinophils (~100% purity), as has been recently reported for other types of immune cells.²² As shown in Fig 5, RT-PCR of RNA isolated from each human eosinophil preparation indicated pronounced expression of mRNA for P2X₁, P2X₅, and P2Y₂ ($n = 5$). The expressions for P2X₄ and P2Y₁ were less intense, whereas that for P2X₇ (P2Z) was not detected. For comparison, similar experiments ($n = 3$) were performed with nearly homogeneous human neutrophils (~100% purity). In each experiment, neutrophils clearly expressed mRNA for P2X₁ and P2Y₁, whereas mRNA for P2X₄, P2X₅, and P2Y₂ were weakly expressed, and that of P2X₇ (P2Z) was not detected. Thus eosinophils and neutrophils differ in their P2 receptor mRNA expression, which may underlie the differential response of signal transduction perturbations in these cells (Figs 3 and 4).

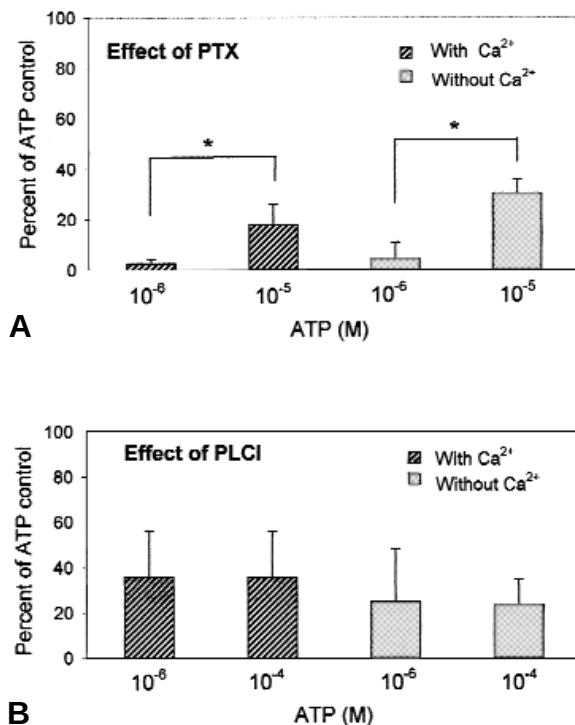


FIG 3. Effect of PTX and the PLC inhibitor (PLCI) U73122 on the ATP-stimulated increase in $[Ca^{2+}]_i$ in eosinophils. **A**, Purified eosinophils were preincubated $\pm$ 500 ng/mL PTX for 2 hours at 37°C. During the last 40 minutes of the preincubation, cells were loaded with FURA-2-AM. Differences in values at data points marked with asterisks are statistically significant ($P < .05$). **B**, Purified eosinophils were preincubated with $\pm$ 2 μ M U73122 for 3 minutes before ATP was added. The response to ATP (mean $\pm$ SE, $n = 4$) was measured as in Figs 1 and 2. Percent of ATP control implies the percentage of the effects of ATP only at respective ATP concentrations.

IFN- γ -induced P2X₇ mRNA expression in eosinophils

Because human peripheral blood eosinophils express receptors for the proinflammatory T_H1-type cytokine, IFN- γ ,²³ and IFN- γ has been reported to upregulate the expression of P2X₇ receptor in human macrophages,²⁴ we tested whether overnight culture in IFN- γ has a similar effect on P2X₇ receptor expression in human eosinophils. Effects were compared with those of similar cultures in dexamethasone, IL-5, and GM-CSF. Eosinophil purity was apparently homogeneous at the initiation and conclusion of the culture. Most of the cells cultured overnight with IL-5, IFN- γ , or GM-CSF were viable (>95%), whereas viability of cells cultured in buffer control and dexamethasone were lower (70%-80%). As shown in Fig 6, A, mRNA for P2X₇ was expressed only in eosinophils cultured with IFN- γ and not in those cultured with GM-CSF, IL-5, or dexamethasone. Because of the oversensitivity and qualitative nature of PCR, an alternative method (ie, the RNase protection assay)²⁵ was also performed. This assay confirmed the presence of P2X₇ mRNA only in eosinophils cultured with IFN- γ (Fig 6, B). RNA isolated

