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Pharmacological characterization of the human $P2Y_{11}$ receptor

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1 The human $P2Y_{11}$ receptor is coupled to both the phosphoinositide and the cyclic AMP pathways. A pharmacological characterization of the recombinant human $P2Y_{11}$ receptor has been conducted following stable expression in two different cell lines: the 1321N1 astrocytoma cells for inositol trisphosphate measurements and the CHO-K1 cells for cyclic AMP assays. The rank order of potency of a series of nucleotides was almost identical for the two pathways: ATPγS $\approx$ BzATP > dATP > ATP > ADPβS > 2MeSATP.

2 ADPβS, AMPαS and A3P5PS behaved as partial agonists of the human $P2Y_{11}$ receptor. At high concentrations, these three nucleotides were able to partially inhibit the ATP response.

3 Suramin was a more potent antagonist than reactive blue 2, whereas pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid was completely inactive. The $P2Y_{11}$ receptor proved to be sensitive to suramin in a competitive way with an apparent K_i value of 0.82 ± 0.07 μM.

4 The ATP derivative AR-C67085 (2-propylthio-β, γ-dichloromethylene-D-ATP), a potent inhibitor of ADP-induced platelet aggregation, was the most potent agonist of the $P2Y_{11}$ receptor, among the various nucleotides tested.

5 The pharmacological profile of the recombinant human $P2Y_{11}$ receptor is closely similar to that of the cyclic AMP-coupled P_2 receptor recently described in HL-60 cells, suggesting that it is the same receptor.

Keywords: $P2Y_{11}$ receptor; adenine nucleotide receptor; inositol trisphosphate; cyclic AMP; suramin

Abbreviations: ADPβS, adenosine 5′-O-(2-thiodiphosphate), AMPαS, adenosine 5′-O-thiomonophosphate; AP_4A, AP_5A and AP_6A, diadenosine polyphosphates; A2P5P, adenosine 2′,5′-diphosphate; A3P5P, adenosine 3′,5′-diphosphate; A3P5PS, adenosine 3′-phosphate 5′-phosphosulphate; ATPγS, adenosine 5′-O-(3-thiotriphosphate); BzATP, (2′- and 3′-O-(4-benzoyl-benzoyl)adenosine 5′-triphosphate; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HPLC, high performance liquid chromatography; IP_3, inositol trisphosphate; 2MeSATP, 2-methylthio-ATP; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid; RB-2, reactive blue 2; 8-p-SPT, 8-(p-sulphophenyl)theophylline

Introduction

The receptors activated by extracellular nucleotides are called P2 receptors (Burnstock, 1972). They have been divided in two groups: P2X receptors which are ligand-gated cation channels (Abbracchio & Burnstock, 1994) and P2Y receptors coupled to G proteins (Fredholm *et al*., 1994). A large number of subtypes have been cloned for these two families. Inside the P2Y family, the $P2Y_1$ and $P2Y_{11}$ are specifically activated by adenine nucleotides (Webb *et al*., 1993; Communi *et al*., 1997). Other subtypes are responsive to uracil nucleotides. Rat and human $P2Y_6$ receptors, as well as their avian ortholog previously called $P2Y_3$ (Barnard *et al*., 1994; Li *et al*., 1998), are preferentially activated by UDP (Chang *et al*., 1995; Communi *et al*., 1996a). The human $P2Y_4$ receptor is a UTP receptor (Communi *et al*., 1995a; 1996b; Nguyen *et al*., 1995; Nicholas *et al*., 1996), whereas its rat ortholog is activated by both ATP and UTP (Webb *et al*., 1998; Bogdanov *et al*., 1998), like the $P2Y_2$ and $P2Y_8$ receptors (Lustig *et al*., 1993; Bogdanov *et al*., 1997). There is no functional evidence at that time that the $p2y_5$, $p2y_9$ and $p2y_{10}$ receptors are nucleotide receptors (Li *et al*., 1997; Janssens *et al*., 1997) whereas the $p2y_7$ receptor is definitely a leukotriene B_4 receptor (Yokomizo *et al*., 1997).

Preliminary pharmacological data have shown that the $P2Y_{11}$ receptor is preferentially activated by ATP and is coupled to both the phosphoinositide and the adenylyl cyclase pathways (Communi *et al*., 1997). The coupling of this receptor to adenylyl cyclase stimulation is a unique feature among the P2Y family. The aim of this study was to provide a full characterization of the human $P2Y_{11}$ receptor in terms of rank order of potency of a range of nucleotides and sensitivity to antagonists, to detect possible discrepancies between the two signalling pathways and to compare these features to the pharmacological profile of the cyclic AMP-linked P_2 receptor recently described on HL-60 leukaemia cells (Conigrave *et al*, 1998). That characterization was performed in two distinct cell lines stably expressing the $P2Y_{11}$ receptor. Since 1321N1 cells express an adenosine receptor coupled to adenylyl cyclase stimulation, the cyclic AMP experiments were performed in CHO-K1 cells; reciprocally, the inositol phosphate experiments were performed in 1321N1 cells, since CHO-K1 cells have an endogeneous $P2Y_2$ receptor coupled to phospholipase C (Iredale & Hill, 1993).

