P2 purinergic receptors of human eosinophils: characterization and coupling to oxygen radical production

Davide Ferrari[a,1,*], Marco Idzko[b,1], Stefan Dichmann[c], Daniela Purlis[c], Christian Virchow Jr.[b], Johannes Norgauer[c], Paola Chiozzi[a], Francesco Di Virgilio[a,d], Werner Luttmann[b]

[a] *Department of Experimental and Diagnostic Medicine, Section of General Pathology, University of Ferrara, via L. Borsari 46, I-44100 Ferrara, Italy*
[b] *Department of Pneumology, University of Freiburg, Hugstellter-str. 55, Freiburg D-79106, Germany*
[c] *Department of Dermatology, University of Freiburg, Hugstellter-str. 55, Freiburg D-79106, Germany*
[d] *Center of Biotechnology, University of Ferrara, via Fossato di Mortara 64b, I-44100 Ferrara, Italy*

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Abstract Extracellular nucleotides elicit multiple responses in eosinophils but no information on expression of purinergic receptors in these cells is available so far. In the present study we show that human eosinophils express the following P2Y and P2X subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, and $P2X_1$, $P2X_4$, $P2X_7$, whose stimulation results in intracellular Ca^{2+} increase and production of large amounts of reactive oxygen intermediates. These events are stimulated or inhibited, respectively, by P2 receptor agonists or antagonists.

Key words: Inflammation; Eosinophil; Extracellular ATP; Purinoceptor; Oxygen radical

1. Introduction

Eosinophils play a key role in several diseases including asthma [1–4], allergy [5,6] and infections by helminths [7–10]. Physiologic functions of eosinophils are related to their ability to produce, store and release many biologically active molecules such as major basic protein [11] and eosinophil cationic protein [12]. Secretion of granular enzymes and production of mediators is stimulated by different agents such as unopsonized zymosan [13], IgG-Sepharose [14], synthetic peptides mimicking soluble factors produced by bacteria, the complement protein C5a, or the calcium ionophore A23187 [13,15]. Previous reports showed that extracellular nucleotides elicit multiple Ca^{2+}-dependent processes in human eosinophils, ranging from activation of chemotaxis to secretion of intracellular mediators such as leukotriene C_4 [16–18], or *N*-acetyl-β-D-glucosaminidase [19].

Extracellular nucleotides have been recognized as important mediators in many systems, where they trigger different responses via activation of plasma membrane receptors known as P2 purinoceptors [20,21]. On the basis of pharmacological, functional and cloning data, two P2 receptor subfamilies are so far recognized: P2X and P2Y [22–24].

P2X receptors are identified with plasma membrane channels selective for monovalent and divalent cations which are directly activated by extracellular ATP without requiring hydrolysis of the nucleotide or generation of intracellular second messengers [25,26]. These channels were originally identified in mammalian sensory neurons [27], and then also found in smooth muscle cells, mouse fibroblasts and thymocytes [28–30]. Stimulation of P2X receptors causes Ca^{2+} and Na^+ influx with the accompanying plasma membrane depolarization. An interesting member of the P2X subfamily is $P2X_7$. This receptor differs from the other P2X receptors for an extended carboxy-terminal domain that endows $P2X_7$ with the ability to form large plasma membrane pores permeable to small hydrophilic molecules [31].

P2Y receptors are seven membrane-spanning, G-protein-coupled receptors. Their activation triggers generation of inositol 1,4,5-trisphosphate and release of Ca^{2+} from intracellular stores [32–36]. P2Y receptors are ubiquitous, being expressed by monocytes, macrophages, neurons, smooth and striated muscle cells, epithelia and endothelia.

Although different nucleotides have been shown to trigger a Ca^{2+} response in eosinophils, no information on P2 receptor subtype expression is available in these cells, and besides a recent report [37], there is no hint on the possible physiological significance of nucleotide-dependent stimulation. Thus, we decided to perform a functional, pharmacological and biochemical characterization of P2 receptors in eosinophils.

2. Materials and methods

2.1. Reagents

ATP, UTP, CTP, GTP and adenosine were purchased from Boehringer Mannheim (Mannheim, Germany). 2′,3′-(4-Benzoyl)-benzoyl-ATP (BzATP), ATPγS, 2-methylthio-ATP, recombinant human C5a and Ficoll were obtained from Sigma Chemical Co. (Deisenhofen, Germany). Periodate-oxidized ATP (oATP) was a kind gift of Dr. Stefania Hanau, University of Ferrara (Ferrara, Italy). The anti-CD16 monoclonal antibody was from Immunotech (Marseille, France). Immunomagnetic Dynabeads M-450 were obtained from Dianova (Hamburg, Germany).

2.2. Solutions

Fluorescence measurements were performed either in a saline solu-

*Corresponding author. Fax: (39)-0532-247 278.
E-mail: dfr@dns.unife.it

[1] The first two authors contributed equally to the paper.

