Adenosine inhibits the release of interleukin-1β in activated human peripheral mononuclear cells

Sandor Sipka a,*, Ildikó Kovács a, Sándor Szántó a, Gyula Szegedi a, László Brugóš b, Geza Bruckner c, A. József Szentmiklósi d

a 3rd Department of Internal Medicine, University of Debrecen, Hungary
b Department of Pulmonology, University of Debrecen, Hungary
c Division of Clinical Nutrition, University of Kentucky, USA
d Department of Pharmacology and Pharmacotherapy, University of Debrecen, Hungary

Received 23 December 2004; received in revised form 16 March 2005; accepted 4 May 2005

Abstract

The effects of adenosine and subtype-specific activators of adenosine receptors (A1, A2A, A2B and A3) were studied on the release of interleukin-1β (IL-1β) from peripheral mononuclear cells, monocytes and lymphocytes. In the cells activated by the protein kinase C specific phorbol ester (phorbol 12-myristate 13-acetate) and Ca2+ ionophore (A23187) both adenosine and the subtype-specific receptor agonists, CPA (A1), CGS 21680 (A2A) and IB-MECA (A3) induced a concentration-dependent inhibition of IL-1β release. The rank order of potency in the inhibition of IL-1β release was CPA > CGS 21680 > IB-MECA > adenosine > NECA (in the presence of A1, A2A and A3 receptor inhibitors). The inhibitory actions of CPA, CGS 21680 or IB-MECA were significantly reduced in the presence of DPCPX, ZM 243185 or MRS 1191 as subtype-specific antagonists on A1, A2A and A3 adenosine receptors, respectively. It can be concluded that adenosine inhibits the release of IL-1β from the activated human peripheral mononuclear cells. In this process A1, A2A and A3 receptors are involved.

Keywords: Adenosine; Adenosine receptors; Interleukin-1β; Human mononuclear cells

1. Introduction

IL-1 is one of the most important proinflammatory cytokines. Monocytes and macrophages are considered to be the major cell types responsible for IL-1 production. In acute phase reactions, it promotes the proliferation of lymphocytes, the production of antibodies and IL-6, the activation of phagocytes and endothelial cells as well as induces fever [1,2]. In monocytes and macrophages, IL-1β is produced primarily through stimulation by bacterial lipopolysaccharides (LPS) and tumor necrosis factor-α (TNF-α). These proinflammatory stimuli are capable of inducing the synthesis of the precursor protein of IL-1β with a molecular mass of 31 kDa (pro-IL-1β). During the process of maturation, an enzyme, caspase-1, cleaves pro-IL-1β, and forms the biologically active form, the mature IL-1β with a molecular mass of 17 kDa. This active form of the cytokine can be rapidly released from the cells by a non-identified mechanism [3].

A number of endogenous and exogenous substances have been described to decrease the release of IL-1β, leading to the mitigation of inflammatory processes. Adenosine, an endogenous mediator plays a prominent role as an anti-inflammatory substance. A series of earlier studies showed that signals mediated by A2A
receptors decrease the release of TNF-α, IL-6 and IL-8 [4,5]. Therefore, blocking these receptors has become a primary goal toward diminishing the inflammatory response [6–8]. Numerous studies reported the action of adenosine on the release of cytokines [9] including IL-12, IFNγ [10–12] and IL-10 [13]. To our knowledge, the action of adenosine on the release of interleukin-1β from monocytes has not been clearly clarified to date [5]. IL-1β was reported to be an inducer of TNF-α release in experiments testing A1 and A2 receptor agonists [4], in addition, the agonists of A3 receptors were analysed from the aspect of IL-1 production in concert with other cytokines [9,13].

It is widely accepted that adenosine mediates its action on the cells through at least four G-protein-coupled receptor subtypes, which have been classified as A1, A2A, A2B and A3 [14–17].

In our present study, we investigated the effects of adenosine and various adenosine receptor agonist and antagonist molecules on the release of IL-1β from the suspensions of peripheral mononuclear cells (PBMC), monocytes and lymphocytes activated by phorbol 12-myristate 13-acetate (PMA) and calcium ionophore (A23187). This work is the first to describe the direct inhibition of interleukin-1β release by adenosine from human activated mononuclear cells and the involvement of the various subtypes of adenosine receptors in this process.

