Adenosine-induced neuroprotection

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Chapter 3

Adenosine A3 receptor-induced CCL2 synthesis in cultured mouse astrocytes

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Summary

During neuropathological conditions high concentrations of adenosine are released stimulating adenosine receptors in neurons and glia cells. It has recently been shown that stimulation of adenosine receptors in glia cells induces the release of neuroprotective substances like NGF, S100B and interleukin-6 (IL-6). It has therefore been suggested that glial adenosine receptors are involved in neuroprotection. Since recently neuroprotective effects of the chemokine CCL2 (formerly known as MCP-1) have been reported, we investigated the possible effect of adenosine receptor stimulation on glial CCL2 synthesis. Here we show that stimulation of cultured murine astrocytes with the selective adenosine A3 receptor agonist 2-chloro-N6-(3-iodobenzyl)-N-methyl-5’-carbamoyladenosine (Cl-IB-MECA) induced the release of CCL2. Specific ligands for adenosine A1 or A2 receptors did not affect CCL2 release. Furthermore, Cl-IB-MECA-induced CCL2 synthesis was inhibited by adenosine A3 receptor antagonists. These results show that stimulation of adenosine A3 receptors in astrocytes induced the release of CCL2, thus supporting the assumption that adenosine receptors in glia cells regulate the synthesis of neuroprotective substances.

Introduction

Adenosine is essentially involved in the coupling of cellular metabolism to energy supply. In order to control metabolic rate, adenosine, for example, suppresses neuronal firing and increases blood flow [18, 31, 50]. The effects of adenosine are mediated by four types of G-protein coupled adenosine receptors; A1, A2A, A2B and A3 [27]. In the brain adenosine acts as a physiological neuromodulator [16, 66]. Under pathological conditions like seizures, ischaemia and hypoxia the extracellular adenosine concentration rapidly rises from nanomolar to micromolar levels [20, 57]. This large amount of adenosine has a significant neuroprotective effect [17, 57, 58, 64, 67] mainly by stimulating neuronal adenosine A1 receptors [27]. In neurons, adenosine inhibits the release of excitatory neurotransmitters and causes hyperpolarization of the membrane potential [20, 57]. Recent evidence however, suggests that stimulation of glial adenosine receptors leads to the synthesis of various neuroprotective substances. It has for example been shown that adenosine A1 receptor stimulation in astrocytes induces release of nerve growth factor and S100B protein [13]. Moreover, it was shown that stimulation of adenosine A2B receptors in astrocytes induces synthesis and release of interleukin-6 (IL-6) [23, 59]. The induction of IL-6 synthesis might also contribute to the neuroprotective actions of adenosine since there is accumulating evidence that IL-6 is a neuroprotective cytokine [45-47, 53, 63]. Thus the neuroprotective properties of adenosine might be direct in neurons and indirect in glia cells by stimulating the synthesis of neuroprotective substances. Chemokines are small chemotactic cytokines of approximately 10kDa, that are essential elements in the peripheral immune system (for review see [5, 51, 56]).
It is now clear that chemokines are also found in brain where they are predominantly expressed in glia cells [3, 4, 6].

The expression of CCL2 in glia cells, which is evident in most neurodegenerative conditions, controls the infiltration of the brain by blood leukocytes [25, 37, 38, 60]. On the other hand, the presence of functional chemokine receptors in all types of endogenous brain cells (neurons, astrocytes, oligodendrocytes and microglia) led to the assumption that chemokines may function as intercellular messengers in the brain (for review see [7, 36]). In line with this assumption are the effects of CCL2 on astroglial synthesis of matrix metalloproteinases and cytokine release [10, 15, 19]. Moreover, recent results show direct neuroprotective properties of CCL2 stimulation in NMDA or HIV-tat-treated neurons [11, 21].

Although it is clear that astrocytes express and release CCL2, little is yet known on the regulation of glial CCL2 synthesis. The findings, that astroglial adenosine receptors stimulate expression of various neuroprotective substances and that CCL2 has direct neuroprotective properties, prompted us to investigate possible effects of glial adenosine receptor stimulation on CCL2 synthesis.

