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$P2Y_{11}$ receptor expression by human lymphocytes: evidence for two cAMP-linked purinoceptors

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Abstract

The effects of extracellular ATP, ADP, AMP and adenosine on cAMP accumulation have been studied in freshly isolated B-lymphocytes from patients with chronic lymphocytic leukemia. Extracellular ATP and several nucleotide analogs stimulated cAMP accumulation with the following order of potency: ATP ($EC_{50} = 120 \pm 20$ μM) > ADP ≫ AMP. ADP was less effective than ATP and may be a partial agonist. AMP exhibited variable but generally weak activity. The stable analog of ATP, α,β-methylene ATP ($EC_{50} = 110 \pm 15$ μM) also stimulated cAMP accumulation and exhibited similar efficacy to ATP. The $P2Y_2$ receptor agonist, UTP had no effect on intracellular cAMP levels. Adenosine and the A_{2A}/A_{2B} receptor agonist, 5′-*N*-ethylcarboxamidoadenosine (NECA) also stimulated cAMP accumulation in CLL lymphocytes. Adenosine deaminase inhibited the cAMP response to adenosine but had no effect on the ATP-induced cAMP response. On the other hand, the AMP analog, adenosine 5′-thiomonophosphate, (AMPS; 1.0 mM) inhibited ATP-induced and α,β-methylene ATP-induced cAMP production but had no effect on adenosine-induced cAMP production. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed the presence of $P2Y_{11}$ receptor as well as A_{2A} and A_{2B} receptor mRNA in chronic lymphocytic leukemia lymphocytes. However, A_{2B} receptors would appear to be relatively ineffective because the A_{2A} selective agonist, CGS-21680 exhibited comparable efficacy to NECA. Furthermore, the A_{2A}-selective antagonist 8-(3-chlorostyryl)-caffeine (CSC) right-shifted the concentration–response curve for NECA. Taken together, the data indicate that ATP induces cAMP accumulation via the activation of $P2Y_{11}$ receptors whereas adenosine induces cAMP accumulation via the activation of A_{2A} receptors. Coordinate activation of $P2Y_{11}$ and A_{2A} receptors may influence the developmental fate of normal B-lymphocytes. © 2001 Published by Elsevier Science B.V.

Keywords: $P2Y_{11}$ receptor; cAMP; ATP; B-lymphocytes, human; Adenosine A_2 receptor; Chronic lymphocytic leukemia

1. Introduction

Nucleotides, such as ATP, ADP and the nucleoside, adenosine exert a wide range of physiological effects by activating two families of cell surface purinoceptors. Based on pharmacological properties, these receptors have been divided into the P1 and P2 receptors (Burnstock, 1996; Dubyak and El-moatassim, 1993). P1 receptors have been further subdivided into three main types: A_1 adenosine receptors, which inhibit adenylyl cyclase, A_{2A} and A_{2B} receptors, which stimulate adenylyl cyclase and A_3 receptors (Jacobson et al., 1996). The P2 family includes P2X receptors which form ionic channels and the P2Y receptors which, in general, couple via G-proteins to phospholipase-C. Cloning studies have revealed seven subtypes of P2X receptors, the most recent member, $P2X_7$, being a unique pore-forming receptor which is strongly expressed on human macrophages and lymphocytes (Falzoni et al., 1995; Rassendren et al., 1997; Wiley and Dubyak, 1989). P2Y receptors are also expressed on cells of the immune system since $P2Y_2$ (formerly termed P2U) is found on neutrophils and monocyte-macrophages (Cowen et al., 1989). In a recent RT-PCR based survey of haematological cells, several phospholipase C-linked P2Y receptors were detected including $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors (Jin et al., 1998). Lymphocytes, however, are thought to lack P2Y receptors or their associated PLC signalling pathway since extracellular nucleotides fail to elicit release of Ca^{2+} from intracellular stores (Wiley and Dubyak, 1989). However,

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the $P2Y_{11}$ receptor which couples either to phospholipase-C or adenylyl cyclase is expressed at high levels in the spleen (Communi et al., 1997) raising the possibility that this receptor is normally expressed by B- and/or T-lymphocytes.