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tion containing 125 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM Na_2HPO_4, 5.5 mM glucose, 5 mM $NaHCO_3$, 1 mM $CaCl_2$, and 20 mM HEPES (pH 7.4 with NaOH), or in a Na^+-free saline solution containing 300 mM sucrose, 1 mM $MgSO_4$, 1 mM K_2HPO_4, 5.5 mM glucose, 1 mM $CaCl_2$ and 20 mM HEPES (pH 7.4 with KOH). For oxygen radical production, cells were maintained in Hanks' bovine serum albumin (BSA) solution (1 mg/ml BSA).

2.3. Measurement of Ca^{2+} transients

Changes in the intracellular Ca^{2+} were measured with the fluorescent indicator fura-2/AM, using a Zeiss microscope (Oberkochem, Germany) equipped with the digital fluorescence unit Attofluor. For fura-2/AM loading, eosinophils (1×10^6/ml) were incubated in Hanks' BSA solution, in the presence of 4 μM fura-2/AM and 250 μM sulfinpyrazone to block fluorescent dye extrusion, and incubated at 37°C for 30 min. Cells were then washed twice in normal or Na^+-free saline solution and plated in 24 well plates for Ca^{2+} measurements. Ca^{2+} transients were determined by multiple cell acquisitions with the 340/380 excitation ratio at an emission wavelength of 505 nm, curves shown are representatives of the whole cell population.

2.4. Eosinophil purification

Eosinophils were obtained from blood of healthy donors, and negatively selected by magnetic separation with anti-CD16 antibody-conjugated Dynabeads to remove neutrophils, as previously reported [61]. Purity of isolated eosinophils was >96% (Pappenheim staining).

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

RNA was isolated by using QIAshredder and RNeasy kits from Qiagen GmbH (Hilden, Germany). 1 μg/ml RNA was reverse-transcribed using $pd(N)_6$ primers and M-MLV reverse transcriptase purchased from Gibco BRL (MD, USA). Control reactions in the absence of reverse transcriptase were also carried out. 2 μl cDNA was amplified in a PCR (35 cycles) with 1 μl of a 20 μM primers' mix, in a final volume of 50 μl. The sequence specific primers used for P2Y and P2X receptors were: $P2Y_1$ (445 bp) forward, 5′-TGCCGCCGTCTCCTCGTCGTTC-3′, 5′-CGCCACCACCACAATGAGCCACAC-3′ reverse, $P2Y_2$ (432 bp) forward, 5′-CCTCAAGACCTGGAATGCGT-3′, and 5′-TGACTGAGCTGTAGGCCACG-3′ reverse primers. $P2Y_4$ (303 bp) forward, 5′-GCCATGGCCAGTACAGAGTC-3′, and 5′-GTGGTTGTGGGCTGCATAAT-3′ reverse primers. $P2Y_6$ (392 bp) forward, 5′-GTGGCTGGCCCGTGACAACC-3′, and 5′-CCGCTGCAAAGCCCTCCAATAC-3′ reverse primers. $P2Y_{11}$ (238 bp) forward, 5′-GTGGTTGAGTTCCTGGTGGC-3′, and 5′-CCAGCAGGTTGCAGGTGAAG-3′ reverse primers. $P2X_1$ (248 bp) forward, 5′-CGCCTTCCTCTTCGAGTATG-3′, and 5′-GGAAGACGTAGTCAGCCACA-3′ reverse primers. $P2X_4$ (484 bp) forward, 5′-CCTGTTCGAGTACGACACGC-3′, and 5′-GTGTGTGTCATCCTCCACCG-3′ reverse primers. $P2X_7$ (399 bp) forward, 5′-AGATCGTGGAGAATGGAGTG-3′, and 5′-TTCTCGTGGTGTAGTTGTGG-3′ reverse primers. β2-Microglobulin (259 bp), forward 5′-CCTTGAGGCTATCCAGCGTA-3′, and 5′-GTTCACACGGCAGGCATACT-3′ reverse primers. The obtained PCR products (7 μl+3 μl loading buffer) were separated in a 1.5% agarose, ethidium bromide-containing gel. Appropriate controls to exclude contamination with genomic DNA were also run.

2.6. Oxygen radical production

Production of reactive oxygen intermediates by eosinophils was determined by measuring lucigenin-dependent chemiluminescence as previously described [38]. Briefly, 5×10^4 cells were resuspended in a 200 μM lucigenin-containing Hanks' BSA-balanced salt solution and triggered by different stimuli. Light emission was measured for 60 min and values were expressed as intensity integral counts.

Experiments with BAPTA/AM were performed as follows: eosinophils were incubated in the presence of 100 μM of the intracellular Ca^{2+} chelator, then rinsed two times with Hanks' BSA and challenged with 1 mM nucleotide.

2.7. Data presentation

Data shown are from individual experiments each replicated at least five times with similar results. Blood samples were drawn from 10 different healthy subjects. Statistical significance was assessed by the Tuckey's multiple comparison test (ANOVA).

3. Results

3.1. ATP and UTP trigger Ca^{2+} changes in human eosinophils

Stimuli as different as bacterial peptides, IgE cross-linking agents, the complement protein C5a, recombinant human eotaxin and extracellular nucleotides [18,38,39] induce Ca^{2+} changes in eosinophils and basophils. It is well known in other cell types that ATP and other nucleotides trigger Ca^{2+} increases by stimulating purinoceptors of the P2X and P2Y subtypes.