Methods

Materials

Trypsin was from Flow Laboratories (Bioggio, Switzerland). Culture media and foetal bovine serum (FBS) were purchased

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from GIBCO–BRL (Grand Island, NY, U.S.A.). Myo-D-[2-^{3}H]-inositol (17.7 Ci mmol^{-1}) was from Amersham (Ghent, Belgium). Dowex AG1X8 (formate form) was from Bio-Rad Laboratories (Richmond, CA, U.S.A.). ATP, GTP, CTP, TTP, ITP, ATPγS (adenosine 5-O-(3-thiotriphosphate)), ADPβS (adenosine 5′-O-(2-thiodiphosphate)), AMPαS (adenosine 5′-O-thiomonophosphate), BzATP (2′- and 3′-O-(4-benzoyl-benzoyl)adenosine 5′-triphosphate), A2P5P (adenosine 2′,5′-diphosphate), A3P5P (adenosine 3′,5′-diphosphate), A3P5PS (adenosine 3′-phosphate 5′-phosphosulphate), the diadenosine polyphosphates (AP_4A, AP_5A and AP_6A) and pertussis toxin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Indomethacin was from Merck. 2-Methylthio-ATP (2MeSATP), suramin, reactive blue 2 (RB-2), pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and 8-(p-sulphophenyl)theophylline (8-p-SPT) were from Research Biochemicals International (Natick, MA, U.S.A.). Rolipram was a gift from the Laboratoires Jacques Logeais (Trappes, France). AR-C67085 (2-propylthio-β, γ-dichloromethylene-D-ATP) was a generous gift of Drs J.D. Turner and P. Leff (Astra Charnwood).

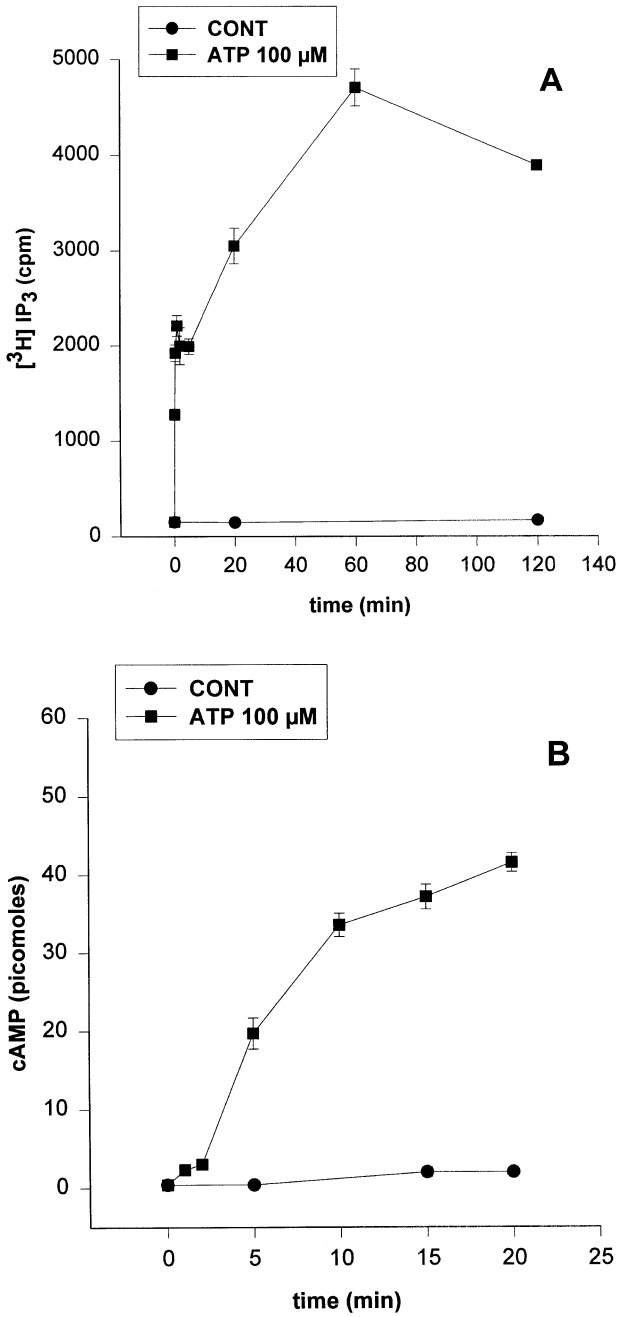

Figure 1 Time course of IP_3 and cyclic AMP accumulation respectively in 1321N1 or CHO-K1 cells expressing the human P2Y$_{11}$ receptor. ^{3}H-inositol labelled 1321N1 cells (A) or CHO-K1 cells (B) were incubated for different times in the presence of ATP (100 μM) or without ATP (CONT). The data represent the mean$\pm$s.d. of triplicate experimental points and are representative of three independent experiments.