**Materials and methods**

All cell culture media and supplements were obtained from Gibco-BRL Life technologies (Breda, the Netherlands). Reverse transcriptase enzyme and buffer were purchased from Promega (Madison, WI, USA). Taq-polymerase and buffer for PCR were obtained from Eppendorf (Boulder, CO, USA). N6-cyclopentyladenosine (CPA), N-ethylcarboxamidoadenosine (NECA), 1,3-dimethyl-8-phenylxanthine (8PT) and 3-propyl-6-ethyl-5-[(ethylthio)carbonyl]-2 phenyl-4-propyl-3-pyridine carboxylate (MRS 1523), Lipopolysaccharide (E.Coli, 0128:B12) and pertussis toxin were obtained from Sigma-Aldrich (St.Louis, MO, U.S.A.), 2-chloro-N6-(3-iodobenzyl)-N-methyl-5'-carbamoyladenosine (Cl-IB-MECA) and 2-[p-(2-carboxyethyl)phenyl-ethylamino]-5'-N-ethylcarboxamidoadenosine (CGS21680) were purchased from Tocris Cookson (Ellisville, MO, USA).

**Glial cultures**

Astrocyte cell cultures were established as described previously [8]. In brief, mouse cortex was dissected from new-born mouse pups (<1 day). Brain tissue was gently dissociated by trituration in phosphate-buffered saline (PBS) and filtered through a cell strainer (70 mm Ø, Falcon) into Dulbecco’s Modified Eagle medium (DMEM). After two washing steps (200 x g for 10 min), cells were seeded in culture flasks (75 cm², Greiner; 10 x 10⁶ cells/flask). Cultures were maintained up to 4 weeks in DMEM containing 10% fetal calf serum with 0.01%
penicillin and 0.01% streptomycin in a humidified atmosphere (5% CO₂) at 37°C. Culture medium was changed the second day after preparation and every 6 days thereafter. For in situ hybridization mixed astrocyte cultures were incubated with 0.25% w/v porcine trypsin at 37°C for 10 min and seeded on poly-L-lysine-coated glass coverslips in 6-well culture dishes. Floating microglia were harvested from confluent mixed astrocyte cultures and plated on new culture dishes. Microglia cultures were pure (> 95%) as tested by cell specific markers (F4/80 and Mac-1). For in situ hybridization microglia were seeded on poly-L-lysine-coated glass coverslips in 6-well culture dishes.

CCL2 ELISA
For ELISA experiments glia cells were seeded directly after preparation in 24-well dishes at a density of 1.0~1.5 x 10⁵ cells/well and experiments were performed 12-14 days thereafter. Microglia were removed from these mixed astrocytes cultures by tapping and rinsing the plates yielding cultures that contained > 90% astrocytes. Harvested microglia were plated in 24-well plates at a density of 1.0 x 10⁵ cells/well. The medium was changed two hours before stimulation with DMEM containing 0.1% fetal calf serum supplemented with 0.01% penicillin and 0.01% streptomycin. Cells were incubated 24 hours with adenosine receptor agonists, which were added to the culture medium. Antagonists were added 30 minutes before agonist treatment and were left in the medium the following 24 hours. Control cells (unstimulated) received solvent only. In some experiments, cells were pre-incubated 18 hours with 200 ng/ml pertussis toxin. The medium was then changed followed by incubation with the adenosine agonist. Supernatants were then removed, centrifuged at 10.000 g for 2 minutes and stored at –80°C until analysis.

A commercial ELISA kit (R&D systems, Minneapolis, MN, USA) was used to determine CCL2 levels. For ELISA analysis supernatants from astrocytes were diluted 1:4 in stimulation medium (DMEM + 0.1% fetal calf serum, 0.01% penicillin and streptomycin); supernatants from microglia were not diluted. ELISA experiments were carried out according to the manufacturer’s protocol with some modifications due to the low activity of the streptavidin horseradish peroxidase supplied in the ELISA kit. After incubation with the secondary biotinylated antibody, plates were washed and incubated for 30 min with streptavidin conjugated to poly-horseradish peroxidase (Strepta-E from Centraal Laboratorium voor Bloedtransfusie, Amsterdam, The Netherlands) at a concentration of 125 ng/ml in incubation buffer. A final washing step was followed by the chromogen reaction: 1 mg of 3’, 3’, 5’, 5’-tetramethylbenzidin (Roth, Germany) in 0.3 ml 96% EtOH was added to 11 ml 0.1 M Na-acetate, pH 6, and 0.004% H₂O₂ and plates were incubated for ca. 5 min with 0.1 ml of the chromogen solution until the reaction was stopped by addition of 1 M H₂SO₄.
The absorption at 450 nm minus 570 nm was measured in a microplate reader (Labsystems©, Breda, The Netherlands), and the concentrations were calculated using SOFTmax PRO software (Molecular Devices, Sunnyvale, CA).