Intracellular cAMP levels exert a powerful influence on the fate of B-lymphocytes. For example, elevated cAMP enhances proliferation of B-lymphocytes in the presence of the key growth factor interleukin-4 (Gantner et al., 1998). However, in the absence of growth factors, elevated cAMP promotes apoptosis of B-lymphocytes (Mentz et al., 1996, 1999; Kim and Lerner, 1998). Lymphocyte cAMP may be influenced by many physiological factors including plasma adenosine. Thus, incubation with adenosine elevates cAMP levels in peripheral blood lymphocytes (Smith et al., 1971; Wolberg et al., 1975) presumably by A_{2A} receptors which have been identified recently on human T-lymphocytes (Koshiba et al., 1999). In the current work, we set out to determine whether purinoceptors might also stimulate cAMP accumulation in circulating B-lymphocytes from subjects with CLL. The work provides evidence for the expression of cAMP-coupled P1 and P2 receptors.

2. Materials and methods

2.1. Materials

Ficoll–Paque (d.1.077) was obtained from Pharmacia (Uppsala, Sweden). ATP, ADP, adenosine, UTP, α,β-methylene ATP, EGTA, forskolin, IBMX (3-isobutyl-1-methyl xanthine) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). Xanthine amine congener, 8-(p-sulfophenyl) theophylline, 1-[*N*, *O*-bis(5-isoquinoline-sulphonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN62), the A_{2A}/A_{2B} agonist 5′-*N*-ethylcarboxamidoadenosine (NECA), the A_{2A}-selective agonist CGS-21680 and the A_{2A}-selective antagonist 8-(3-chlorostyryl) caffeine were from Research Biochemicals (Natick, MA, USA). The tracer, adenosine 3′,5′-cyclic phosphoric acid 2′-*O*-succinyl 3′- [^{125}1] iodotyrosine methyl ester was from Amersham (Australia).

2.2. Lymphocyte preparation

Peripheral blood lymphocytes were obtained from patients with B-cell chronic lymphocytic leukemia (CLL) as described previously (Wiley et al., 1998) in accordance with a protocol approved by the Human Ethics Committee of the University of Sydney. Venous blood (20 ml) was added to heparin anti-coagulant and diluted with two volumes HEPES-buffered saline (145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ 5 mM HEPES-CI, pH 7.4, 5 mM D-glucose, 1 g/l BSA). Mononuclear cells were separated by density gradient centrifugation over Ficoll–Paque. Cell counts were performed using a Coulter Counter (Model ZBI, Hertz, UK) and adjusted to 5×10^6 cells/ml for the incubations below. Immunophenotype analysis of CLL samples showed that $94 \pm 4\%$ of the lymphocytes were B-cells.

2.3. Accumulation of intracellular cAMP

Lymphocytes (in 0.2 ml aliquots) were stimulated with several different nucleotides (0–20 min, 37 °C) at various concentrations. In some experiments, the cells were preincubated with IBMX (0.5 mM) for 10 min at 37 °C. The reactions were stopped by transfer to an ice bath and the cells were centrifuged (5 min, 4 °C, $12\,000 \times g$). Following centrifugation, the supernatant was discarded and the cell pellet was lysed by the addition of ice cold 90% ethanol plus 1 mM EDTA followed by vortexing. The tubes were centrifuged again and the supernatant was collected. Supernatants were evaporated and the precipitates re-dissolved in 0.2 ml of cAMP assay buffer (50 mM Na acetate, 25 mM $CaCl_2$, 0.1% BSA, pH 6.2). The radio-immunoassay for cAMP was performed using the radioactive tracer adenosine 3′,5′cyclic phosphoric acid 2′-*O*-succinyl 3, [^{125}I] iodotyrosine methyl ester as described previously (Luttrell and Henniker, 1991). The addition of 1 mM ATP to control samples had no effect on the cAMP levels determined using the radio-immunoassay.