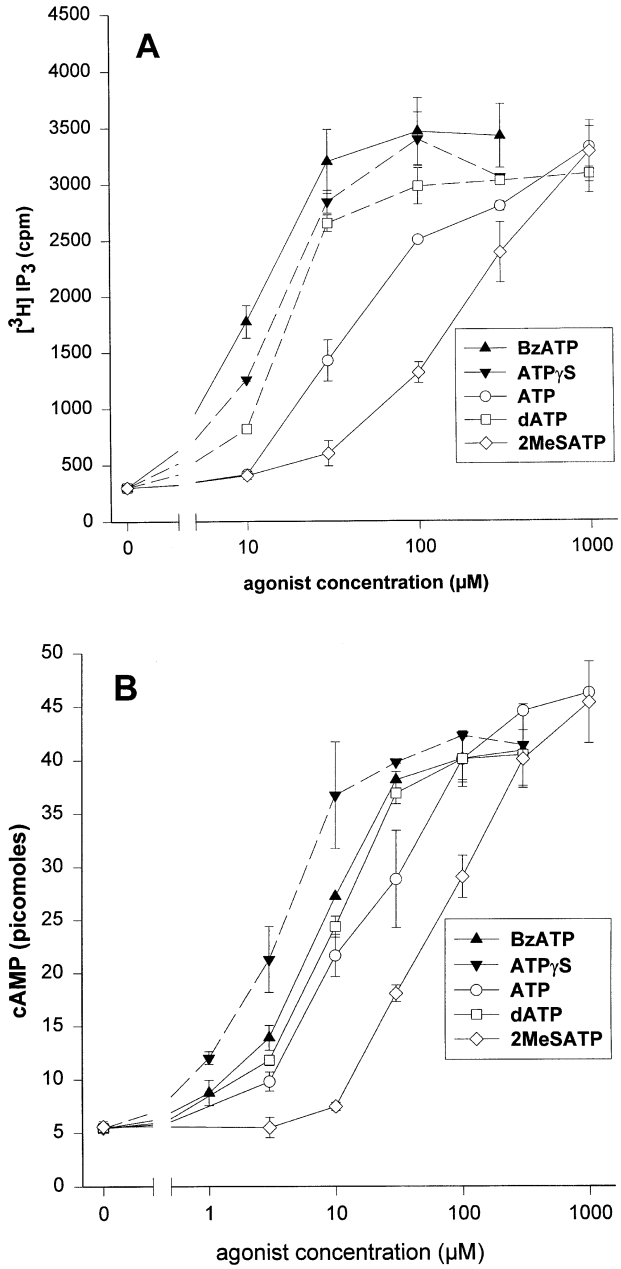

Figure 2 Concentration-action curves of ATP and ATP derivatives on the IP_3 and cyclic AMP accumulation in P2Y$_{11}$-transfected 1321N1 or CHO-K1 cells. The transfected cells were incubated in the presence of various concentrations of nucleotides for 30 s (A) or 15 min (B). The data represent the mean$\pm$s.d. of triplicate experimental points obtained in one representative experiment of three. The EC_{50} values shown in Table 1 were determined by curve fitting (Sigma plot: version 2.0).

Cell culture and transfection

The 1321N1 and CHO-K1 cells transfected with the $P2Y_{11}$-pEFIN3 recombinant vector as previously described (Communi *et al*., 1997) were cultured in the following medium: 10% FCS, 100 units ml^{-1} penicillin, 100 $\mu g\ ml^{-1}$ streptomycin, 2.5 $\mu g\ ml^{-1}$ amphotericin B, 400 $\mu g\ ml^{-1}$ G418 and 1% sodium pyruvate in respectively Dulbecco's modified Eagle's medium (DMEM) or Ham's F-12 medium.

Inositol trisphosphate (IP_3) measurements

1321N1 cells (200,000 cells/35 mm-dish) were labelled for 24 h with 10 $\mu Ci\ ml^{-1}$ [^{3}H]-inositol in inositol-free MEM containing 5% FCS and antibiotics. Cells were then incubated in Krebs-Ringer HEPES (KRH) buffer of the following composition (mM) NaCl 124, KCl 5, $MgSO_4$ 1.25, $CaCl_2$ 1.45, KH_2PO_4 1.25, HEPES (pH:7.4) 25 and glucose 8) for 30 min. The cells were then challenged by various nucleotides in the presence or the absence of various antagonists for different times (30 s in most experiments). The incubation was stopped by the addition of an ice cold 3% perchloric acid solution. Inositol phosphates were extracted and separated on Dowex columns as previously described (Communi *et al*., 1995b). EC_{50} and IC_{50} values were obtained by curve fitting (Sigma plot: version 2.0). Slope and pA_2 values were obtained from a linear regression of a Schild plot analysis (Sigma plot: version 2.0).

Table 1 Rank order of potency of various adenine nucleotides as agonists of inositol triphosphates and cyclic AMP production in $P2Y_{11}$ transfected cells

	EC_{50} (IP_3)	*EC_{50} (cyclic AMP)*
ATPγS	13.5±2.7 μM	3.4±0.3 μM
BzATP	10.5±0.3 μM	7.2±0.5 μM
dATP	16.3±0.7 μM	8.9±0.6 μM
ATP	65±12 μM	17.4±6.1 μM
ADPβS	174±28 μM	29.7±2.7 μM
2MeSATP	210±6 μM	50±4 μM

Transfected 1321N1 cells or CHO-K1 cells were stimulated by various nucleotides respectively for 30 s (IP_3) or 15 min (cyclic AMP). EC_{50} values (mean±s.d. of at least three independent experiments) were obtained by curve fitting (Sigma Plot: version 2.0)

Cyclic AMP measurements

CHO-K1 transfected cells were spread on Petri dishes (150,000 cells/35-mm dish) and cultured in Ham's F12 medium containing 10% FCS, antibiotics, amphotericin, sodium pyruvate and 400 $\mu g\ ml^{-1}$ G418. Cells were then preincubated for 30 min in KRH buffer with 25 μM rolipram and incubated in the same medium for different times in the presence of the agonists and in the presence or the absence of various antagonists (15 min in most experiments). The incubation was stopped by the addition of 1 ml HCl 0.1 M. The incubation medium was dried up, resuspended in water and diluted as required. Cyclic AMP was quantified by radioimmunoassay after acetylation as previously described (Brooker *et al*., 1979). EC_{50} and IC_{50} values were obtained by curve fitting (Sigma plot: version 2.0).

Results

We have first studied the time course of the ATP response (Figure 1A,B). The IP_3 response reached a first peak after 1 min, but the response was already significant after 15 s. The response was maintained and even increased at longer times (1 h), despite the absence of lithium. We have chosen 30 s as stimulation time for all the following experiments in order to minimize agonist degradation. Concerning the time course of cyclic AMP production, the ATP response was already significant after 1 min: increase from 0.4 to 3 pmoles. The half-maximum response was obtained at 5 min. We have chosen 15 min as stimulation time for all the following experiments.