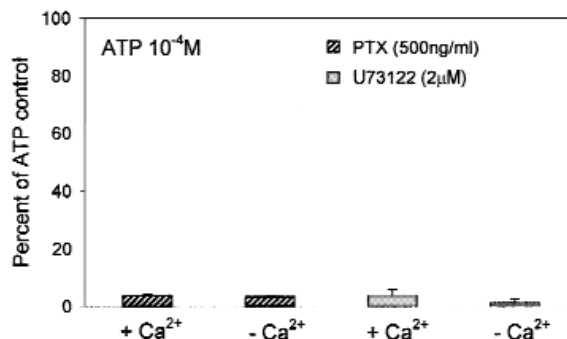


FIG 4. Effect of PTX and the PLC inhibitor U73122 on the ATP (10^{-4} mol/L)-stimulated increase of $[Ca^{2+}]_i$ in neutrophils ($\pm$ SE, $n = 3$). Neutrophils were similarly treated with PTX and PLC inhibitor, as was done with eosinophils in Fig 3. The presence (+ Ca^{2+}) and absence (- Ca^{2+}) of extracellular Ca^{2+} are indicated.

from human lung macrophages and peripheral blood lymphocytes of patients with chronic lymphocytic leukemia as positive controls^{26,27} showed the presence of mRNA for P2X₇ as expected. RT-PCR analysis of mRNA expression for P2X₁, P2X₄, P2X₅, P2X₇, P2Y₁, and P2Y₂ on eosinophils cultured under the above conditions have also been performed. Results show downregulation of mRNA expression for P2X₄ and P2Y₁ in cells cultured with GM-CSF, for P2Y₁ only in cells cultured with IL-5, and for P2X₄ only in cells cultured with dexamethasone; for the rest of the receptors and conditions, mRNA expressions remained unaltered ($n = 3$, data not shown).

DISCUSSION

Eosinophil activation, degranulation, and oxidative burst play a major role in asthma and allergic diseases,^{4,28-31} and activation of the respiratory burst in eosinophils requires Ca^{2+} fluxes.³² ATP and UTP function as extracellular signaling molecules,¹³ and extracellular ATP has been implicated in the cause of asthma.³³ ATP is a potent enhancer of human lung mast cell histamine release.³⁴ Inhaled ATP produces bronchoconstriction at a lower concentration in patients with asthma than in subjects without asthma,³⁵ and intravenous ATP causes pronounced bronchoconstriction in anesthetized dogs.³⁶ The mechanisms of these actions are not fully known.

Our study shows that ATP, UTP, and ITP stimulate human peripheral blood eosinophils causing a pronounced and transient rise in $[Ca^{2+}]_i$. The dose-response relationship of the effect of ATP and other nucleotides in human eosinophils manifested the following potency rank order: UTP $\geq$ ATP $>$ ITP $\gg \gg$ 2mS-ATP $>$ α , β mATP = β , γ mATP = CTP = 0. This pattern suggests the presence of a functional P2Y₂ receptor subtype in these cells.^{14,37} UTP can act on the P2Y₄ receptor subtype; however, it is unlikely to be functional in human eosinophils because ATP is reported to be an antagonist at this receptor.³⁸ The lack of response to α , β mATP suggests a lack of functional receptors for either P2X₁ or P2X₃ despite mRNA expression being found for the for-

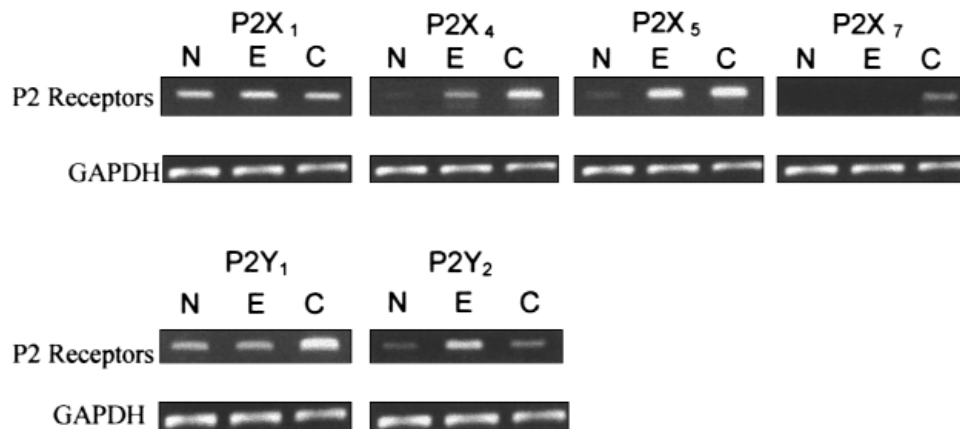


FIG 5. P2 Receptor mRNA expression in human eosinophils and neutrophils as detected by RT-PCR. *N*, Neutrophil; *E*, eosinophil; *C*, control.