2.4. RT-PCR analysis

Total RNA was isolated from cells using RNA isolation reagent (Advanced Biotechnologies, UK) according to the manufacturer's instructions. For cDNA synthesis, 1 pg of total RNA was used in reverse transcription reaction with 0.5 μg of primer Oligo (dT) with 200 units SUPERSCRIPT II Reverse Transcriptase (Life Technologies,

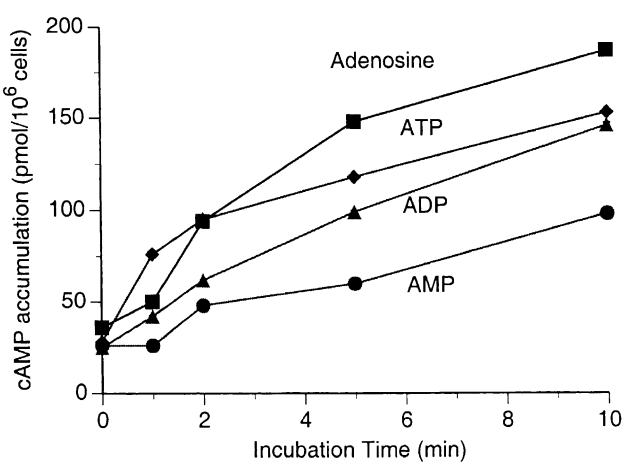

Fig. 1. The effect of extracellular ATP and breakdown products on cAMP accumulation in human CLL lymphocytes. CLL lymphocytes were exposed to 0.5 mM adenine nucleotides and adenosine for various times in physiological saline at 37 °C after pre-incubation with 0.5 mM IBMX. Zero-time samples were obtained by the addition of stop solution before agonist. The data were obtained from a single human subject. For clarity, only means (SEMs all $\leq 20\%$; $n = 3$) are shown.

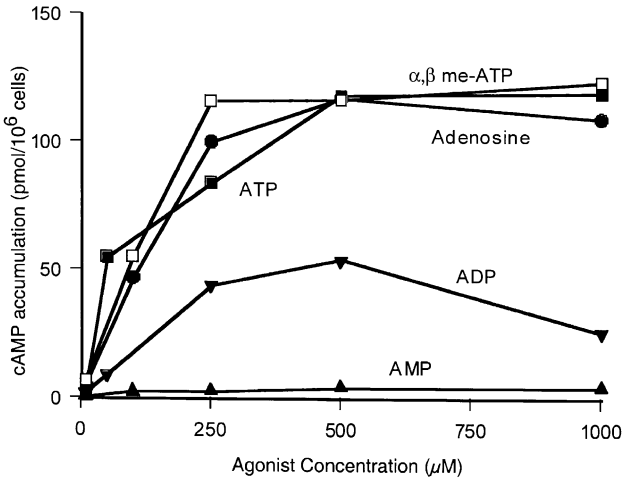

Fig. 2. Concentration dependence of the effects of ATP and ATP analogs on cAMP accumulation in human CLL lymphocytes. CLL lymphocytes from a single human subject were pre-incubated for with 0.5 mM IBMX and then exposed to various concentrations of ATP or ATP analogs in physiological saline for 5 min at 37 °C. For clarity, only means (SEMs all $\leq 20\%$, expecting ADP 1000 μM; $n = 3$) are shown.