In human eosinophils incubated in the presence of extracel-

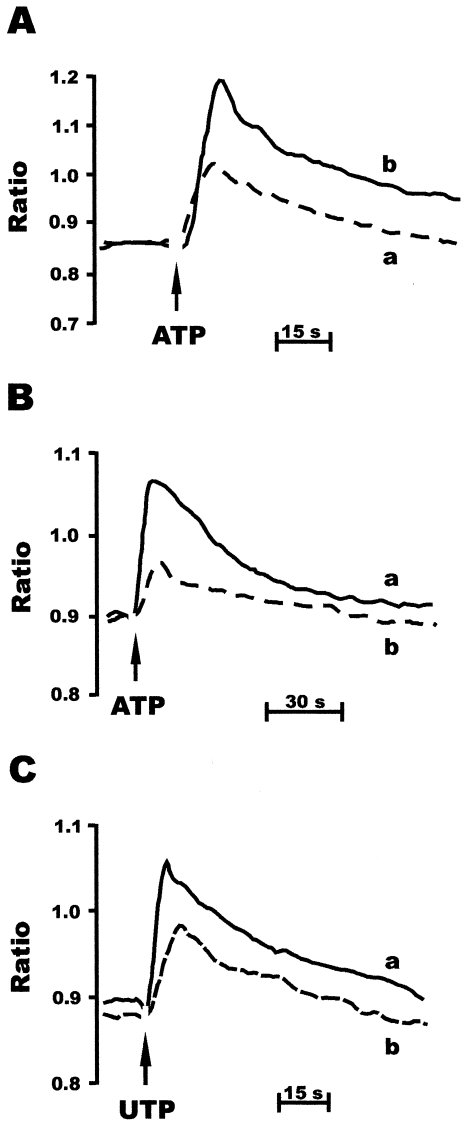

Fig. 1. ATP (A) induces an increase in the intracellular Ca^{2+} of eosinophils which is higher in the absence than in the presence of extracellular Na^+. Eosinophils were loaded with the Ca^{2+} indicator fura-2/AM, as specified in Section 2, and then stimulated with ATP in a Na^+-containing saline solution (A, a) or in a medium where Na^+ was replaced with isotonic sucrose (A, b). Sucrose solution was also used to perform all the subsequent Ca^{2+} measurements. B: Kinetics of ATP-triggered Ca^{2+} changes in Ca^{2+}-containing (B, a) or Ca^{2+}-free, 0.5 mM EGTA-containing medium (B, b). C: Kinetics of UTP-triggered Ca^{2+} changes in Ca^{2+}-containing (C, a) and Ca^{2+}-free, 0.5 mM EGTA-containing medium (C, b). All nucleotides were added at the concentration of 1 mM. Traces are from a single experiment representative of five similar.

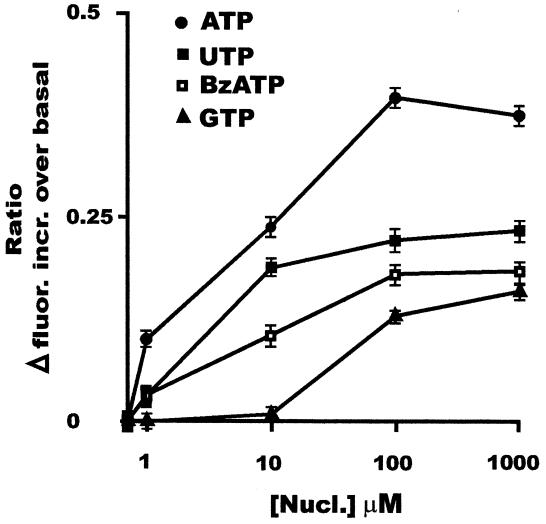

Fig. 2. ATP, UTP, GTP, BzATP dose-dependency curves for Ca^{2+} changes. Cells were loaded with fura-2/AM, and then stimulated with different nucleotide concentrations, in a Na^+-free, Ca^{2+}-containing saline solution. Data are means ± S.D. of triplicate determinations.

lular Ca^{2+}, ATP triggered a rapid and dose-dependent Ca^{2+} spike followed by a slowly decreasing plateau (Fig. 1A, trace a). We and others have shown that in mouse and human lymphocytes Na^+ competes with Ca^{2+} for entry through the P2X receptor [29,40,41]. This was also the case for the ATP receptor of human eosinophils. In Fig. 1A, trace b, cells were stimulated with ATP in a Na^+-free, sucrose-containing, Ca^{2+}-supplemented solution. Under this condition, the Ca^{2+} rise elicited by ATP was about twice as high as in the presence of extracellular Na^+, suggesting a competition between Na^+ and Ca^{2+} also in the case of the eosinophil ATP receptor. In order to maximize the Ca^{2+} response, the following experiments, unless indicated otherwise, were performed in Na^+-free medium. To discriminate whether the Ca^{2+} rise was due to P2Y or P2X receptor activation or both, eosinophils were stimulated with ATP in the virtual absence of extracellular Ca^{2+}, i.e. in a Ca^{2+}-free, 0.5 mM EGTA-containing solution. Fig. 1B, trace b, shows that the Ca^{2+} spike elicited by 1 mM ATP was also present in the absence of the extracellular cation, although with a lower amplitude, an indication that eosinophils express P2Y purinoceptors coupled to Ca^{2+} release from intracellular stores. Fig. 1C, trace a, shows that the shape and amplitude of the Ca^{2+} spike triggered by UTP were similar to those elicited by ATP, a result not surprising as at least at one P2Y receptor, $P2Y_2$, UTP is equipotent as ATP.

