Potential Therapeutic Applications of Adenosine A2A Receptor Ligands and Opportunities for A2A Receptor Imaging

van Waarde, Aren; Dierckx, Rudi A. J. O.; Zhou, Xiaoyun; Khanapur, Shivashankar; Tsukada, Hideo; Ishiwata, Kiichi; Luurtsema, Gert; de Vries, Erik F. J.; Elsinga, Philip H.

Published in:
Medicinal research reviews

DOI:
10.1002/med.21432

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Potential Therapeutic Applications of Adenosine A$_{2A}$ Receptor Ligands and Opportunities for A$_{2A}$ Receptor Imaging

Aren van Waarde,1 Rudi A. J. O. Dierckx,1,2 Xiaoyun Zhou,1 Shivashankar Khanapur,1 Hideo Tsukada,3 Kiichi Ishiwata,4,5,6 Gert Luurtsema,1 Erik F. J. de Vries,1 and Philip H. Elsinga1

1University of Groningen, University Medical Center Groningen, Department of Nuclear Medicine and Molecular Imaging, 1, 9713 GZ Groningen, The Netherlands
2Department of Nuclear Medicine, University Hospital, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium
3Central Research Laboratory, Hamamatsu Photonics K.K., Hamakita, Hamamatsu, Shizuoka 434–8601 Japan
4Research Institute of Cyclotron and Drug Discovery Research, Southern TOHOKU Research Institute for Neuroscience, 7–115 Yatsuyamada, Koriyama, 963–8052 Japan
5Department of Biofunctional Imaging, Fukushima Medical University, 1 Hikarigaoka, Fukushima, 960–1295 Japan
6Research Team for Neuroimaging, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo, 173-0015 Japan

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/med.21432

Abstract: Adenosine A$_{2A}$ receptors (A$_{2A}$Rs) are highly expressed in the human striatum, and at lower densities in the cerebral cortex, the hippocampus, and cells of the immune system. Antagonists of these receptors are potentially useful for the treatment of motor fluctuations, epilepsy, postischemic brain damage, or cognitive impairment, and for the control of an immune checkpoint during immunotherapy of cancer. A$_{2A}$R agonists may suppress transplant rejection and graft-versus-host disease; be used to treat inflammatory disorders such as asthma, inflammatory bowel disease, and rheumatoid arthritis; be locally applied to promote wound healing and be employed in a strategy for transient opening of the blood–brain barrier (BBB) so that therapeutic drugs and monoclonal antibodies can enter the brain. Increasing A$_{2A}$R signaling in adipose tissue is also a potential strategy to combat obesity. Several radioligands for positron emission tomography (PET) imaging of A$_{2A}$Rs have been developed in recent years. This review article presents a critical overview of the potential therapeutic applications of A$_{2A}$R ligands, the use of A$_{2A}$R imaging in drug development, and opportunities and limitations of PET imaging in future research.

Key words: Positron emission tomography (PET) imaging; adenosine A$_{2A}$ receptors; antagonists; agonists; drug development

Correspondence to: Aren van Waarde, Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713GZ Groningen, The Netherlands. E-mail: a.van.waarde@umcg.nl.

Medicinal Research Reviews, 38, No. 1, 5–56, 2018
© 2017 Wiley Periodicals, Inc.
I. INTRODUCTION

The purine nucleoside adenosine (Fig. 1) is a precursor for the synthesis of adenosine triphosphate (ATP), the main molecule for cellular energy storage, and an intermediate for the synthesis of nucleic acids such as deoxyribonucleic acid (DNA). Extracellular adenosine is an important regulator of many physiological functions, such as vasodilation and blood flow,1,2 contraction of the heart,3 the formation of new blood vessels,4,5 inflammation6 and wound healing,7-9 sleep and arousal,10-13 and learning and memory.14,15 Within the brain, both neurons and glial cells release adenosine. Extracellular adenosine modulates neuronal excitability16-19 the release and uptake of several neurotransmitters (e.g., glutamate20-23), and the plasticity of synapses.24-26