NSW, Australia). The primers for $P2Y_{11}$ were designed from the human $P2Y_{11}$ cDNA sequence (GenBank accession number AF030335) using the computer program Primer 3 (version 3, Whitehead Institute, USA). The forward primer was 5′-ACTGGTGGTTGAGTTCCTGG-3′ (nucleotide positions 93–112), and the reverse primer was 5′-TCAGGTGGGAGAAGCTGAGT-3′ (nucleotide positions 502–483). The primers for A_{2A} and A_{2B} were also designed from the human cDNA sequences (GenBank accession numbers NM_000675 and NM_000676, respectively) using Primer3. For A_{2A}, the forward primer was 5′-CTGCTCATGCTGGGTGTCTAT-3′ (nucleotide positions 852–872) and the reverse primer was 5′-TTGAAAGGTTCTTGCTGCCTC-3′ (nucleotide positions 1225–1205). For A_{2B}, the forward primer was 5′-ATGGAACCACGAATGAAAGC-3′ (nucleotide positions 808–827) and the reverse primer was 5′-GCTGGCTGGAAAAGAGTGAC-3′ (nucleotide positions 1117–1098). Controls, in which reverse transcriptase was omitted from the RT-incubations, were routinely performed to exclude the possibility of PCR amplification from contaminating genomic DNA. To perform PCR, a 25-μl reaction contained $1 \times$ PCR buffer, 1.5 mM $MgCl_2$, 100 μM dNTPs (dATP, dGTP, dCTP and dTTP), 10 pmol of each $P2Y_{11}$ primer, 0.75 U Taq DNA polymerase (Promega, Australia), and 100 ng of cDNA sample. The PCR was carried out with a thermocycling program as follows: initial denaturation at 95 °C for 5 min, then 35 cycles of 95 °C for 45 s, 57 °C for 45 s, and 72 °C for 1 min; the final extension step was performed at 72 °C for 10 min. Conditions for the PCR for A_{2A} and A_{2B} were identical except for the annealing step which was 52 °C for 45 s. Detection of PCR products was performed by electrophoresis in a 2% agarose gel followed by ethidium bromide staining. The sizes of PCR fragments were determined with a 100-bp DNA molecular weight ladder.

3. Results

3.1. Effects of extracellular ATP, ATP analogs and ATP breakdown products

Basal levels of cAMP in B-lymphocytes from 17 patients with chronic lymphocytic leukemia ranged from 13.1 to 29.2 pmol/10^6 cells with a mean value of 19.9 ± 5.8 (SD) pmol/10^6 cells. The addition of extracellular ATP (1 mM) induced a rapid, approximately fivefold, increase in intracellular cAMP levels that reached a maximum value after 10 min (Fig. 1); half-maximal responses occurred at approximately 2 min. Although there was variability between patient samples, the ATP-induced elevation in cAMP level reached 59 ± 20 (SD, $n = 13$) pmol/10^6 cells at 20 min ($P < 0.001$) in the absence of the phosphodiesterase inhibitor IBMX. The effect of ATP was amplified approximately threefold after 10 min preincubation with 0.5 mM IBMX (not shown). The effects of various concentrations (10–1000 μM) of nucleotides and adenosine were determined after 10 min pre-incubation with 0.5 mM IBMX, followed by a 5-min incubation with agonist. ATP, ADP, adenosine and the stable ATP analog, α,β-methylene ATP (Fig. 2) all produced an accumulation of cAMP over basal

Table 1
Effects of potential inhibitors on cAMP accumulation

Treatment	Control (%)	AMPS (%)	Ado Deaminase (%)	8-SPT (%)	XAC (%)	Indomethacin (%)
Forskolin (100 μM)	127 ± 34	124 ± 30	84 ± 23	87 ± 17	82 ± 19	135 ± 10
ATP (500 μM)	100	$35 \pm 7^{***}$	136 ± 26	68 ± 15	74 ± 10	115 ± 12
Adenosine (500 μM)	80 ± 16	116 ± 39	$43 \pm 21^{*}$	$30 \pm 5^{**}$	$19 \pm 3^{***}$	99 ± 21

Accumulation of cAMP was measured in lymphocytes (5×10^6/ml) exposed to the indicated agonists in the absence ($n = 11$) or presence ($n = 3$) of the putative inhibitors for 20 min (5 min in the case of adenosine deaminase). The inhibitor concentrations were as follows: AMPS (1 mM), adenosine deaminase (4 U/ml), 8-SPT (50 μM), XAC (20 μM) or indomethacin (50 μM). All cellular cAMP concentrations have been expressed as a percentage of the response to 500 μM ATP. All values are the mean of at least three separate experiments from different subjects and are expressed as means ± SEM.

$^{*}P < 0.05$.

$^{**}P < 0.02$.