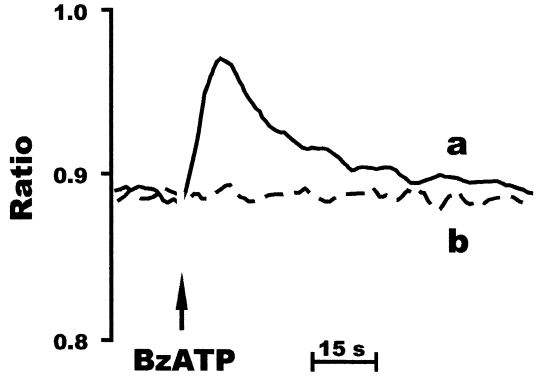

Fig. 3. Ca^{2+} changes induced by BzATP stimulation. Cells were pulsed with 1 mM BzATP, either in a Ca^{2+}-containing (a) or in a Ca^{2+}-free, 0.5 mM EGTA-containing sucrose solution (b).

3.2. Dose-dependency curves for Ca^{2+} changes induced by different nucleotides

Fig. 2 shows dose–response curves for ATP, UTP, BzATP, and GTP. A Ca^{2+} increase was observed at an ATP concentration as low as 1 μM, and a plateau was reached at 100 μM. The other nucleotides tested (UTP, GTP, and BzATP) were significantly less potent than ATP. ADP, 2-methylthio-ATP, ATPγS and CTP were also active in the high micromolar

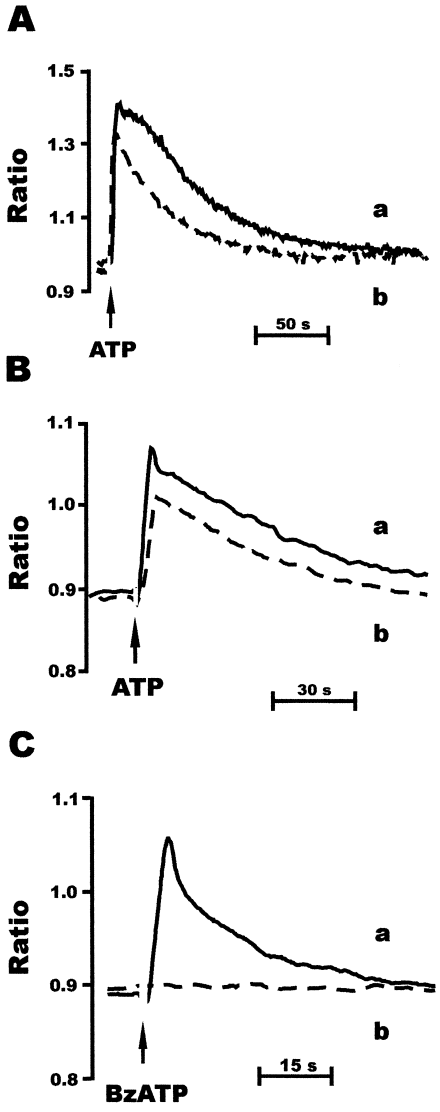

Fig. 4. Effects of $P2X_7$ blockers on the ATP-induced Ca^{2+} increase. A: Eosinophils were pretreated with 300 μM oATP for 2 h at 37°C, rinsed and then challenged with ATP (A, b); A, a: controls. B and C: KN-62 (25 nM) was added to eosinophils 10 min prior to ATP or BzATP stimulation and left in the samples throughout the experiment, b; a, controls. Nucleotide concentration was 1 mM. Traces are from a single experiment representative of five similar. Traces are from a single experiment representative of five similar.