We have then tested the effect of a series of nucleotides on the accumulation of both IP_3 and cyclic AMP (Figure 2A,B). The rank order of agonist potency was almost identical for the two pathways: ATPγS≈BzATP>dATP>ATP>ADPβS>2MeSATP (Table 1). The only differ-

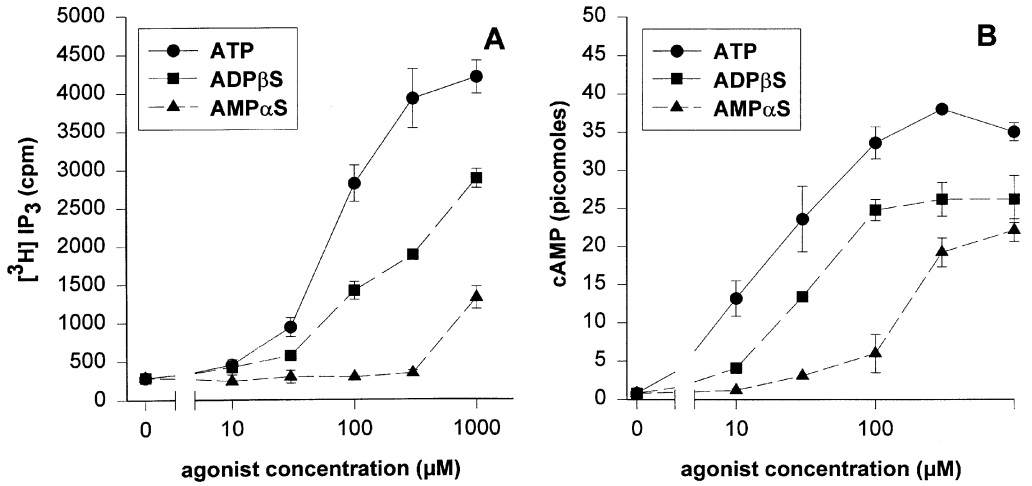

Figure 3 Concentration-action curves of ATP, ADPβS and AMPαS on the IP_3 and cyclic AMP accumulation in 1321N1 or CHO-K1 cells expressing the human $P2Y_{11}$. The cells were incubated with ATP, ADPβS and AMPαS at various concentrations for 30 s (A) or 15 min (B). The data represent the mean±s.d. of triplicate experimental points and are representative of three independent experiments.

ence was that ATPγS was slightly more potent than BzATP to stimulate the cyclic AMP pathway, whereas the opposite was true for the phosphoinositide pathway.

In CHO-K1 cells, ADPβS and AMPαS clearly behaved as partial agonists of the P2Y$_{11}$ receptor and had a lower potency than ATP itself (Figure 3B). At a concentration of 300 μM, where the cyclic AMP response had reached a plateau, the effects of ADPβS and AMPαS represented respectively 70$\pm$7% and 63$\pm$16% of the ATP response (mean$\pm$s.d. of three independent experiments). This could not be evaluated in the 1321N1 cells, since a plateau was not reached even at a 1 mM concentration of these nucleotides (Figure 3A). We have then tested the potential inhibitory effect of these two thiophosphorylated agonists on the ATP response (Figure 4). At a concentration of 1 mM ADPβS or AMPαS, an inhibition of respectively 35$\pm$5% and 56$\pm$6% of the IP$_3$ response induced by ATP 100 μM was observed (mean$\pm$s.d. of three independent experiments). These inhibitions have been reproduced on the cyclic AMP pathway but they were observed at higher concentrations: 19$\pm$4% inhibition for ADPβS 3 mM and 37$\pm$6% for AMPαS 3 mM (mean$\pm$s.d. of three independent experiments).

We have also tested three known nucleotide antagonists of the human P2Y$_1$ receptor (Boyer *et al*., 1996): A2P5P, A3P5P and A3P5PS (Figure 5A,B). A2P5P and A3P5P had no