$^{***}P < 0.01$.

values ($n = 3$ for all). ATP, adenosine and α,β-methylene ATP all gave similar maximal accumulation of cAMP (ca.120 pmol/10^6 cells for the subject shown in Fig. 2) while ADP gave a lower maximal accumulation, suggesting that it may be a partial agonist. AMP was only a weak activator of cAMP accumulation under these conditions (Figs. 1 and 2 showing data from two different subjects). The $P2Y_2$ agonist UTP (1 mM; not shown), was without effect. Maximum levels of cAMP accumulation by forskolin, which directly stimulates adenylyl cyclase, and extracellular ATP and adenosine were comparable (Table 1).

3.2. Effects of putative inhibitors of cAMP accumulation

The effects of several potential inhibitors of cAMP accumulation were investigated. The classical P2 receptor inhibitor, suramin (0.5 mM) inhibited ATP-induced cAMP accumulation by 70–80% but suramin also inhibited adenosine-induced cAMP accumulation by 50% (results not shown). AMPS (1 mM), which inhibits the $P2Y_{11}$ receptor (Communi et al., 1999; Conigrave et al., 1998), inhibited ATP-induced cAMP accumulation by about 60% ($P < 0.01$) but had no effect on forskolin, or adenosine-induced cAMP accumulation (Table 1). One mM AMPS also inhibited 100 μM α,β-methylene ATP-induced cAMP accumulation by about 50% ($n = 3$; $P < 0.01$; not shown). Inclusion of adenosine deaminase (4 U/ml) significantly inhibited adenosine-induced cAMP accumulation ($P < 0.01$) without reducing ATP-induced cAMP accumulation. The P1 receptor antagonists 8-sulfophenyl theophylline (8-SPT, 50 μM) and xanthine amine congener (XAC, 20 μM) had no significant effect on ATP-stimulated cAMP but significantly inhibited adenosine-stimulated cAMP accumulation ($P < 0.02$ and $P < 0.01$, respectively; Table 1). The cyclo-oxygenase inhibitor, indomethacin (50 μM) had no effect on ATP- or adenosine-induced cAMP accumulation (Table 1), thereby excluding a role for autocrine-dependent signalling via prostaglandin E_1 or E_2.

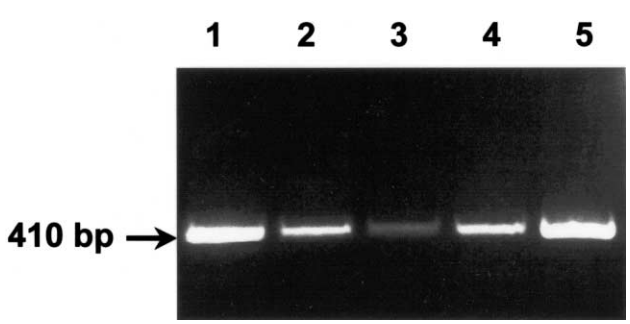

Fig. 3. RT-PCR analysis for the $P2Y_{11}$ receptor in human CLL lymphocytes. PCR was performed using $P2Y_{11}$ specific primers and cDNA prepared from CLL lymphocyte RNA samples from five separate patients (lanes 1–5). In control reactions, no bands were obtained after omission of reverse transcriptase thereby excluding the possibility of genomic DNA contamination of the RNA preparations.

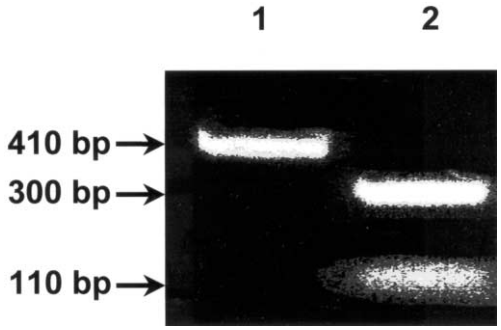

Fig. 4. Restriction digest of PCR product. The predicted $P2Y_{11}$ 410 bp PCR product contained a single Pvu-II restriction site. Restriction digest of the purified 410 bp PCR product with Pvu-II yielded the predicted fragment sizes of 300 and 110 bp. Lane 1, full length PCR band. Lane 2, DNA fragments obtained after digestion by Pvu-II.