2. Results

2.1. Effect of adenosine and the subtype-specific adenosine receptor agonists on the release of IL-1β from activated human peripheral mononuclear cells

In the suspensions of PBMC activated by phorbol ester and calcium ionophore, the action of adenosine and the subtype-specific adenosine receptor agonists (CPA, CGS 21680 and IB-MECA) as selective activators of A1, A2A and A3 receptors, respectively) were studied on the release of IL-1β. In order to elicit an A2B specific stimulation, NECA, a non-specific A2 receptor agonist was applied, and the A2A receptors were blocked by 0.1 μM ZM 243185, in addition, the potential A1 and A3 receptor related actions were also blocked by adding 0.3 μM DPCPX and 10 nm MRS 1191, respectively. Adenosine and the receptor agonists were tested in the concentration range of 10 pM–1 μM (n = 32).

For the activation of peripheral blood mononuclear cells, the PKC specific phorbol ester (phorbol 12-myristate 13-acetate, PMA) and the Ca2+ ionophore, A23187 were used in parallel for 4 h, as the mechanisms regarding their actions on signal transduction are biochemically and pharmacologically well defined [18,19]. Mean PMA + A23187-induced IL-1β concentration was 82.6 ± 9.2 pg/ml (n = 32) before the exposition to the adenosine receptor agonists. Values are expressed as the mean ± S.E.M. of 5–7 experiments.

2.2. Action of specific adenosine receptor agonists on the inhibition of IL-1β release from activated human peripheral mononuclear cells in the presence of various adenosine receptor subtype-specific antagonist agents

In these experiments, the actions of the various subtype-specific antagonists, DPCPX (A1), ZM 243185 (A2A) and MRS 1191 (A3) were studied on the CPA (A1), CGS 21680 (A2A) and IB-MECA (A3) induced inhibition of IL-1β release from activated PBMC. Each antagonist significantly mitigated the percent of inhibition caused...
Inhibitory effects of selective activators of various adenosine receptor subtypes on IL-1β production in activated human peripheral mononuclear cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activation of adenosine receptor subtype</th>
<th>pD₂</th>
<th>E_max</th>
<th>nᵢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>A₁/A₂A/A₃</td>
<td>7.43 ± 0.11*</td>
<td>72 ± 8</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>CPA</td>
<td>A₁</td>
<td>10.27 ± 0.14**</td>
<td>80 ± 6</td>
<td>0.7 ± 0.3**</td>
</tr>
<tr>
<td>CGS 21680</td>
<td>A₂A</td>
<td>9.78 ± 0.62**</td>
<td>82 ± 6</td>
<td>0.8 ± 0.3**</td>
</tr>
<tr>
<td>NECA*</td>
<td>A₂B</td>
<td>9.35 ± 0.12**,*</td>
<td>70 ± 6</td>
<td>1.2 ± 0.2**</td>
</tr>
<tr>
<td>IB-MECA</td>
<td>A₃</td>
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</table>

Values are expressed as the mean ± S.E.M. of 5–7 experiments.

*p < 0.05 compared with CPA.

**p < 0.05 compared with adenosine.

Analysis of statistical significance was done using variance analysis (ANOVA) with a Newman–Keuls post hoc test.

pD₂, half maximum inhibition.

E_max, maximum inhibition.

nᵢ, Hill coefficient.

* NECA was administered in the presence of A₁ and A₂A adenosine receptor antagonists (0.3 μM DPCPX and 0.1 μM ZM 243185, respectively).

by the corresponding agonist (CPA 56.0% versus CPA + DPCPX 19.1%; CGS 21680 62.6% versus CGS 21680 + ZM 243185 18.1%; IB-MECA 66.5% versus IB-MECA + MRS 1191 19.4%; Fig. 2). Mean PMA + A23187-induced IL-1β concentration was 87.5 ± 10.3 pg/ml (n = 12) before the exposition to the adenosine receptor agonists. IL-1β concentrations in the presence of 0.1 μM DPCPX, 0.1 μM ZM 243185 or 10 nM MRS 1191 were 92.4 ± 11.4 (n = 4), 83.1 ± 9.8 (n = 4) and 81.3 ± 12.5 pg/ml (n = 4), respectively. These values are not significantly different from the pre-drug concentration (P > 0.05).

3. Discussion

The present study is the first to describe the direct inhibition of interleukin-1β release by adenosine and various subtype-selective adenosine receptor agonists in human mononuclear cells activated by phorbolester and Ca²⁺ ionophore. The combination of the activating agents used in these experiments was selected based on the role of (PMA sensitive) protein kinase C enzymes in the stimulation of A₂A receptors [20] and on the importance of elevated intracellular Ca²⁺ level in the secretion of IL-1β [21,22]. The release of IL-1β derived from the “plastic surface” (cell cultivation plate) activated cells [23] but not stimulated by PMA and Ca²⁺ ionophore was also taken into correction in every case. For testing the involvement of the various receptors of adenosine, selective agonists were applied, CPA for A₁, CGS 21680 for A₂A and IB-MECA for A₃ receptors. In order to determine the specific effects of the adenosine A₃B receptors, the A₁/A₂ receptor specific NECA was used in the presence of DPCPX and ZM 243185, as selective antagonists of A₁ and A₂A receptors to produce an A₃B specific response. For the exclusion of the potential A₃ type effect of NECA, the selective inhibitor of A₃ receptors, MR 1191 used. The specificity of changes related to the A₁, A₂ and A₃ receptors was also tested in the presence of their selective antagonists.