The concentration of adenosine in the extracellular space is determined by the balance between adenosine formation and removal. Cells can release and take up adenosine via equilibrative nucleoside transporters. Adenosine is generated from ATP by the action of ATPases, adenylyl kinase, apyrase, alkaline phosphatase, and ecto-5'-nucleotidase. Formed adenosine is either removed via a salvage pathway (phosphorylation by adenosine kinase) or degraded to inosine by adenosine deaminase. Under normal conditions, the levels of extracellular adenosine in the brain are low (in the 10\textsuperscript{-8} to 10\textsuperscript{-7} M range), but these levels rise during periods of neuronal activity and particularly under pathophysiological circumstances such as inflammation, hypoxia, or ischemia, when adenosine concentrations may reach the micromolar range.30-35 The half-life of extracellular adenosine is short (ranging from less than one to a few seconds).36 Because of this short half-life, adenosine exerts local or regional (but not global) effects in the brain.

The actions of extracellular adenosine are mediated through four subtypes of G-protein coupled adenosine receptors (ARs) called A\textsubscript{1}R, A\textsubscript{2A}R, A\textsubscript{2B}R, and A\textsubscript{3}R. These subtypes have different regional and tissue distributions, agonist and antagonist specificities, and intracellular signaling mechanisms. A\textsubscript{1}Rs and A\textsubscript{3}Rs inhibit, whereas A\textsubscript{2A}Rs and A\textsubscript{2B}Rs stimulate adenylyl cyclase. A\textsubscript{1}Rs and A\textsubscript{2A}Rs have fairly high affinities for adenosine (in the 10\textsuperscript{-7} M range), whereas A\textsubscript{2B}Rs and A\textsubscript{3}Rs are occupied only at higher adenosine concentrations (in the 10\textsuperscript{-6} to 10\textsuperscript{-5} range).
Thus, A1Rs and A2A Rs may already be activated under normal physiological conditions, whereas A2B Rs and A3 Rs will only be stimulated after tissue damage.

The regional expression of A1Rs and A2A Rs in the brain can be studied noninvasively, since positron-emitting ligands for PET imaging of these receptors have been developed. However, although many attempts were made to develop radioligands for A2B Rs and A3 Rs, the imaging of these subtypes has remained challenging. The limited success in imaging of A2B Rs and A3 Rs may be due to low levels of target expression, insufficient target affinity of the probes, or rapid degradation of the probes in the mammalian body. The present review is focused on potential therapeutic applications of A2A R-binding drugs and the use of PET imaging of A2A Rs in drug development, since A1 R imaging has been discussed in a previous publication and PET imaging of A2B Rs and A3 Rs is still in the infant stage.

2. WHERE ARE A2A Rs LOCATED?

Using in situ hybridization, messenger ribonucleic acid (mRNA) for the A2A R could be detected in the striatum, nucleus accumbens, and olfactory tubercle of the rat, but not in other areas of the brain. The regional pattern of A2A R gene expression in mouse brain is very similar to that in brain of the rat, mRNA being detected only in the striatum, nucleus accumbens, and olfactory tubercle. Using the more sensitive reverse transcriptase-polymerase chain reaction (RT-PCR), very low levels of A2A R mRNA were detected in all brain areas. Expression of the A2A R gene is thus not restricted to the basal ganglia. By the RT-PCR method, moderate or low levels of A2A R mRNA were also detected in several peripheral tissues of the rat with the following rank order of transcription: eye and skeletal muscle > heart, lung, bladder, and uterus > aorta, spleen, stomach, testis, skin, kidney, and liver.

mRNA for the A2A R is abundant in human striatum and nucleus accumbens, but present at very low levels in other areas of the human brain. In the human body, transcripts of the A2A R gene are predominantly detected in immune tissues (thymus, peripheral leukocytes, spleen), to a lesser extent in heart and lung and at very low levels in small intestine and kidney.