3.3. $P2X_7$ is not involved in cAMP accumulation

The potent $P2X_7$ inhibitor, KN62 (100 nM; Gargett and Wiley, 1997) failed to inhibit ATP or adenosine-induced accumulation of cAMP (not shown). Removal of extracellular Ca^{2+} ions using the Ca^{2+}-chelator EGTA (0.1 mM) also had no effect on the accumulation of cAMP induced by ATP or adenosine (not shown). These data indicate that neither $P2X_7$ receptors nor Ca^{2+} influx was required for the activation of adenylyl cyclase by ATP.

3.3.1. RT-PCR analysis for $P2Y_{11}$ receptor

RT-PCR was carried out on cDNA prepared from B-lymphocyte RNA of five patients. A 410-bp band obtained in all cases from samples prepared in the presence, but not the absence, of reverse transcriptase confirmed the expression of $P2Y_{11}$ mRNA transcripts (Fig. 3). Further investigation of this PCR fragment using restriction enzyme digestion (Fig. 4) and DNA sequencing (not shown) confirmed that the PCR fragment derived from these patient samples arose from $P2Y_{11}$.

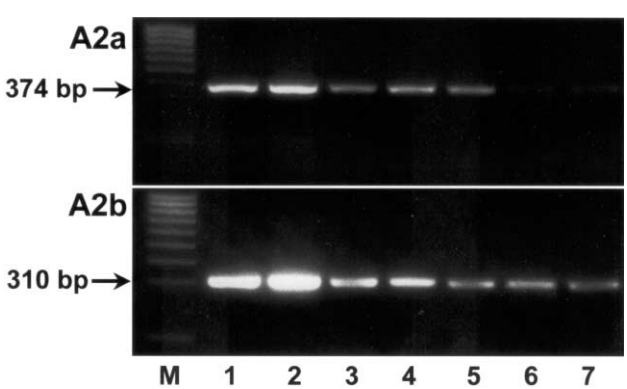

Fig. 5. RT-PCR analysis for the A_{2A} and A_{2B} receptors. PCR was performed using adenosine A_{2A} and A_{2B} receptor specific primers and cDNA prepared from CLL lymphocytes from seven separate patients (lanes 1–7). The top panel shows a single PCR product of the predicted 374 bp size for A_{2A} and the bottom panel a product of 310 bp size predicted for the A_{2B} receptor.

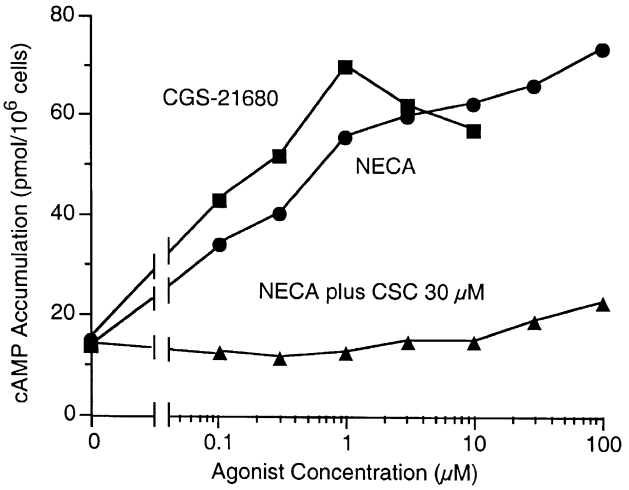

Fig. 6. Pharmacological analysis of A_2 receptors. CLL lymphocytes (no preincubation with IBMX) were exposed to various concentrations of the A_{2A}/A_{2B} agonist NECA as well as the A_{2A}-selective agonist CGS-21680. The effect of the A_{2A}-selective antagonist CSC (30 μM) on the NECA concentration response is also shown. Incubation time was 5 min.

3.3.2. Expression of A_2 receptors by CLL lymphocytes

The finding that adenosine stimulated cAMP production indicates that one or both of the recognised A_2 receptor subtypes is expressed by CLL lymphocytes. RT-PCR analysis of cDNA from CLL lymphocytes from seven individuals revealed the presence of both A_{2A} and A_{2B} receptors (Fig. 5).