Reverse transcription-polymerase chain reaction (RT-PCR)

Cultured glia cells were lysed in guanidinium isothiocyanate/mercaptoethanol buffer and total RNA was extracted with one phenol/chloroform step, precipitated according to Chomczynski and Sacchi [12]. Subsequently, RNA was treated with RNase free DNAse (Sigma-Aldrich, Bornhem, Belgium) for 2 hours and precipitated. Reverse transcription (RT): 1 µg of total RNA was transcribed into cDNA as described [8]. The quality of the cDNA was controlled using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers [9] and potential contamination by genomic DNA was checked for by running the reactions without reverse transcriptase and using GAPDH primers in subsequent polymerase chain reaction (PCR) amplifications.

PCR: 2 µl of the RT reaction product was used in the subsequent PCR amplification as described [8]. Primer sequences used are listed in table 3.1. Cycle numbers were 35 and annealing temperature was 58ºC for adenosine receptor primers and 28 cycles and 60ºC for GAPDH primers.

Table 3.1. Primer sequences for mouse adenosine receptors and GAPDH

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5’-3’)</th>
<th>PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>ACTTCTTCGTCTGGGTGCTG</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>AGAAAGGTGACCCGGAACCTT</td>
<td></td>
</tr>
<tr>
<td>A2A</td>
<td>TCATGTACCTGGCCATCATC</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>TGCTCCTGGTAAAGAAGCTC</td>
<td></td>
</tr>
<tr>
<td>A2B</td>
<td>ATGTGGTCCCCCATGAGCTAC</td>
<td>351</td>
</tr>
<tr>
<td></td>
<td>AGGCATAGACAATGGGGTTG</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>ATGGAAGCCGACAACACC</td>
<td>309</td>
</tr>
<tr>
<td></td>
<td>CAGCAAGGACATGATGGAGA</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>CATCCTGCACCACTAGCTTAG</td>
<td>312</td>
</tr>
<tr>
<td></td>
<td>GCCTGCTTCACCCACCTTGTAG</td>
<td></td>
</tr>
</tbody>
</table>

In situ hybridization

For in situ hybridization PCRII vectors containing mouse adenosine receptor PCR products were linearized with BamHI or XhoI. Adenosine A3 receptor sense and antisense probes were synthesized by run off transcription and the use of digoxigenin-conjugated UTP according to the manufacturer’s protocol (Boehringer Mannheim, Mannheim, Germany).
Cultured glia cells were seeded on poly-L-lysine-coated glass cover-slides and fixed in 4% paraformaldehyde for 30 min. The in situ hybridization procedure was carried out as described earlier [14]; alkaline phosphatase-conjugated sheep antidigoxigenin was used for the immunological detection of the digoxigenin- labeled RNA-RNA complex. Control experiments included hybridization with digoxigenin-labeled sense probes and hybridization with β-actin probes.

Intracellular calcium measurements

For calcium measurements, astrocytes were plated on glass coverslips two days before the experiment. Cells were loaded 1 hour at 37ºC with Fura-PE3 (TEF Labs, Austin, USA) in loading buffer containing 120 mM NaCl, 5 mM HEPES, 6 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 5 mM glucose, 22 mM NaHCO₃, 0.005 mM Fura; pH 7.4. Subsequently, the coverslips were fixed in a perfusion chamber and mounted on an inverted microscope. Fluorimetric measurements were done using a sensicam CCD camera supported by AxolabR 2.1 imaging software. Digital images of the cells were obtained at an emission wavelength of 510 nm using paired exposures to 340 and 380 nm excitation wavelength sampled at a frequency of 1 Hz. Fluorescence values representing spatial averages from a defined pixel area were recorded on-line. Increases in intracellular calcium concentrations were expressed as the 340/380 ratio of the emission wavelengths. Compounds were administered with a pipette directly into the medium covering the cells. In pertussis toxin experiments, astrocytes were pre-incubated with 200 ng/ml pertussis toxin overnight before the calcium measurements.

Data analysis

The results of the ELISA experiments are presented as mean ± S.E.M. of 3-4 experiments per ligand. Statistical comparisons were performed using ANOVA followed by Tukey post hoc test. Significance refers to results where p < 0.05 was obtained.