Both in the human and rat brain, the A2A R protein is most abundant in the striatum (Table I). Lower (but still quite considerable) levels of gene expression are detected in the nucleus accumbens and the olfactory tubercle (Table I). Receptor densities in other brain regions such as hippocampus and cerebral cortex are at least tenfold lower than those in striatum. Old data based on binding of the agonist [3H] CGS 21680 (Fig. 1) should be interpreted with caution, since [3H] CGS 21680 binds to A2A Rs in the striatum but to additional non-A2A R sites in other areas of the brain. For this reason, Table I lists only data for antagonist radioligands ([3H]KF17837S, [3H]SCH 58261, [3H]ZM 241385, [3H]MSX-2 [where MSX-2 is 3-hydroxypropyl-7-methyl-8-(3-methoxystyryl)-1-propargylxanthine], Fig. 2).

Detection of the A2A R protein in extrastriatal areas is difficult and results may be ligand-dependent. Using [3H]ZM 241385, an A2A R antagonist with an affinity of about 1 nM, British investigators noted that tracer binding in rat hippocampus and frontal cortex was of low density and failed to saturate. An American group confirmed that in brain areas other than caudate/putamen, nucleus accumbens and olfactory tubercle, the receptor density was below the limit of detection. However, when the in vivo binding of the antagonist tracer [3H] SCH 58261 in the brain of wild-type and A2A R knockout mice was compared, significant differences were detected not only in the striatum, but also in the cerebral cortex, whereas no differences were noted in hippocampus, hypothalamus, pons, and cerebellum. This finding suggests that cortical A2A Rs may be detectable with suitable radioligands.
Table I. Adenosine A<sub>2A</sub>R Expression in Mammalian Tissues

<table>
<thead>
<tr>
<th>Brain region/tissue</th>
<th>Species</th>
<th>B&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
<th>Average B&lt;sub&gt;max&lt;/sub&gt;</th>
<th>B&lt;sub&gt;max&lt;/sub&gt; / K&lt;sub&gt;d&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatum</td>
<td>Rat</td>
<td>1300</td>
<td>[52]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>310</td>
<td>[53]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1680</td>
<td>[54]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1150</td>
<td>[55]</td>
<td>884</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>335</td>
<td>[56]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>953</td>
<td>[57]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>997</td>
<td>[58]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>344</td>
<td>[59]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>Rat</td>
<td>1300</td>
<td>[55]</td>
<td>371</td>
<td>34</td>
</tr>
<tr>
<td>Olfactory tubercle</td>
<td>Rat</td>
<td>47–60</td>
<td>[55]</td>
<td>54</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>[60]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Rat</td>
<td>26</td>
<td>[61]</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29</td>
<td>[58]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>Rat</td>
<td>26</td>
<td>[62]</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>Striatum</td>
<td>Human</td>
<td>840–870&lt;sup&gt;*&lt;/sup&gt;</td>
<td>[63]</td>
<td>850</td>
<td>78</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>Human</td>
<td>580&lt;sup&gt;*&lt;/sup&gt;</td>
<td>[63]</td>
<td>580</td>
<td>53</td>
</tr>
<tr>
<td>White matter</td>
<td>Human</td>
<td>180&lt;sup&gt;*&lt;/sup&gt;</td>
<td>[63]</td>
<td>180</td>
<td>16</td>
</tr>
<tr>
<td>Myocardium</td>
<td>Human</td>
<td>135</td>
<td>[64]</td>
<td>135</td>
<td>12</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>Human</td>
<td>44</td>
<td>[65]</td>
<td>102</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>[66]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>82</td>
<td>[67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Human</td>
<td>49</td>
<td>[64]</td>
<td>49</td>
<td>4</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Human</td>
<td>55</td>
<td>[64]</td>
<td>55</td>
<td>5</td>
</tr>
</tbody>
</table>

Listed values are data for binding of antagonist radioligands.