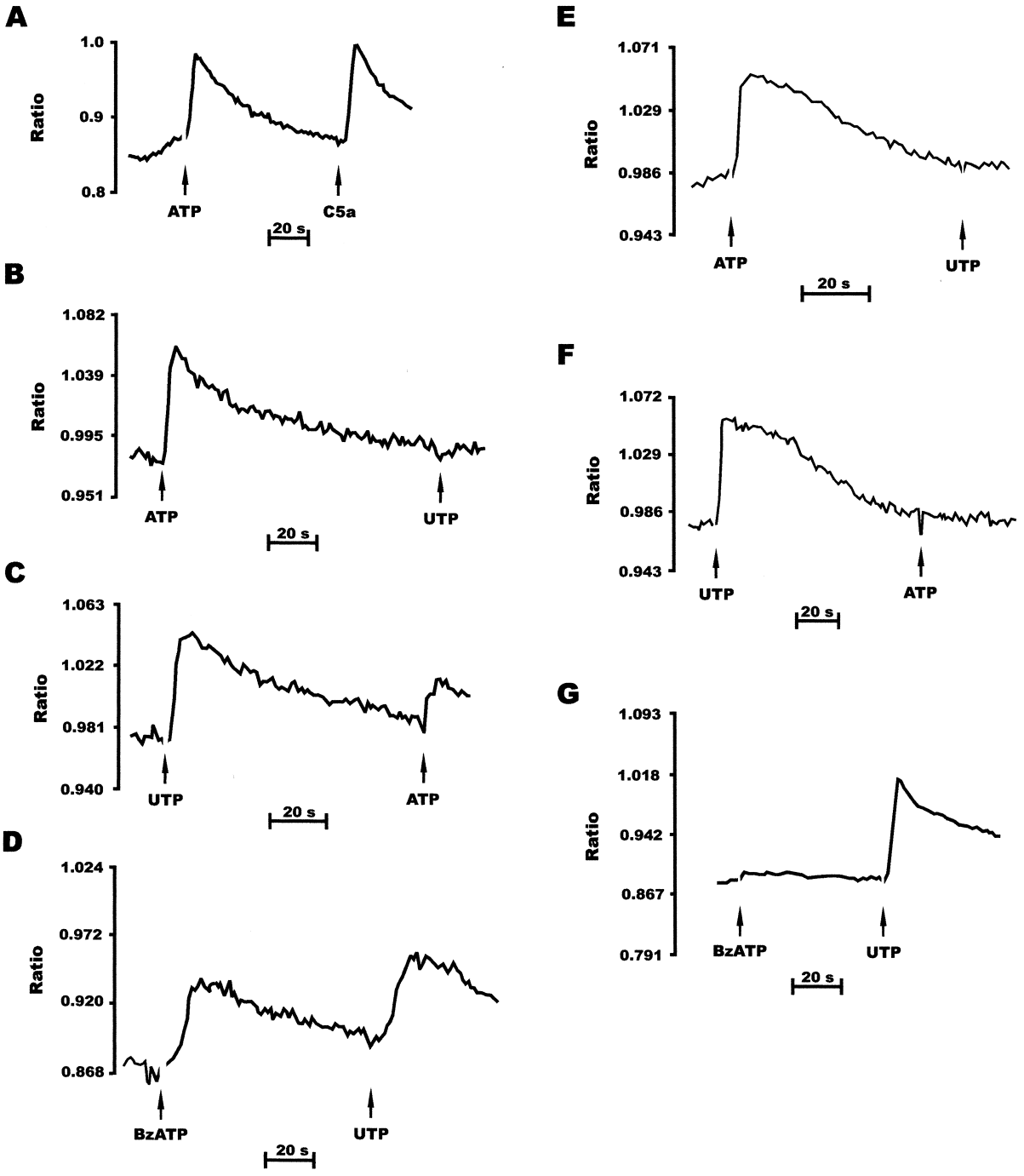

Fig. 5. ATP completely blocks the UTP-induced Ca^{2+} rise, both in the presence or in the absence of extracellular Ca^{2+}. Eosinophils were subjected to sequential stimulation with two different nucleotides. Nucleotide concentration was 1 mM. C5a was 10^{-7} M. A, B, C, D: Ca^{2+}-containing sucrose solution; E, F, G: Ca^{2+}-free, 0.5 mM EGTA-containing sucrose solution.

range (not shown). As reported in Fig. 3, the ATP pharmacological analogue BzATP was a Ca^{2+} mobilizing stimulus, however, at variance with the other nucleotides, BzATP was an effective stimulus only in the presence of extracellular Ca^{2+} (compare Fig. 3, traces a and b). Despite an early claim that BzATP activates P2Y receptors [42], it is now well established that BzATP is a selective agonist for the P2X receptors [43,44]. Thus, this experiment suggests that eosinophils express functional P2X receptors and that these receptors contribute to the ATP-dependent Ca^{2+} rise. The $[Ca^{2+}]_i$ rise cannot be unequivocally assigned to a given P2X receptor due to the fact that BzATP could also activate other P2X receptor subtypes besides $P2X_7$. We found that αβ-methylene ATP was also an agonist, albeit only at concentrations above 300 μM.

3.3. *P2X receptor blockers reduced ATP-induced Ca^{2+} increase*

To further investigate the contribution of the $P2X_7$ receptor, we took advantage of two inhibitors, the 2′,3′-dialdehyde ATP derivative oATP [36,43,45] and KN-62, a compound

originally described as a Ca^{2+}/calmodulin-dependent protein kinase II inhibitor that however turned out to be a potent and selective blocker of the human $P2X_7$ receptor [46–48]. Pretreatment of eosinophils with 300 μM oATP reduced the ATP-induced Ca^{2+} increase (Fig. 4A). The fast initial Ca^{2+} transient, due to a release from intracellular stores, was less affected than the second declining phase. The residual Ca^{2+} rise in the presence of oATP is likely due to P2Y receptors, as oATP is ineffective at these receptors [45]. Similarly to oATP, KN-62 reduced the amplitude of the ATP-induced Ca^{2+} increase (Fig. 4B), while completely abrogated that due to stimulation with BzATP (Fig. 4C).