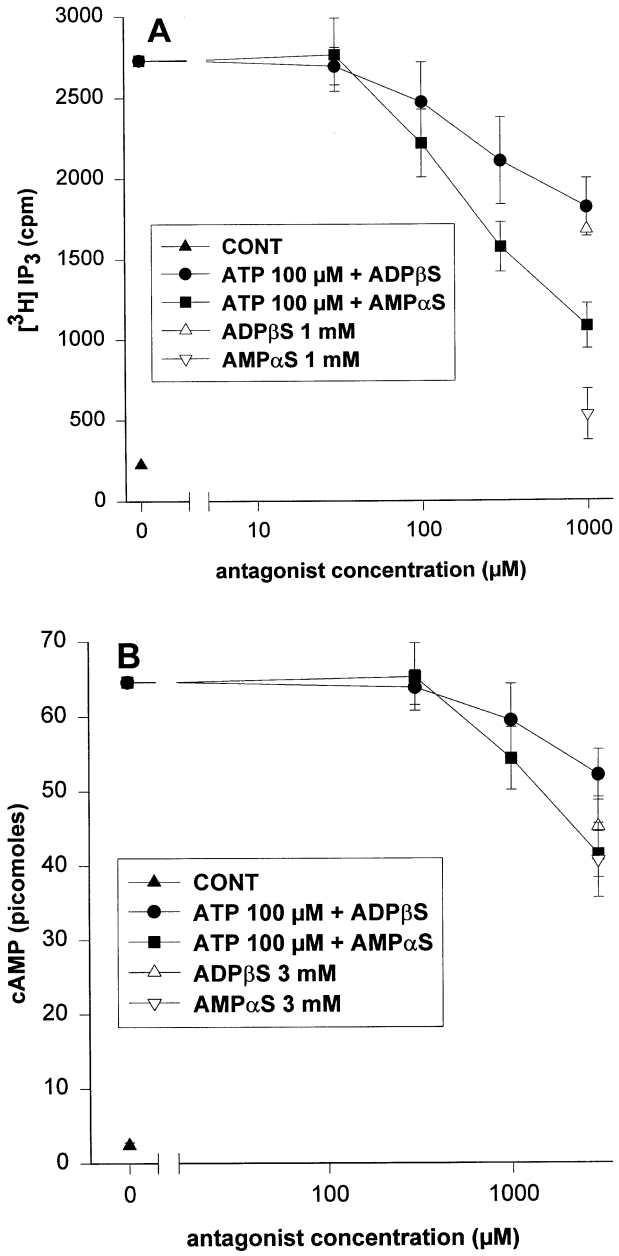

Figure 4 Concentration-inhibition curves of ADPβS and AMPαS on the IP$_3$ or the cyclic AMP accumulation by ATP respectively in 1321N1 or CHO-K1 cells expressing the human P2Y$_{11}$ receptor. Transfected cells were incubated in the presence or the absence (CONT) of ATP (100 μM) and various concentrations of ADPβS or AMPαS for 30 s (A) or 15 min (B). The data are the mean$\pm$s.d. of triplicate experimental points obtained in an experiment representative of three.

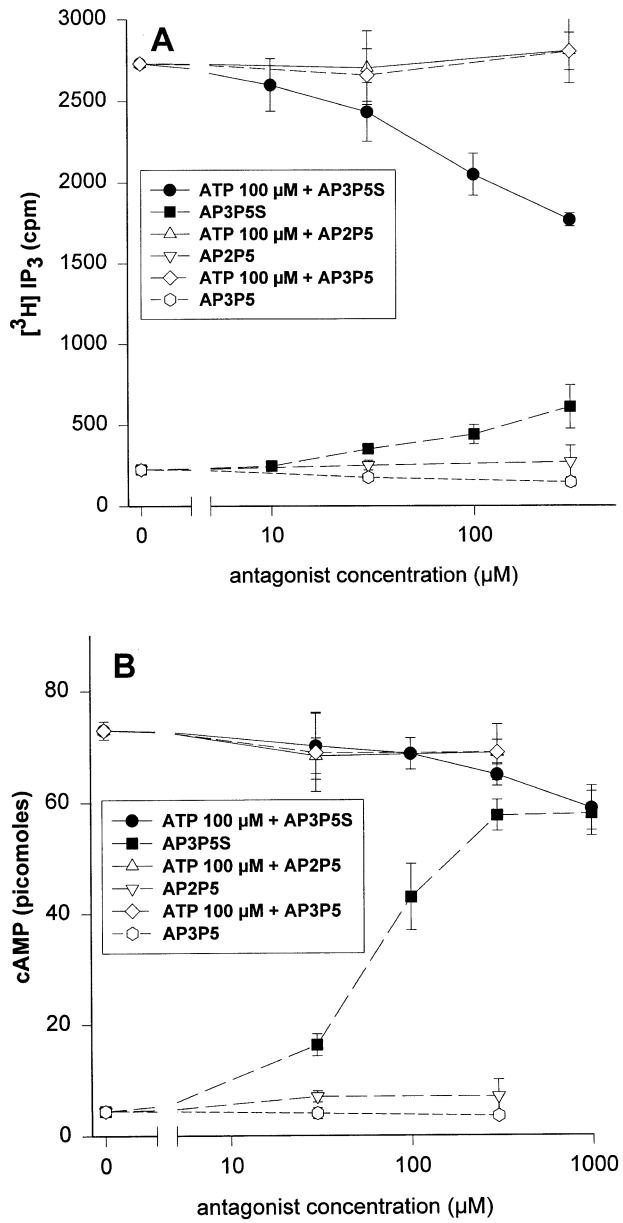

Figure 5 Concentration-action and -inhibition curves of A2P5P, A3P5P and A3P5PS on the IP$_3$ and the cyclic AMP accumulation respectively in 1321N1 or CHO-K1 cells expressing the human P2Y$_{11}$ receptor. Transfected cells were incubated in the presence of various concentrations of A2P5P, A3P5P and A3P5PS alone or in the presence of ATP (100 μM) for 30 s (A) or 15 min (B). The data are the mean$\pm$s.d. of triplicate experimental points obtained in an experiment representative of three.

significant effect on the $P2Y_{11}$ receptor. At a concentration of 300 μM, the A3P5PS IP_3 response represented $24 \pm 8\%$ of the maximal ATP response and A3P5PS was able to produce a $37 \pm 9\%$ inhibition of the ATP response (Figure 5A) (mean s.d. of three independent experiments). A significant effect of A3P5PS was also observed at lower concentrations on the cyclic AMP response (Figure 5B). A3P5PS behaved as an agonist of the $P2Y_{11}$ receptor with a maximal effect representing $78 \pm 4\%$ of the maximal ATP response and with an EC_{50} of 61 ± 5 μM (mean $\pm$ s.d. of three independent experiments). At a concentration of 1 mM, A3P5PS produced an inhibition of $20 \pm 4\%$ of the response to ATP 100 μM, consistent with its partial agonist nature (Figure 5B) (mean $\pm$ s.d. of three independent experiments).

A series of non-adenine triphosphonucleotides (UTP, GTP, CTP, TTP, ITP) and several diadenosine polyphosphates (AP_4A, AP_5A, AP_6A) were completely unable to activate the $P2Y_{11}$ receptor, as evaluated both by IP_3 and cyclic AMP measurements (data not shown).