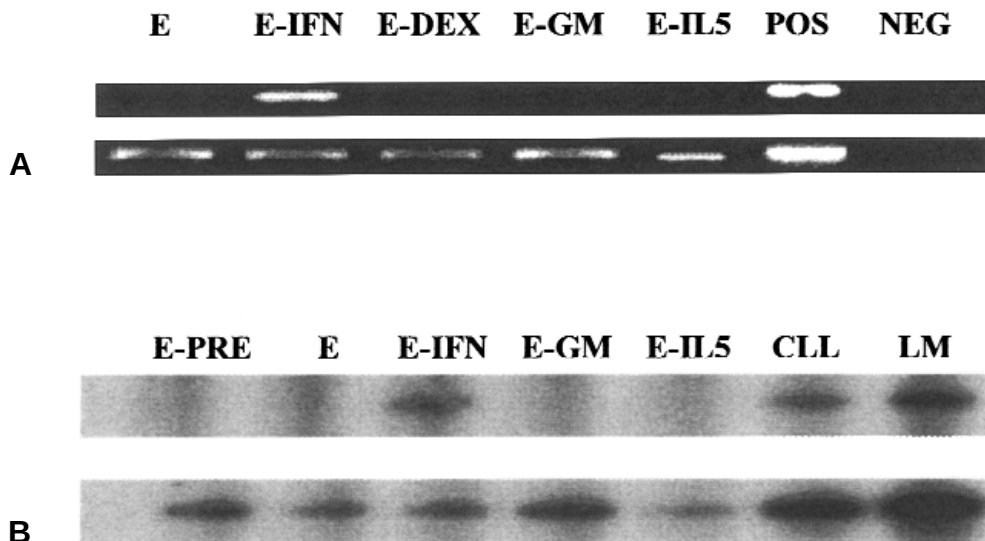


FIG 6. RT-PCR (**A**) and RNase protection assay (**B**) showing P2X₇ mRNA expression in eosinophils cultured with medium alone (*E*), IFN- γ (*E-IFN*), dexamethasone (*E-DEX*), GM-CSF (*E-GM*), and IL-5 (*E-IL5*). In RT-PCR, *POS* and *NEG* indicate PCR of cDNA prepared from total RNA of normal human peripheral blood mononuclear cells (positive control) and no cDNA (negative control), respectively. In the RNase protection assay, total RNA from eosinophils before culture (*E-PRE*) served as one of the negative controls, and that from chronic lymphocytic leukemia lymphocytes (*CLL*) and lung macrophages (*LM*) served as positive control for the expression of P2X₇.

mer receptor subtype. These results do not exclude the possible presence of functional P2X₄ and P2X₅ receptors because these are not sensitive to α,β mATP. The ATP-induced $[Ca^{2+}]_i$ changes in eosinophils in the presence versus the absence of extracellular Ca^{2+} are in contrast with previous findings involving nonnucleotide ligands. We showed earlier that removal of external Ca^{2+} has no effect on histamine or PGD₂-stimulated increases in $[Ca^{2+}]_i$ in eosinophils.³⁹ Others have also shown that the absence of external Ca^{2+} has no effect on the calcium-mobilizing activity of the eosinophil chemotactic factors platelet-activating factor (PAF), chemokine regulated upon activation normal T cell expressed and secreted

(RANTES), or complement-derived anaphylatoxin peptide (C5a).^{32,40} These results suggest that ATP has a unique mechanism of action, different from mechanisms of other activators of eosinophils studied heretofore.