3.4. ATP completely abrogated the UTP-induced Ca^{2+} rise

To better characterize the contribution to the Ca^{2+} rise of the different purinoceptor subtypes, we also performed cross-desensitization experiments. Fig. 5A shows that ATP did not have any effects on the C5a-triggered Ca^{2+} rise, on the contrary stimulation with ATP completely abrogated the Ca^{2+} rise induced by UTP (Fig. 5B). A reduction in the amplitude of the ATP-elicited signal was seen when eosinophils were stimulated with UTP prior to ATP (Fig. 5C), suggesting that ATP, differently from UTP, acts at two receptor subtypes. Finally, BzATP did not desensitize the UTP-induced response (Fig. 5D), suggesting that the two nucleotides act on different receptors. These findings were confirmed by experiments performed in Ca^{2+}-free conditions (Fig. 5E,F,G). In the absence of the extracellular cation, receptors activated by ATP and UTP were reciprocally blocked, and again BzATP did not interfere with the UTP-induced Ca^{2+} increase. These findings suggest that the ATP-induced Ca^{2+} rise was due to the activation of both P2Y and P2X subtypes, while UTP only activated the P2Y subtype. Good candidates for this response are $P2Y_2$ and $P2Y_4$ as ATP and UTP are equipotent at these receptors, although $P2Y_4$ can probably be excluded since ATP is reported to be inactive at the human $P2Y_4$ subtype [49].

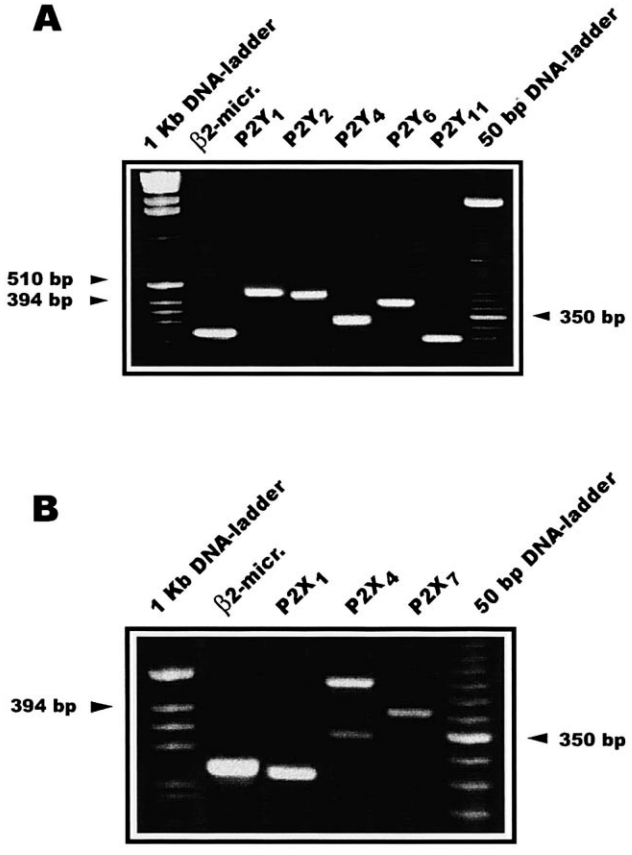

Fig. 6. P2 receptors expressed by human eosinophils. P2Y receptors (A). P2X receptors (B). RT-PCR was performed as described in Section 2.

3.5. Human eosinophils express P2Y and P2X purinoceptor subtypes

No antibodies against P2Y receptors are available as yet, thus we checked receptor expression by RT-PCR. Fig. 6A shows that human eosinophils express $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$. Fig. 6B shows that in the P2X subfamily, only the $P2X_1$, $P2X_4$, and $P2X_7$ subtypes are expressed.

3.6. Oxygen radical generation induced by extracellular nucleotides

Stimulation of human eosinophils and neutrophils with recombinant eotaxin [38], or C3a [50] activates oxygen radical (ROIs) generation. Furthermore, ATP was identified as the soluble factor released by platelets that induces oxygen radical production by neutrophils [51]. Therefore we asked whether ATP could also be able to trigger the generation of oxygen intermediates from human eosinophils. Using a lucigenin-dependent assay, we show in Fig. 7 that ATP triggered the production of ROIs to a level comparable to that obtained with C5a (Fig. 7A). ROIs due to ATP, but not that due to C5a, were partially blocked by oATP (Fig. 7A) and KN-62 (Fig. 7B), this inhibitor completely blocked ROIs production due to BzATP stimulation (Fig. 7C); in support of the specificity of KN-62 as a $P2X_7$ blocker, C5a- (Fig. 7C) and UTP-stimulated production of ROIs (not shown) were not inhibited by this antagonist. Among P1 agonists, we tested adenosine, that however lacked any effect.

Finally, we investigated whether other P2 receptors, besides P2X, could be involved in ROIs production. Fig. 7D shows that UTP, ATP, and GTP (C5a was used as a positive control) triggered the generation of ROIs, in the absence of extracellular Ca^{2+}. This is a strong indication that also P2Y receptors participate in ROIs production due to stimulation by extracellular nucleotides. Lack of activity of BzATP under these conditions further stresses the P2X selectivity of this compound. The intracellular Ca^{2+} chelator BAPTA/AM at a concentration of 100 μM was used to check whether ATP-induced ROIs production was mediated by an increase in the $[Ca^{2+}]_i$. BAPTA/AM completely obliterated ROIs generation, showing that intracellular Ca^{2+} was involved (not shown).