Results

Adenosine A₃ receptor stimulation leads to CCL2 release from astrocytes

Similar to previous reports [30, 34], constitutive synthesis of CCL2 was observed in unstimulated astrocyte cultures. The CCL2 levels varied from 900-1800 pg/ml between various preparations. The adenosine A₁ and A₂A receptor agonists CPA and CGS 21680 (10 nM – 1 µM) did not affect the constitutive CCL2 synthesis in these cultures (Figure 3.1A). A significant increase in CCL2 protein release in astrocyte cultures was observed in response to stimulation with the selective A₃ adenosine receptor agonist Cl-IB-MECA and the non-selective adenosine receptor
Figure 3.1. Effect of adenosine receptor agonists and antagonists on CCL2 release in cultured murine astrocytes and microglia. A) Effect of various concentrations (10 nM – 10 µM) of adenosine receptor agonists on CCL2 release in cultured mixed astrocytes. Data represented as percentage of control (unstimulated) value, which varied between 900 pg/ml and 1800 pg/ml in different preparations. *, p < 0.05 for Cl-IB-MECA induced CCL2 release versus control value. ^, p < 0.05 for NECA induced CCL2 release versus control value. B) Effect of adenosine receptor antagonists on CCL2 release in cultured mixed astrocytes induced by 100 nM Cl-IB-MECA. Data represented as percentage of value stimulated with 100 nM Cl-IB-MECA. Data are given as mean ± S.E.M. (n=4). *, significantly different from control p < 0.05. C) Lack of effect of various adenosine receptor agonists on CCL2 release in pure microglial cultures. Stimulation cultured microglia for 24h with 100 nM or 1 µM adenosine receptor ligands did not change basal CCL2 release (300 pg/ml), whereas stimulation with LPS (1 ng/ml for 24h) pronouncedly induced CCL2 in these cultures. Data are given as mean ± S.E.M. (n=4).
agonist NECA, respectively (Figure 3.1A). Significant induction of CCL2 release by Cl-IB-MECA was observed at a concentration of 100 nM (p < 0.05). At concentrations 10 nM and 1 µM CCL2 induction just failed to reach significance. The non-selective adenosine receptor antagonist NECA stimulated CCL2 release at a concentration of 1 µM.

The specific induction of CCL2 release with Cl-IB-MECA suggested the involvement of adenosine A3 receptors. In subsequent experiments astrocyte cultures were pre-incubated with different concentrations (10 nM, 100 nM and 1 µM) of the antagonists 8-PT and MRS 1523 and then stimulated with 100 nM Cl-IB-MECA (Figure 3.1B). The non-selective adenosine antagonist 8-PT, which has a very low affinity for the adenosine A3 receptor, did not influence Cl-IB-MECA-induced CCL2 release. In contrast, the selective A3 receptor antagonist MRS 1523 significantly inhibited Cl-IB-MECA-induced CCL2 release (p < 0.05) (Figure 3.1B), whereas CCL2 levels in unstimulated cells were unaffected by treatment with 1 µM MRS 1523 (116 ± 18% from unstimulated controls).

No involvement of adenosine A3 receptors in CCL2 release from microglia

Since astrocyte cultures used in our experiments still contained microglia cells, similar ELISA experiments were performed in pure microglia cultures in order to investigate whether adenosine receptor stimulation would induce CCL2 release in these cells. CCL2 ELISA analysis revealed a basal release of CCL2 in cultured microglia that was strongly induced by stimulation with LPS (1 ng/ml) (Figure 3.1C). In contrast to the situation in astrocyte cultures, CCL2 release from pure microglia was not significantly changed by stimulation with CPA, NECA, CGS21680 or Cl-IB-MECA (Figure 3.1C).

Adenosine receptor mRNA expression in glia cells

In order to check whether adenosine A3 receptor mRNA is expressed both in astrocyte cultures and pure microglia cultures, RT-PCR experiments were performed. Results of the RT-PCR experiments (Figure 3.2A) showed mRNA expression of the adenosine A1, A2B and A3 receptor subtypes in astrocyte cultures. Expression of adenosine A2A receptor mRNA was not detectable (Figure 3.2A). In pure microglial cultures mRNA expression for adenosine A1, A2B and A3 adenosine receptor mRNA were found. Adenosine A2A receptor mRNA expression in cultured microglia was only detected after 2 hour stimulation with bacterial lipopolysaccharide (LPS) (100 ng/ml). In contrast to microglia, LPS did not induce the expression of A2A receptor mRNA in mixed astrocyte cultures (data not shown). Since adenosine A3 receptor mRNA was detected in both mixed astrocyte- and pure microglial cultures in situ hybridization experiments using a probe for adenosine A3 receptor mRNA were performed. In situ hybridization analysis, using the antisense probe for adenosine A3 receptor mRNA, showed
positive staining in cultured astrocytes (Figure 3.2B) and microglia (Figure 3.2D). Approximately 50% of the microglia and 75% of the astrocytes expressed adenosine A3 receptor mRNA. No staining was found using the sense control probe in astrocytes (Figure 3.2C) or microglia (Figure 3.2E).
Cl-IB-MECA-induced CCL2 release from astrocytes is not blocked by pertussis toxin (PTX)