The effects of lower concentrations of ATP (10⁻⁶ mol/L) on eosinophils were sensitive to PTX (Fig 3, A), giving additional support to the conclusion that the functional P2Y receptors are coupled to G proteins ($G_{\alpha i/o}$). However, at higher concentration of ATP (10⁻⁵ mol/L), a PTX-insensitive component remains, which did not change even after the removal of extracellular Ca^{2+} . These findings could be explained by a dose-dependent activation of different signal transduction pathways. Specific

ly, at a relatively low concentration ($\sim 10^{-6}$ mol/L), ATP activates a P2Y receptor coupled to a $G_{\alpha i/o}$ protein, and at higher concentrations ($\geq 10^{-5}$ mol/L), it activates a P2Y receptor coupled to a PTX-insensitive G protein, which might be linked to a different PLC.⁴¹ The effect of PTX on the ATP response in eosinophils is somewhat weaker than its effect on ATP-induced increases in $[Ca^{2+}]_i$ in human neutrophils (Fig 4). In addition, the effects of the PLC inhibitor U73122 on ATP-induced $[Ca^{2+}]_i$ increases in eosinophils and neutrophils are markedly different. Although the response of neutrophils to even high concentrations of ATP (10^{-4} mol/L) is totally blocked by 2 μ mol/L U73122 (Fig 4), there is only a partial inhibition of the ATP-stimulated increase of $[Ca^{2+}]_i$ in eosinophils (Fig 3, B). This could suggest that ATP activates more than one type of PLC in eosinophils, one of which is linked to a PTX-insensitive P2Y receptor.

Results of RT-PCR performed on the total RNA isolated from purified eosinophils and neutrophils suggest the presence of mRNAs for P2X₁, P2X₄, P2X₅, P2Y₁, and P2Y₂. The intensity of bands for P2X₄, P2X₅, and P2Y₂ mRNA are consistently different in eosinophils and neutrophils (Fig 5), whereas P2X₇ mRNA is absent in both cell types.

Data of RT-PCR and RNase protection assays indicate that the T_H1 cytokine IFN- γ induces eosinophils to express mRNA for the P2X₇ receptor (Fig 6), a receptor functionally linked to apoptosis and cell death.^{42,43} In human asthmatic airways, multiple lines of evidence implicate the accumulation of T_H2 cytokines that support eosinophil survival, including GM-CSF and IL-5. Conversely, asthmatic T cells have reduced production of IL-12 and IFN- γ , at levels that correlate with disease activity.⁴⁴ Treatment of asthmatic patients with allergen immunotherapy or corticosteroids increases IFN- γ expression, which might have shifted the airways from a T_H2 bias to a T_H1 bias. This shift may also have implications in the downregulation of IgE-mediated responses that ultimately occur in the natural history of allergic reactions.⁴⁵⁻⁴⁷ Our current data may support one critical mechanism by which IFN- γ controls eosinophil survival.

While this manuscript was being prepared, another study on ATP-induced increase in $[Ca^{2+}]_i$ in eosinophils from human nonatopic donors was published by Dichmann et al,⁴⁸ who claimed that these cells express both functional P2X and functional P2Y receptors. Our results in eosinophils from human donors with a history of allergy and/or asthma add important information regarding the modulation of $[Ca^{2+}]_i$ by extracellular ATP in these cells. Specifically, our wider dose-response range (10^{-8} - 10^{-3} mol/L) established 2 components of ATP's action: one independent and the other dependent on extracellular Ca^{2+} . Results similar to those of the latter component were reported by Dichmann et al, who used 10^{-5} to 10^{-3} mol/L ATP.⁴⁸ In this study we have shown for the first time the effect of UTP suggesting the presence of P2Y₂ receptor, PLC inhibition data suggesting 2 different signal transduction pathways, and mRNA expression data for purinergic receptors in human eosinophils. Furthermore, our data indicate that eosinophils derived from human

subjects with a history of allergy and asthma do not express P2X₇ mRNA, whereas Dichmann et al reported modulation of $[Ca^{2+}]_i$ in response to ATP by oxidized ATP, an agent touted as a P2X₇ antagonist.⁴⁹ However, a recent report suggested that oxidized ATP blocked nitric oxide synthase upregulation and nitric oxide production in lipopolysaccharide-stimulated or bacilli Calmette-Guerin-stimulated macrophages derived from P2X₇ gene-disrupted mice.⁵⁰ Thus oxidized ATP may act through a different mechanism, and its effect on human eosinophils needs further investigation.

These data on human eosinophils, together with other recent in vitro studies in human mast cells³⁴ and in vivo data in airway challenges,^{35,36,51} suggest that action of extracellular ATP and the relevant signal transduction pathways through P2 purinergic receptors may be potential targets in the pharmacologic/therapeutic modulation of human hypersensitivity reactions.

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