We have then tested the effect of three non-specific antagonists which have already been used to discriminate some P_2 receptors : suramin, RB-2 and PPADS (Boyer *et al*., 1994; Brown *et al*., 1995). PPADS had no effect on IP_3 nor on cyclic AMP (data not shown). On the contrary, suramin and RB-2 were able to produce a full inhibition of the IP_3 accumulation induced by ATP. Suramin was the most potent antagonist (Figure 6A) (IC_{50} (suramin) $= 1.00 \pm 0.07$ μM and

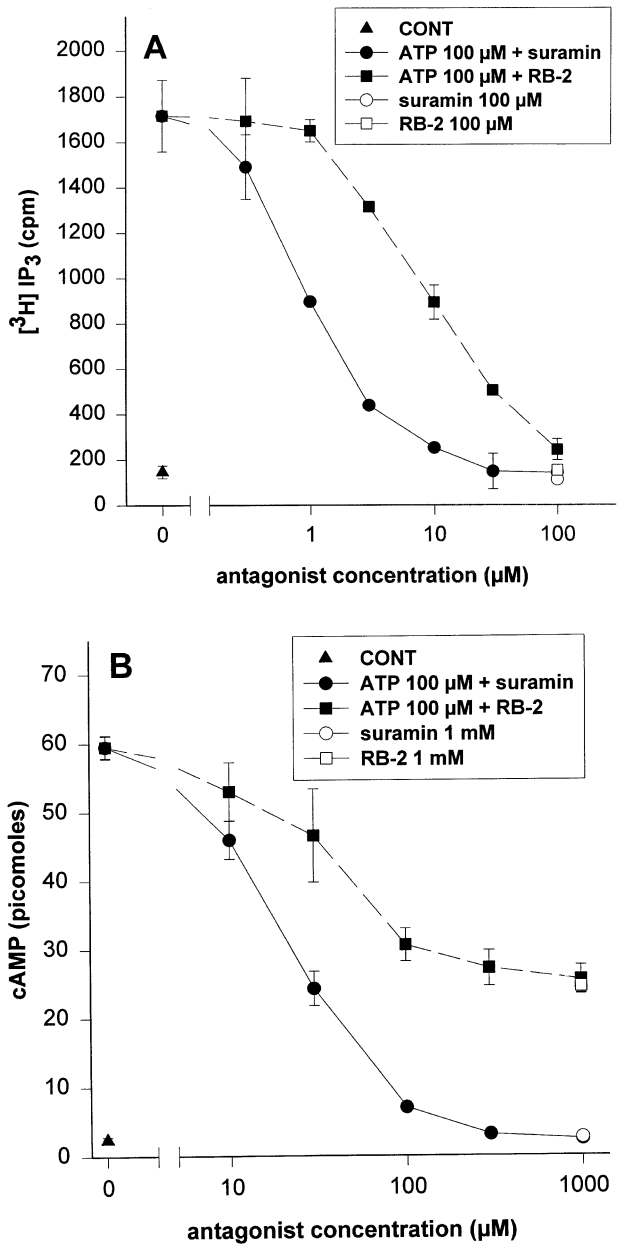

Figure 6 Concentration-inhibition curves of suramin and reactive blue 2 on the IP_3 and cyclic AMP accumulation induced by ATP respectively in 1321N1 of CHO-K1 cells expressing the human $P2Y_{11}$ receptor. Transfected cells were incubated in the presence of various concentrations of suramin or reactive blue 2 and with or without ATP (100 μM) (CONT) for 30 s (A) or 15 min (B). The data are the mean $\pm$ s.d. of triplicate experimental points and are representative of three experiments.

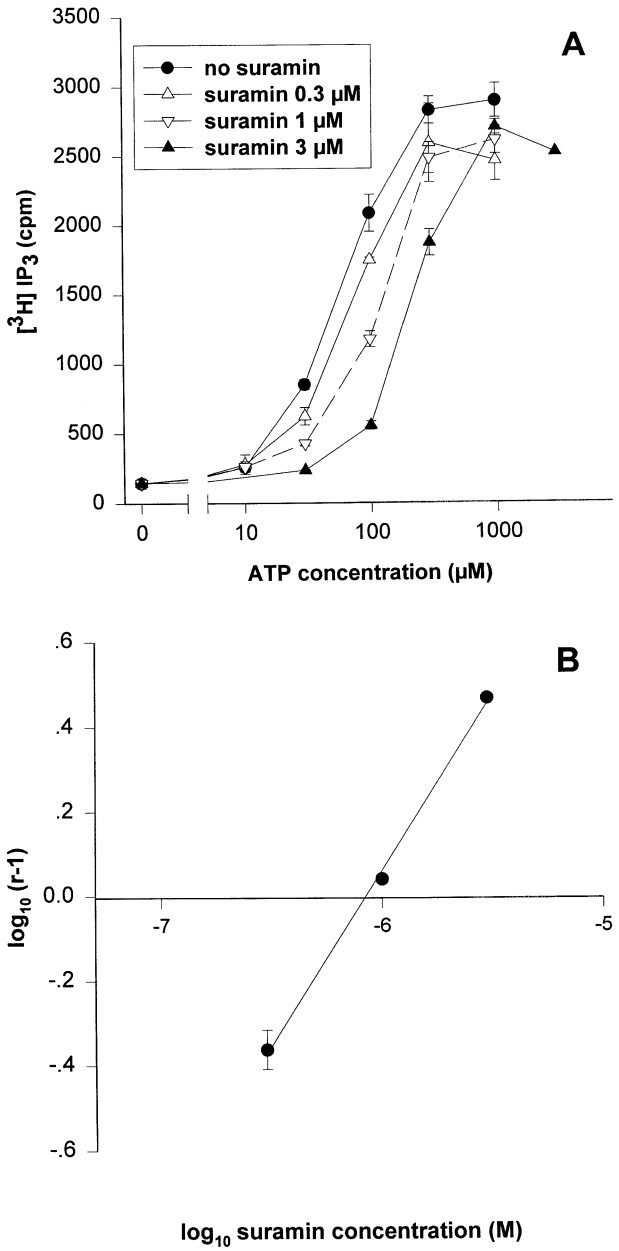

Figure 7 Competitive antagonism of suramin on the IP_3 response induced by ATP in 1321N1 cells expressing the human $P2Y_{11}$ receptor. Transfected 1321N1 cells were incubated in the presence of various concentrations of ATP in the presence of increasing concentrations of suramin for 30 s (A). A Schild plot has been derived from the data shown in panel A (B). The data are the mean $\pm$ s.d. of triplicate experimental points and are representative of three independent experiments.