Overnight pre-incubation with PTX (200 ng/ml for 16h) did not effect Cl-IB-MECA-induced CCL2 release (Figure 3.3). Surprisingly, PTX treatment tended to increase CCL2 release from astrocytes stimulated with 100 nM Cl-IB-MECA and unstimulated cells (Figure 3.3). This increase, however, was not significant. PTX treatment did not affect CCL2 release in LPS (1 ng/ml) stimulated astrocyte cultures. The average amount of CCL2 protein in those samples was $7.4 \times 10^3$ pg/ml for LPS alone versus $6.6 \times 10^3$ pg/ml for LPS preceded by PTX treatment (n=3).

Figure 3.3. Lack of effect of pertussis toxin pre-treatment (200 ng/ml for 16h) on Cl-IB-MECA (100 nM) induced CCL2 release in cultured mixed astrocytes. Data are given as mean ± S.E.M. (n=4). * indicates a significant difference (p < 0.05).

Cl-IB-MECA-induced calcium transients in astrocytes are not affected by pertussis toxin

In order to gain more information on the G-protein that couples to adenosine A3 receptors, studies on intracellular calcium transients in cultured astrocytes were performed. It was found that approx. 50% of the investigated cells (141 out of 271) responded with increases in intracellular calcium after stimulation with 10 µM Cl-IB-MECA (see Figure 3.4A for a typical response). Intracellular calcium transients in cultured astrocytes were also found in response to 10 µM of the adenosine A1 receptor specific ligand CPA, however, this was found less frequent since approx. 30% (46 out of 133) of the investigated cells responded to CPA stimulation (see fig. 3.4B for a typical response). Pre-incubation with PTX (200 ng/ml) had no influence on the calcium transients caused by Cl-IB-MECA stimulation; neither the rise in ratio nor the number of responding cells (110 out
of 177) was changed after PTX treatment (Figure 3.4C and 3.4D). In striking contrast pre-treatment with PTX (200 ng/ml) completely abolished the induction of intracellular calcium transients in astrocytes in response to 10 µM CPA (Figure 3.4D). Only 4 out of 151 investigated cells still responded and showed a smaller rise in ratio compared to untreated cells (Data not shown).

Figure 3.4. Effect of adenosine receptor stimulation and PTX treatment on intracellular calcium transients in cultured murine astrocytes. A) Typical intracellular calcium response induced by 10 µM CI-IB-MECA. B) Stimulation with 10 µM CPA also induced intracellular calcium transients in murine astrocytes. C) Typical intracellular calcium response induced by 10 µM CI-IB-MECA after pre-treatment with PTX (200 ng/ml). D) Bar graph showing the percentage of cells responding to CI-IB-MECA and CPA in the presence or absence of PTX pre-treatment. Data in D are represented as mean ± S.E.M. from 6-12 experiments. *, significant effect of PTX treatment (p < 0.05). The arrow in A-C indicates the moment of stimulation.
Discussion