A pharmacological analysis of A_2 receptor activity was undertaken in one patient to determine whether the effect of adenosine was primarily due to A_{2A} or A_{2B} receptors (Fig. 6). In the absence of IBMX, the non-selective A_{2A}/A_{2B} agonist NECA activated cAMP accumulation in a concentration-dependent fashion. The concentration–response curve for NECA was right-shifted by greater than two orders of magnitude in the presence of the A_{2A} selective antagonist 8-(3-chlorostyryl) caffeine (30 μM). Consistent with this finding, the A_{2A} selective agonist, CGS-21680 was a potent activator of cAMP accumulation (Fig. 6) and the maximal effects of CGS-21680 and NECA were comparable. Taken together, the data indicate that A_{2A} receptors are largely responsible for adenosine-induced cAMP accumulation in CLL lymphocytes.

4. Discussion

cAMP is an important second messenger for the control of lymphocyte proliferation, differentiation and apoptosis (Gantner et al., 1998; Mentz et al., 1995). Hence, receptors that stimulate cAMP accumulation have potentially important roles in the control of lymphocyte maturation, disposition and cell density. The novel cAMP-linked $P2Y_{11}$ receptor was shown to be expressed at high levels in the spleen (Communi et al., 1997) suggesting the hypotheses that lymphocytes express $P2Y_{11}$ receptors at high levels and that extracellular ATP may play a role in lymphocyte biology. Therefore, in the present study, human CLL lymphocytes were examined for ATP-stimulated cAMP accumulation and $P2Y_{11}$ receptor expression. Human lymphocytes responded acutely to extracellular ATP as well as its breakdown product adenosine with a rapid accumulation of cAMP. In addition, $P2Y_{11}$ receptor mRNA expression was observed in all CLL samples studied by RT-PCR. The data indicate that two cAMP-linked purinoceptors are present on CLL lymphocytes—one receptor for ATP ($P2Y_{11}$) and a second receptor for adenosine (A_2). Such an arrangement would permit the coordinated activation of adenylyl cyclase in the event of ATP release into the local environment of lymphocytes (e.g., in the spleen). Thus, the acute activation of P2 receptors might be followed by the delayed activation of adenosine receptors as ATP breakdown yielded successively, ADP, AMP and adenosine. Interestingly, however, AMP elicited little or no cAMP accumulation in the majority of patients investigated (e.g., Fig. 2) although some variability in AMP response was observed (compare Fig. 1 and Fig. 2). This behaviour indicates that AMP is only a weak activator of $P2Y_{11}$ or A_{2A} receptors in its own right and that its conversion to adenosine by 5′-nucleotidase on the surface of CLL lymphocytes is relatively slow. Expression of ecto-5′-nucleotidase activity on the surface of B-lymphocytes is significantly reduced in CLL but a subset of patients with normal or elevated expression has also been described (Silber et al., 1975; Rosi et al., 1998).

Several different receptor-dependent mechanisms may account for ATP-activated cAMP accumulation. One potential mechanism operates via the generation of adenosine by the concerted action of ectonucleotidases and 5′-nucleotidase followed by the activation of A_2 receptors. Such a mechanism may explain ATP—as well as adenosine-induced cAMP accumulation in aortic endothelial cells (Cote et al., 1993), cerebral capillary endothelial cells (Anwar et al., 1999) and NG108-15 neuronal cells (Ohkubo et al., 2000). This mechanism requires careful consideration in CLL lymphocytes because adenosine, like ATP, also stimulated cAMP accumulation in the present study and because A_{2A} receptors have been recently demonstrated in T-lymphocytes (Koshiba et al., 1999). It seems unlikely, however, that this mechanism was responsible for the *acute* stimulatory effect of ATP on cAMP accumulation in CLL lymphocytes because the ATP breakdown product and adenosine precursor, AMP did not mimic the effect of ATP and the stable ATP analog, α,β-methylene ATP was approximately equipotent with ATP. α,β-Methylene ATP is a recognised agonist of the cloned human $P2Y_{11}$ receptor (Van der Weyden et al., 2000a,b). We were further able to differentiate between the effects of adenosine and ATP because the A_2 receptor antagonists 8-sulfophenyl-theophylline and xanthine amine congener inhibited adenosine-induced cAMP accumulation but had no signifi-

cant effect on ATP-induced cAMP accumulation. In addition, adenosine deaminase, which converts adenosine to the inactive metabolite inosine, had no effect on ATP-induced cAMP accumulation but inhibited adenosine-induced cAMP accumulation by about 50%.