IC_{50} (RB-2) = 9.0 ± 1.3 μM; mean ± s.d. of three independent experiments). Suramin produced a complete inhibition of the cyclic AMP response, with an IC_{50} of 16.0 ± 4.2 μM (mean ± s.d. of three independent experiments); with RB-2, the inhibition of the cyclic AMP response to ATP was incomplete and reached a maximum of 51 ± 3% (Figure 6B) (mean ± s.d. of three independent experiments). This apparently partial inhibitory effect is explained by the observation that high concentrations of RB-2 increase *per se* the cyclic AMP level in CHO-K1 cells expressing the $P2Y_{11}$ receptor (Figure 6B), as well as in non-transfected CHO-K1 and 1321N1 cells (data not shown). We have then tested the effect of various concentrations of suramin on the ATP concentration-action curve (Figure 7A). Each suramin concentration generated a shift in the curves to the right without affecting the maximum effect of ATP. A linear Schild plot has been derived from these data: it was characterized by a slope of 0.81 ± 0.10 and a K_i value of 0.82 ± 0.07 μM (pA_2 = 6.09 ± 0.52) (mean ± s.d. of three independent experiments) (Figure 7B).

We have also tested AR-C67085, an ATP analogue known to inhibit ADP-induced platelet aggregation. Not only had AR-C67085 no antagonist activity, but, among the nucleotides tested, it proved to be the most potent agonist of the $P2Y_{11}$ receptor. The EC_{50} values of AR-C67085 for IP_3 and cyclic AMP accumulation were respectively 8.9 ± 1.2 μM and 1.5 ± 0.4 μM, as compared to respectively 72 ± 8 μM and 17.4 ± 6.1 μM for ATP (mean ± s.d. of three independent experiments) (Figure 8).

Pretreatment of the cells with 50 ng ml^{-1} pertussis toxin during 24 h had no effect on the IP_3 formation induced by ATP (data not shown). To exclude the possibility that the cyclic AMP response to ATP was a consequence of prostaglandins release due to an increase in the intracellular Ca^{2+} concentration, we have tested the effect of a preincubation of the cells with indomethacin (10 μg ml^{-1}) during 30 min. There was no effect on the cyclic AMP response induced by ATP 30 μM. 8-p-SPT (100 μM), an antagonist of adenosine receptors, was also unable to inhibit this response (data not shown).

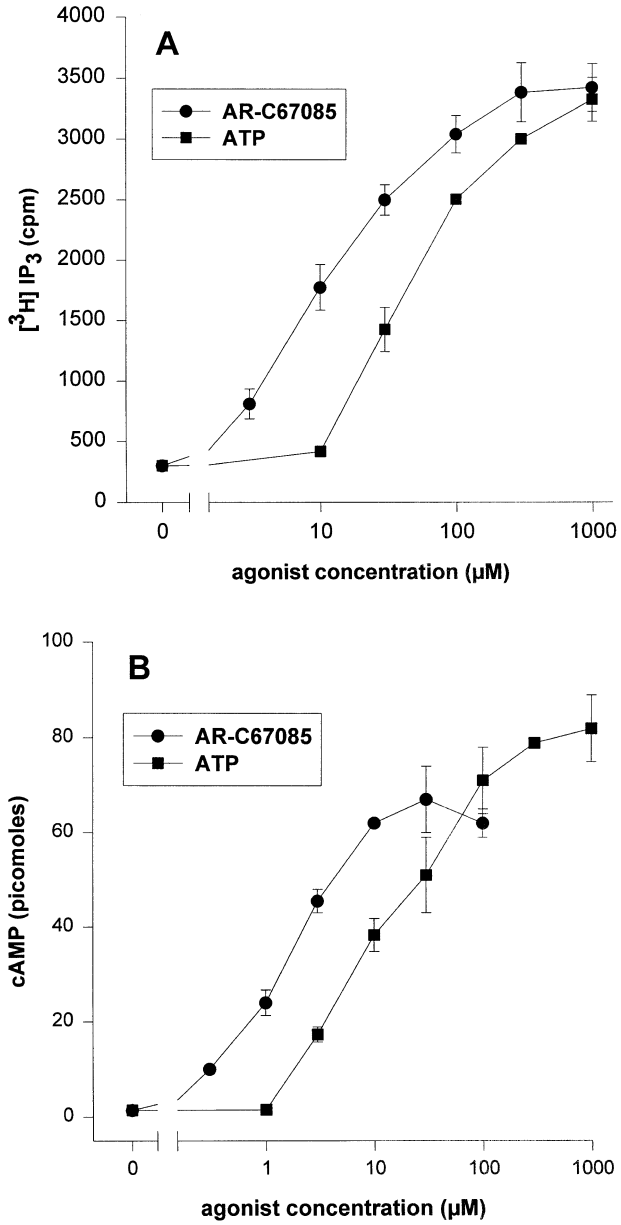

Figure 8 Effect of the AR-C67085 compound and ATP on the IP_3 and the cyclic AMP accumulation respectively in 1321N1 of CHO-K1 cells expressing the human $P2Y_{11}$ receptor. Transfected cells were incubated in the presence of various concentrations of AR-C67085 (2-propylthio-β, γ-dichloromethylene-D-ATP) or ATP for 30 s (A) or 15 min (B). The data are the mean ± s.d. of triplicate experimental points and are representative of three independent experiments.