The neuromodulator adenosine has prominent neuroprotective effects in neurodegenerative diseases by directly inhibiting excitatory neurotransmitter release and by inducing neuronal hyperpolarization [20, 57]. Recent evidence suggests that neuroprotection by adenosine might also occur indirectly by induction of the release of neuroprotective substances from glia cells [13, 23, 35, 59]. In damaged neural tissue the chemokine CCL2 is predominately expressed in glia cells (see for example [29]) and recently direct neuroprotective effects of this chemokine have been published [11, 21]. However, little is yet known on the regulation of glial CCL2 expression. We therefore investigated the possible effect of adenosine receptor stimulation on glial CCL2 synthesis. We show that stimulation of mixed mouse astrocyte cultures with the selective adenosine A3 receptor ligand Cl-IB-MECA induces a significant release of CCL2. Other selective adenosine receptor agonists (CPA; adenosine A1 receptors and CGS 21680; adenosine A2A receptors) did not have any effect on CCL2 release in mixed astrocytic cultures, indicating that CCL2 release is induced by stimulation of adenosine A3 receptors. Most likely, the effect of the non-selective adenosine agonist NECA is also due to activation of adenosine A3 receptors, since NECA has an intermediate affinity for this receptor subtype [27, 28, 68]. Antagonist studies further corroborated the involvement of adenosine A3 receptors. The non-selective antagonist 8PT, which has high affinity for A1- and A2A-, intermediate affinity for A2B- but very low affinity for A3 adenosine receptors [27], did not affect Cl-IB-MECA-induced CCL2 release. In contrast, 10 nM of the selective adenosine A3 receptor antagonist, MRS 1523 [26, 49, 55], inhibited the effect of Cl-IB-MECA on CCL2 release. The affinity of MRS 1523 at mouse adenosine A3 receptors is not known yet. K_i-values for MRS 1523 at rat and human A3 receptors were 113 and 18.9 nM respectively [44], indicating species dependent differences of the affinity of MRS 1523. Similar to our findings a maximal effect of MRS 1523 already with 10 nM was reported by Reshkin and colleagues [55] in A6 cells, a renal cell line derived from Xenopus Laevis. Thus, MRS 1523 can block adenosine A3 receptor-mediated responses at low concentrations. Taken together the results obtained by RT-PCR analysis and in situ hybridization and the pharmacological data confirm the expression of the adenosine A3 receptor in mouse astrocytes and its involvement in the release of CCL2. The effect of adenosine A3 receptor ligands on the release of CCL2 was cell type specific. Microglia that are known to release CCL2 upon a variety of stimuli (for review see [7]) did not release CCL2 in response to Cl-IB-MECA, although adenosine A3 receptors are expressed in these cells and functional as published recently (this work and [24, 32]).
Cl-IB-MECA-induced CCL2 release in cultured astrocytes was not inhibited by pre-incubation with pertussis toxin, suggesting that Cl-IB-MECA-induced CCL2 release through a Gi protein independent pathway. This is in agreement with previous studies that describe various pertussis toxin insensitive adenosine A3 receptor mediated effects [43, 55]. Adenosine A3 receptors are reported to couple mainly to Gi or Gq proteins [52]. Since the involvement of Gi proteins in Cl-IB-MECA mediated CCL2 release in astrocytes seems to be unlikely, Gq proteins might be important here. The observed effects of Cl-IB-MECA on intracellular calcium transients in astrocytes, which were completely insensitive to PTX treatment, corroborate the assumption that adenosine A3 receptors in murine astrocytes are coupled to Gq proteins. On the other hand we also observed intracellular calcium transients in response to the adenosine A1 receptor agonist CPA. This effect, however, was most likely mediated by Gi proteins since it was completely blocked by PTX pre-treatment.

Little is yet known about the physiological role of adenosine A3 receptors. In peripheral tissues both beneficial as well as harmful effects of adenosine A3 receptor stimulation have been reported [22, 33]. This also is the case in CNS tissues. In animal experiments it has been found that adenosine A3 receptor ligands are neuroprotective in cerebral ischaemia models [65]. These findings have recently been confirmed in adenosine A3 receptor deficient mice [22]. Adenosine A3 receptor stimulation has been reported to regulate the survival of glia cells. It was thus reported that administration of adenosine A3 receptor ligands at nanomolar concentrations protected cultured astrocytes cells from cell death, whereas stimulation with micromolar concentrations induced cell death [40, 48].

Also CCL2 induces both detrimental and beneficial effects in the brain. Thus CCL2 is clearly involved in neuroinflammation and several lines of evidence suggest a function of CCL2 in the pathogenesis of neurodegenerative diseases [37, 41, 42, 54, 61, 62]. On the other hand there are also reports showing direct neuroprotective effects of CCL2 in culture [11, 21].

To what extent the effects of adenosine A3 receptor stimulation in vivo are caused by increased release of CCL2 is still unclear. This should be further investigated in adenosine A3 receptor deficient- or CCL2 deficient mice [1, 2, 39].

In conclusion, the data presented here show that stimulation of adenosine A3 receptors in cultured astrocytes induced the release of CCL2. This effect is most likely mediated via Gq proteins and suggests a new function of adenosine A3 receptors in astrocytes. The here presented results furthermore corroborate the assumption that adenosine receptors in glia cells regulate the release of various neuroprotective substances as it was already shown for NGF and IL-6 [23, 35, 59].
References