ATP may also induce cAMP accumulation via the activation of P2 receptors. First, P2 receptor stimulation may promote cAMP accumulation indirectly via the activation of phospholipase-A_2 leading to the generation of arachidonate followed by its conversion to prostaglandin E_1 or E_2 (PGE). PGE may then, in turn, bind to prostanoid receptors and stimulate adenylyl cyclase. This pathway, which is blocked by the cyclo-oxygenase inhibitor indomethacin, has been implicated, for example, in the generation of cAMP by ATP- or UTP-stimulated $P2Y_2$ receptors in MDCK cells (Post et al., 1996). However, indomethacin had no effect on ATP-induced cAMP accumulation in CLL lymphocytes (Table 1). We also considered the possibility that ATP might stimulate cAMP accumulation via the activation of $P2X_7$ receptors that are known to be present on lymphocytes. However, neither ATP- nor adenosine-induced cAMP accumulation was inhibited by the potent $P2X_7$ antagonist KN62 (Gargett and Wiley, 1997) and a role for Ca^{2+} influx in the stimulation of adenylyl cyclase was also excluded by removal of Ca^{2+} ions and inclusion of the Ca^{2+} chelator EGTA in the incubation solution.

The likely role of G-protein coupled $P2Y_{11}$ receptors in ATP-stimulated cAMP accumulation in the current study is supported by the finding that $P2Y_{11}$ receptor mRNA is expressed by CLL lymphocytes (Figs. 3 and 4). In addition, AMPS, which inhibits ATP-induced cAMP accumulation in cells that stably express the $P2Y_{11}$ receptor (Communi et al., 1999; Van der Weyden et al., 2000a), as well as $P2Y_{11}$ expressing HL-60 cells (Conigrave et al., 1998), suppressed ATP- but not adenosine-induced cAMP accumulation in lymphocytes (Table 1).

The RT-PCR data also indicate that CLL lymphocytes express A_{2A} and A_{2B} receptors (Fig. 5) thereby explaining adenosine-induced cAMP accumulation in CLL lymphocytes as well as the selective inhibitory effects of the P1 antagonists 8-SPT and XAC on adenosine-, but not ATP-induced, cAMP accumulation. Pharmacological analysis of cells from one individual using the selective A_{2A} receptor agonist, CGS-21680 and antagonist CSC indicated that the A_{2A} receptor sub-type could account for all of the response to the non-selective A_{2A}/A_{2B} receptor activator NECA. However, variations in the levels of A_{2A} and A_{2B} receptor expression might lead to variations in pharmacological behaviour in different individuals with CLL.

The data indicate that CLL lymphocytes like HL-60 (Choi and Kim, 1997; Conigrave et al., 1998; Jiang et al., 1997) and NB4 myeloid leukemia cells (Van der Weyden et al., 2000b), express $P2Y_{11}$ receptors. Thus, cells of both the myeloid and lymphoid lines express $P2Y_{11}$ receptors leading us to speculate that ATP-dependent activation of the cAMP signalling pathway by $P2Y_{11}$ receptors contributes to the normal control of white cell maturation. In the special case of B-lymphocytes, coordinate activation of $P2Y_{11}$ and A_{2A} receptors by the local release of ATP followed by its breakdown to adenosine, in a cell rich compartment such as the bone marrow or spleen, may contribute to the determination of cell fate. For example, these receptors may activate cAMP-linked suppression of B-cell maturation (Huang et al., 1995) or promote proliferation (Gantner et al., 1998) depending on the local growth factor environment.

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