<sup>a</sup>Receptor density (B<sub>max</sub>) expressed as femtomole per milligram protein. Values marked with asterisks (*) were reported as femtomole per milligram original tissue weight in the published references (but are here recalculated as femtomole per milligram protein).

<sup>b</sup>Binding potential (B<sub>max</sub> / K<sub>d</sub>) is calculated assuming that 10% of the tissue wet weight represents protein and affinity of the radioligand for A<sub>2A</sub>R (K<sub>d</sub>) is 1.1 nM (this is the K<sub>i</sub> of preladenant at human A<sub>2A</sub>R [68]). B<sub>max</sub> / K<sub>d</sub> represents the maximum value for accumulation of injected radioactivity in tissue due to interaction of the radioligand with receptors. As explained in Section 9 of this review, the actually observed value is frequently much lower.

Most A<sub>2A</sub>Rs in rat striatum are postsynaptic and involved in signal processing by striatopallidal neurons (see below). However, most A<sub>2A</sub>Rs in the hippocampus are presynaptic and involved in the regulation of neurotransmitter release from nerve endings.\(^{58}\)

### 3. A<sub>2A</sub>R EXPRESSION IN CELL LINES

Table II lists data on the expression of A<sub>2A</sub>Rs in various cell lines. Substantial levels of the receptor protein have been detected in the pheochromocytoma cell line PC12 (rat origin). Lower levels were observed in the rat glioma cell line C6 and in various tumor cells of human origin (HeLa cervical cancer, U-87 MG glioblastoma, SH-SY5Y neuroblastoma, and human malignant melanoma cell line [A375]). Expression levels are increased by hypoxia,\(^{71}\) chemotherapy,\(^{72}\) and stimulation with bacterial lipopolysaccharide,\(^{73}\) but the A<sub>2A</sub>R expression decreases during cellular differentiation.\(^{74}\)

*Medicinal Research Reviews* DOI 10.1002/med
4. DISEASE-ASSOCIATED CHANGES OF $A_{2A}$R EXPRESSION

Data concerning changes of $A_{2A}$R expression in human disease and animal models of disease are summarized in Tables III and IV. Many diseases are associated with a considerable upregulation of $A_{2A}$R, but in the published studies increases of receptor density ($B_{max}$) were frequently combined with increases of the dissociation constant ($K_d$) of $A_{2A}$R ligands. Tracer binding potential ($B_{max}/K_d$) shows a smaller change than receptor number in target tissue under such conditions.

The disorders listed in Tables III and IV can be categorized under a few headings:

1. Alterations of the immune system. Diseases with an inflammatory component or with immune system abnormalities are associated with changes of $A_{2A}$R expression. These include amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), normal pressure hydrocephalus, schizophrenia, chronic heart failure, rheumatoid arthritis, and endotoxemia. Since microglia in the brain are activated when neurons die, Huntington’s disease (HD), mild cognitive impairment (MCI), Parkinson’s disease (PD), and Pick’s disease can also be included in this category. The findings in animal models of peritonitis and myasthenia gravis confirm that $A_{2A}$R expression is altered as a consequence of inflammation or in autoimmune disorders. An upregulation of $A_{2A}$Rs is observed in most cases, which can be interpreted as a protective brake limiting activity of the immune system and protecting healthy tissue against attack by overactive immune cells. Anti-inflammatory therapy, such as treatment with pulsed low-frequency electromagnetic fields, is also associated with upregulation of $A_{2A}$Rs. However, in some cases (namely normal pressure hydrocephalus, a subgroup of patients with schizophrenia, and myasthenia gravis) $A_{2A}$Rs are down-regulated. This downregulation may reflect failure of the protective brake and excessive activity of the immune system. In some neurodegenerative diseases (e.g., MCI progressing to Alzheimer disease), $A_{2A}$R expression has been found to be stage-dependent, an upregulation in early, preclinical stages of the disease being followed by a downregulation when MCI progresses to overt dementia. 