The results of the current study clearly show that the inhibitory effect of adenosine on the release of IL-1β from activated monocytes can also be mediated by the A₁, A₂A and A₃ receptors, while the effect of A₃B is negligible. The pharmacological analysis of the concentration–response curves for adenosine and its receptor specific agonists demonstrates that the Hill coefficient
(nH) for adenosine versus specific adenosine receptor activators is different. The slopes of the concentration—response curves for the adenosine receptor selective agonists do not differ from unity. On the other hand, the slope of adenosine is approximately 3, suggesting a strong positive correlation between multiple receptors. In the case of A1 and A3 receptors the inhibition of IL-1β release from the mononuclear cells can be mediated by adenylyl cyclase independent pathways, whereas the involvement of A2 receptors may have connections with adenylyl cyclase and PKC enzymes [21,24–30]. The clearly dose dependent inhibition of IL-1β release via A2 receptors is likely due to some PKC dependent phosphorylation [20]. Otherwise, there exists a specific cross-talk between adenosine and the receptors of various proinflammatory cytokines including IL-1β. These cytokines can up-regulate the expression of A2A receptors, at least in PC12 cells [31]. The slight inhibition of IL-1β release mediated by A3 receptors is most likely a consequence of the relatively low affinity of these receptors for adenosine [32].

In the current study IL-1β was primarily produced by the monocytes in the suspension of peripheral mononuclear cells (PBMC) consisting of both monocytes and lymphocytes [1,2]. The advantage of unfractionated PBMC versus purified mononucle was that the purification procedure could have resulted in some functional damage in the cells [33]. Therefore, the unfractionated non-damaged cell preparation was used for these studies. Furthermore, this approach reflects better the natural situation where circulating monocytes and lymphocytes can both be the sources of IL-1β in the peripheral blood.

The physiological and clinical importance of the present findings is that the inhibitory effect of adenosine and of its receptor specific analogues on the release of IL-1β has been observed in activated human peripheral mononuclear cells. Specifically, A2A receptors may serve as potential anti-inflammatory agents [9,34,35]. It was noteworthy that previously we reported that the average concentration of adenosine in the sera of healthy volunteers was about 40 nM, whereas the level of adenosine reached a concentration of 0.75 µM in patients with septic shock [36]. According to Martin et al. [37], the adenosine concentrations of circulating blood can be 4–10 µM in patients suffering from sepsis. In addition, it has been documented that adenosine concentrations can reach as high as 100 µM in some inflammatory regions [38]. According to our present results, adenosine is able to inhibit the release of IL-1 from the activated mononuclear cells at lower concentrations than previously mentioned. As IL-1 is one of the most effective natural inducer of the hyperthermic reactions [1,2,39], one of the main physiologic roles of adenosine may be the prevention of hyperpyrexia by the inhibition of IL-1 release from the activated monocytes, lymphocytes and macrophages [40]. In addition, it may moderate the hyperactivation of inflammatory cells in general. The capability of adenosine to regulate the actual level of IL-1 in certain part of the organism can play an important role in its action on processes related to innate immunity [41]. In addition, adenosine may also contribute to the anti-inflammatory effects of methotrexate (MTX) [42], e.g. by the inhibition of local release of IL-1 [43].

In summary, the present study demonstrates the inhibition of IL-1β release by adenosine and selective adenosine receptor agonists from activated human peripheral mononuclear cells in vitro. Furthermore, aspects of the physiological and clinical importance of the inhibition of IL-1β release caused by adenosine have been proposed. The observation that A1, A3, but especially A2A receptor activation can reduce the release of IL-1β from the activated mononuclear cells provides additional data to better understand the mechanism(s) of the anti-inflammatory effects of this endogenous purine nucleoside.