Discussion

The pharmacological characterization of the human $P2Y_{11}$ receptor has been conducted in two different cell lines. We have chosen the 1321N1 astrocytoma cells to perform inositol trisphosphate measurements and the CHO-K1 cells to perform cyclic AMP assays. Indeed, a significant endogeneous cyclic AMP response to adenosine, a degradation product of ATP, is obtained in the 1321N1 cells. When this endogeneous response to adenosine was inhibited by 8-p-SPT, a significant cyclic AMP response to ATP was still observed, but only at high concentrations (300 μM), suggesting that the coupling of the $P2Y_{11}$ receptor to adenylyl cyclase has a low efficacy in the 1321N1 cell line (data not shown). This is entirely consistent with the data reported by Kennedy *et al.* (1999). Therefore we did not perform a characterization of the cyclic AMP response to ATP and its derivatives in the 1321N1 cell line. By contrast, the CHO-K1 cells do not possess adenosine receptors coupled to adenylyl cyclase, but they express an endogeneous $P2Y_2$ receptor coupled to the phosphoinositide pathway and which presents a high affinity for ATP (Iredale & Hill, 1993). Following transfection by the $P2Y_{11}$ receptor, the inositol phosphate response to ATP was not significantly enhanced, indicating that this model is not adequate to characterize the coupling of the $P2Y_{11}$ receptor to phospholipase C.

The time course of IP_3 accumulation is particularly sustained in the absence of lithium. This suggests a lack of desensitization of this receptor like it was observed for the $P2Y_6$ receptor transfected in the same cell line (Robaye *et al.*, 1997). This receptor was also coupled to a pertussis toxin-insensitive G protein. The time course of cyclic AMP accumulation was slower but compatible with a direct coupling to a G_s protein. Indeed the TSH receptor, which is a typical G_s-coupled receptor, displayed a similar time course of cyclic AMP accumulation, when expressed in the same cell line and using the same phosphodiesterase inhibitor Rolipram (Perret *et al.*, 1990).

There was no major difference in agonist or antagonist sensitivity between the two signalling pathways. The only discrepancy was that ATPγS was the most potent agonist to

stimulate the cyclic AMP pathway, whereas BzATP was the most potent on IP_3. Since cyclic AMP experiments lasted for 15 min, this difference is likely to reflect the resistance of ATPγS to hydrolysis by ectonucleotidases. EC_{50} of agonists were consistently lower in CHO-K1 cells, while the IC_{50} of antagonists were higher. This could be explained if the level of receptor expression was higher in the CHO-K1 cells, thus providing a larger receptor reserve. It is important to note that the nucleotides have been used without any previous HPLC-purification and that it could have some relevance to long time stimulation like in the cyclic AMP assays.

The hypothesis that the $P2Y_{11}$ receptor could be coupled to additional signaling pathways, possibly activated at lower ATP concentrations than phospholipase C and adenylyl cyclase stimulation, was investigated. No evidence of $P2Y_{11}$ receptor coupling to adenylyl cyclase inhibition, phospholipase A_2 activation (measured by the release of arachidonate) or phospholipase D activation (measured by the release of choline) could be obtained (data not shown).

The observation that AR-C67085 is the most potent agonist of the $P2Y_{11}$ receptor was unexpected, since so far this compound was considered to be a highly selective antagonist of the platelet ADP receptor coupled to adenylyl cyclase inhibition and platelet aggregation (Humphries *et al*., 1995). Although this compound was approximately 10 fold more potent than ATP itself, it must be underscored that its potency at the platelet receptor is several orders of magnitude higher than at the $P2Y_{11}$ receptor.

The $P2Y_{11}$ subtype was sensitive to suramin antagonism. Suramin behaved clearly as a competitive antagonist of the $P2Y_{11}$ receptor with a low K_i value as compared to its effect on other P2Y receptors, except for the $P2Y_1$ receptor. The pA_2 value was indeed 6.09 for the $P2Y_{11}$ receptor, as compared to 5.77 for the $P2Y_1$ subtype and 4.32 for the $P2Y_2$ one (Charlton *et al*., 1996). In addition, suramin is a weak antagonist of the $P2Y_6$ receptor (Robaye *et al*., 1997) and is inactive on the $P2Y_4$ receptor (Communi *et al*., 1996b).

Northern blotting experiments have demonstrated the expression of the $P2Y_{11}$ receptor in the HL-60 human promyelocytic leukaemia cell line (Communi *et al*., 1997). The existence of a cyclic AMP-linked P2 receptor mediating the granulocytic differentiation of these cells has also been reported recently (Jiang *et al*., 1997). The present pharmacological characterization strongly supports that the P2Y receptor coupled to the cyclic AMP pathway in these cells is in fact the $P2Y_{11}$ receptor. Indeed, a lot of pharmacological features of the P2Y receptor coupled to the cyclic AMP pathway in HL-60 cells (Jiang *et al*., 1997; Choi & Kim, 1997; Conigrave *et al*., 1998) are similar to those of the recombinant $P2Y_{11}$ receptor. The most striking similarity was the rank order of potency of agonists, with ATPγS, BzATP and dATP being more potent than ATP itself. Moreover ADPβS and AMPαS behaved as partial agonists, able to partially inhibit the ATP response, in both systems. Finally, the sensitivity of the recombinant $P2Y_{11}$ receptor to suramin is consistent with the observation that 100 μM suramin completely blocked the effect of 300 μM ATP in HL-60 cells (Choi & Kim, 1997). In conclusion our previous demonstration by Northern blotting that $P2Y_{11}$ receptor mRNA is present in HL-60 cells, together with the functional similarities between the cyclic AMP responses triggered by ATP in HL-60 cells and in CHO-K1 cells expressing the human $P2Y_{11}$ receptor observed in the present study, strongly suggest that this receptor is involved in the granulocytic differentiation of HL-60 cells by ATP (Jiang *et al*., 1997; Conigrave *et al*., 1998).

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