4. Discussion

ATP is released from many cell types including macrophages, neurons [52] platelets [53,54], endothelial cells [55], and microglia, in physiological or pathological conditions [56], thus leading to stimulation of multiple cell responses such as proliferation, secretion or cell death. Responses to ATP have been detected also in the lung. Mg-ATP has been used to treat pulmonary hypertension in children [57], and inhalation of aerosolized ATP has been reported to cause a strong bronchoalveolar constriction both in normal and asth-

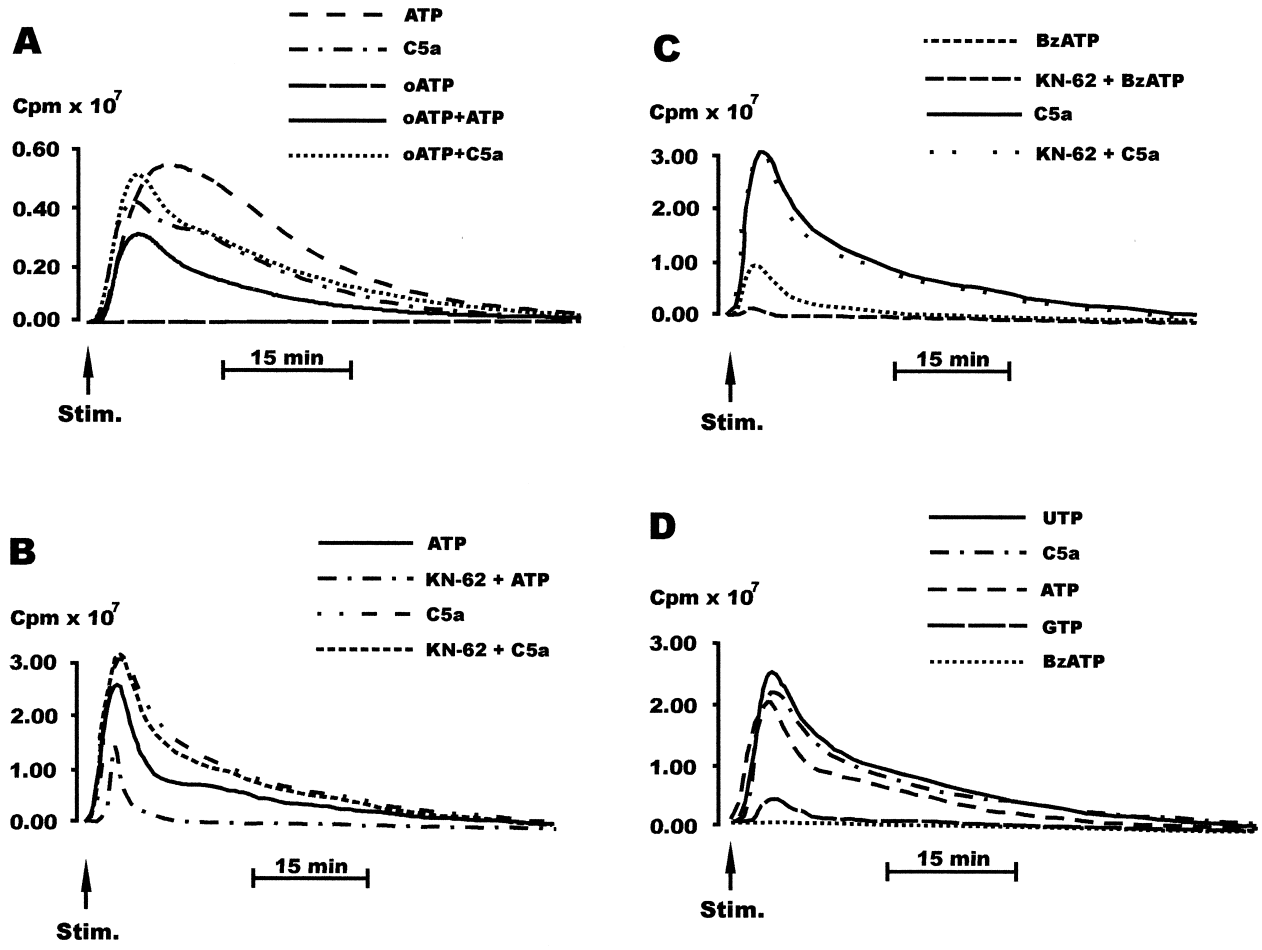

Fig. 7. Extracellular nucleotides trigger ROIs production via P2X and P2Y receptor stimulation. ATP- but not C5a-induced ROIs secretion is reduced by oATP (A) and KN-62 (B). BzATP-stimulated ROIs production is inhibited by KN-62 (C). ROI secretion in Ca^{2+}-free conditions (D). 5×10^6 cells were incubated in 200 μM of a lucigenin-containing Hanks' BSA-balanced salt solution and stimulated with 1 mM ATP, BzATP, UTP, GTP, or 10^{-7} M C5a. Incubation with oATP or KN-62 was performed as described in Fig. 4. Oxygen radical generation was measured by light emission, as specified in Section 2. Measurements were performed for 60 min and values were expressed as intensity integral counts.

matic individuals [58]. Aerosolized UTP is currently used in the therapy of cystic fibrosis with beneficial effects on mucin secretion, ion/water transport and ciliary beat frequency [59].