4. Materials and methods

4.1. Materials

The selective test agonists included the A1 receptor specific ligand N6-cyclopentyladenosine (CPA), A2A adenosine receptor agonist 2-β-[2-carboxethyl]-amino-5'-β-N-ethylcarboxamido-adenosine (CGS 21680), the A3 agent N6-(3-iodobenzyl)-5-(N-methylcarboxamidoadenosine (IB-MECA), the selective A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthin (DPCPX) and the selective A3 receptor antagonist 3-ethyl-5-benzyl-2-methyl-4-phenylethyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS 1191) and were purchased from Research Biochemicals, Inc. (USA). The selective A2A adenosine receptor antagonist 4-(2-[7-aminomethylfuryl][1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino)ethyl)phenol (ZM 241385) was obtained from Tocris (USA). Adenosine, 5'-N-ethylcarboxamidoadenosine (NECA), phorbol 12-myristate 13-acetate (PMA), and calcium ionophore (A23187) were purchased from Sigma Chemicals (USA). The ELISA kit for IL-1β was obtained from Amersham (UK). The RPMI-1640 culture medium and fetal calf serum were obtained from Gibco BRL (UK).

4.2. Preparation of human peripheral blood mononuclear cells (PBMC)

Mononuclear cell suspensions (on average 88–95% lymphocytes, 5–12% monocytes) were prepared from the peripheral blood samples of healthy volunteers according to Boyum's method [44]. The distribution
of the various subsets was detected by flow cytometry. The mean percentage of mononuclear cells was the following CD3⁺: 69.4%, CD19⁺: 13.5%, CD56⁺: 9.8% and CD14⁺: 8.3% (Coulter EPICS XL, flow cytometer, USA).

4.3. Stimulation of PBMC

Cells (5 × 10⁶) per well were cultured in RPMI-1640 medium completed with 10% fetal calf serum of low LPS content (plus with 80 μg/ml gentamycin and 2 mM glutamine) in a humified atmosphere with 5% CO₂ at 37 °C. All the samples were used in triplicates. The stimulation was achieved by the addition of 50 ng/ml of PMA and 5 μM of calcium ionophore (A23187) for 4 h.

4.4. Measurement of IL-1β release in the stimulated suspensions of PBMC

The concentrations of IL-1β were determined in the cell-free supernatants by an ELISA kit (Amersham, G.B.) and the results were given in pg/ml.

4.5. Treatment of PBMC by adenosine, CPA, CGS 21680 and IB-MECA

Various concentrations of adenosine and the selective adenosine receptor agonists were added to the stimulated suspensions of PBMC for 15 min before the stimulation by PMA + calcium ionophore. The adenosine A₂B receptor stimulation was achieved by using various concentrations of NECA (non-specific agonist for A₁ and A₂ receptors) in the presence of 0.3 μM concentrations of DPCPX (inhibitor of A₁ receptors) and ZM 243185 (inhibitor of A₂A receptors) added 15 min prior to NECA. In the experiments using specific inhibitor for A₁ receptors (DPCPX), and for A₂A receptors (ZM 243185) 0.1 μM concentrations of the drugs were used. In the case of A₃ receptors, the antagonist MRS 1191 (10 nM) was added to the cells 15 min before the application of the selective agonist agent CPA (10 nM). The two other agonist molecules, CGS 21680 and IB-MECA, were also applied in the concentration of 10 nM. The concentrations of the antagonists were chosen based on results from preliminary measurements. In the non-stimulated samples, aliquots of culture medium were used which contained the same amounts of dimethyl sulfoxide (DMSO) as the solutions of PMA and calcium ionophore. The amounts of IL-1β released from the cells activated by the “plastic surface” (cell cultivation plates) but not stimulated by PMA and Ca²⁺ ionophore were taken into correction in every case. (The average concentration of IL-1β produced by surface activation was 12.4 ± 2.5 pg/ml.) It is noted that DMSO as solvent, in the concentration applied did not have any significant effect on IL-1β production.

4.6. Measurement of cell viability

The viability of cells treated with various drugs before the measurement of their IL-1β release was determined by trypan blue exclusion test. Only those experimental samples were evaluated in which the viability of cells was higher than 90%.

4.7. Statistical analysis

The alterations in the release of IL-1β induced by adenosine and its agonists in the suspensions of PBMC were expressed as the percent of basal IL-1β production. Individual E/[A] curve data were fitted by the means of a least-square iterative computer program to a logistic function of the form:

\[ E = E_{\text{max}} [A]^{n_{H}} /[A]^{n_{H}} + [EC_{50}]^{n_{H}}, \]

where \( E \) denoted the effect, \( E_{\text{max}} \) was the asymptote, \([A]\) was the concentration of the agonist, \( EC_{50} \) was the concentration producing a half maximal response, and \( n_{H} \) was the midpoint slope parameter.

The data were expressed as means ± S.E.M. Multiple comparison between the experimental groups were performed by one-way analysis of variance (ANOVA) with a Newman–Keuls post hoc test. The criterion for significance was \( P < 0.05 \).

Acknowledgements

This work was supported by grants from the Hungarian Ministry of Health (ETT 111/2003, ETT 202/2003) and the Scientific Research Foundation (OTKA T037531).

References