Eosinophils massively infiltrate lung tissue in asthma and allergic disease and are thought to play a central role in the associated damage. These cells possess several mechanisms able to cause tissue damage, among which generation of activated oxygen species is one of the most important. Local mechanisms driving eosinophil recruitment and activation are incompletely understood although they are clearly of the utmost importance for the development of innovative therapeutic approaches to these diseases. Very little is known about the potential role of nucleotides and nucleotide receptors in the activation of eosinophils and in the ensuing tissue damage. Scattered observations in the past reported an increased release of ATP by activated platelets isolated from atopic asthmatics [60], and given the known eosinophil chemotactic effect of ATP [54], it has been postulated that this nucleotide, among other platelet products, could contribute to lung infiltration by eosinophils in asthma. However, although eosinophils are known to be sensitive to ATP stimulation [18,51] no detailed information on the nucleotide receptor subtypes expressed by human eosinophils was available until recently. Our data show that these cells express most of the P2Y and P2X subtypes so far cloned: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$; $P2X_1$, $P2X_4$, and $P2X_7$. The Ca^{2+} changes triggered by the different nucleotides show that P2Y and P2X receptors are not only expressed but also functional. P2Y receptors are well known for their coupling to phospholipase C and therefore to Ca^{2+} mobilization from intracellular stores. On the other hand, P2X receptors, that are intrinsic ion channels, only mediate Ca^{2+} influx from the extracellular milieu. The fact that in eosinophils nucleotides elicit a Ca^{2+} increase both in the presence and absence of extracellular Ca^{2+} is highly suggestive for the presence of functional P2Y receptors on the eosinophil plasma membrane. By the same token, the ability of BzATP to increase Ca^{2+} only in Ca^{2+}-containing but not in Ca^{2+}-free medium shows that these cells also express functional P2X receptors which contribute in part to the Ca^{2+} rise caused by ATP. Further support to this conclusion comes from the effect of the two inhibitors, oATP and KN-62 that are chemically unrelated and differ widely in the mechanism of action. oATP, introduced as a $P2X_7$ inhibitor by our laboratory 7 years ago, is an irreversible inhibitor that forms

Schiff's bases with available lysines in the vicinity of the ATP binding site. On the contrary, KN-62 is a reversible inhibitor originally introduced as a calmodulin blocker and subsequently shown to powerfully inhibit the human $P2X_7$ receptor [46,61]. These P2X blockers partially reduced the Ca^{2+} rise triggered by ATP. KN-62 completely abrogated the BzATP-dependent Ca^{2+} rise and reduced the ATP-dependent Ca^{2+} increase observed in the presence of extracellular Ca^{2+}, as expected from an inhibitor that fully blocks Ca^{2+} influx through a P2X receptor but has no effect on P2Y. Eosinophil activation by extracellular ATP triggers a large production of reactive oxygen intermediates. This process is likely mediated through both P2Y and P2X receptors as other nucleotides (i.e. UTP and GTP) that are P2Y but not P2X agonists are also effective, in the absence of the extracellular cation. In addition, production of oxygen intermediates was partially blocked by oATP and almost completely abrogated by KN-62.

It is increasingly appreciated that nucleotide receptors could have a central role in inflammation as they stimulate chemotaxis, NADPH oxidase, secretion of lysosomal enzymes and release of inflammatory mediators. The present observation that eosinophils express P2 receptors and are susceptible to activation by extracellular nucleotides is of great potential interest as these cells are involved in diseases with a large impact on public health, yet little is known about factors that mediate their recruitment and activation at the sites of inflammation. ATP, and possibly other nucleotides, can be released by many cells via different mechanisms: platelets secrete large amounts of ATP, stored in their dense granules; endothelial and epithelial cells, that do not accumulate ATP in a secretory compartment, release this nucleotide by a plasma membrane transporter probably belonging to the ABC family; macrophages and microglial cells also release ATP via an unknown pathway stimulated by bacterial compounds. Besides these non-lytic, regulated pathways, ATP is also obviously released as a consequence of plasma membrane damage or cell death. At inflammatory sites it is not at all unlikely that significant ATP concentrations build up in the extracellular space to a level sufficient to activate P2 receptors. Eosinophils, that are increased and activated in pathologic conditions such as parasitic disease, asthma or allergy might easily be exposed to high local concentrations of extracellular nucleotides. Given that these cells express several P2Y receptors in the absence of subtype-selective inhibitors, it is not possible to precisely identify which of the P2 metabotropic receptors is responsible for activation, although P2Y receptors are clearly implicated both in Ca^{2+} rise and in NADPH oxidase activation. On the contrary, the participation of $P2X_7$ is more clearly defined as oATP and KN-62 significantly, albeit incompletely, reduced the Ca^{2+} increase and generation of oxygen intermediates. Further efforts should be directed to study how ATP and its analogues could be used in vivo to modulate eosinophil functions in those diseases where these cells play a major role.

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