---

*Medicinal Research Reviews* DOI 10.1002/med
### Table II. Adenosine A<sub>2A</sub>R Expression in Various Cell Lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>Species</th>
<th>( B_{\text{max}} )</th>
<th>( B_{\text{max}}/K_d )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A375</td>
<td>Melanoma</td>
<td>Human</td>
<td>220</td>
<td>20</td>
<td>[75]</td>
</tr>
<tr>
<td>BON-1</td>
<td>Pancreatic neuroendocrine tumor</td>
<td>Human</td>
<td>Only mRNA data available</td>
<td>—</td>
<td>[76]</td>
</tr>
<tr>
<td>C6</td>
<td>Glioma</td>
<td>Rat</td>
<td>399 (Control)</td>
<td>36</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>655 (Hypoxic)</td>
<td>60</td>
<td>[71]</td>
</tr>
<tr>
<td>CEM</td>
<td>T-lymphoma</td>
<td>Human</td>
<td>Only western blot data available</td>
<td>—</td>
<td>[77–79]</td>
</tr>
<tr>
<td>HeLa</td>
<td>Cervical cancer</td>
<td>Human</td>
<td>326 (Control)</td>
<td>30</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>872 (Azacytidine-treated)</td>
<td>79</td>
<td>[72]</td>
</tr>
<tr>
<td>KRJ-1</td>
<td>Intestinal neuroendocrine tumor</td>
<td>Human</td>
<td>Only mRNA data available</td>
<td>—</td>
<td>[76]</td>
</tr>
<tr>
<td>P493-6</td>
<td>B-cell</td>
<td>Human</td>
<td>Only mRNA data available</td>
<td>—</td>
<td>[80]</td>
</tr>
<tr>
<td>PC12</td>
<td>Pheochromocytoma</td>
<td>Rat</td>
<td>2085</td>
<td>190</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2000</td>
<td>182</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Undifferentiated)</td>
<td>500 (Differentiated)</td>
<td>45</td>
</tr>
<tr>
<td>SH-SY5Y</td>
<td>Neuroblastoma</td>
<td>Human</td>
<td>274 (Control)</td>
<td>25</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>854 (Azacytidine-treated)</td>
<td>78</td>
<td>[72]</td>
</tr>
<tr>
<td>THP-1</td>
<td>Monocyte</td>
<td>Human</td>
<td>Only western blot data available</td>
<td>—</td>
<td>[82]</td>
</tr>
<tr>
<td>U-87 MG</td>
<td>Glioblastoma</td>
<td>Human</td>
<td>333 (Control)</td>
<td>30</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>326 (Azacytidine-treated)</td>
<td>30</td>
<td>[72]</td>
</tr>
<tr>
<td>Wehi-3</td>
<td>Leukemia</td>
<td>Mouse</td>
<td>0 (Control)</td>
<td>0</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>85 (4 hr after LPS)</td>
<td>8</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150 (8 hr after LPS)</td>
<td>14</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>348 (20 hr after LPS)</td>
<td>32</td>
<td>[73]</td>
</tr>
</tbody>
</table>

Listed values are data for binding of antagonist radioligands.

*Receptor density (\( B_{\text{max}} \)) expressed as femtomole per milligram protein.

*Binding potential (\( B_{\text{max}}/K_d \)) is calculated assuming that 10% of cellular wet weight represents protein and affinity of the radioligand for A<sub>2A</sub>R (\( K_d \)) is 1.1 nM.

---

2. Impairment of basal ganglia pathways. Because of the heteromeric interaction and close association of A<sub>2A</sub>Rs and dopamine D<sub>2</sub> receptors, changes in the dopaminergic system result in changes of A<sub>2A</sub>R expression and vice versa. Such interactions are visible in:

(i) Dopaminergic motor pathways. Changes of A<sub>2A</sub>R expression in the brain can be related to motor disturbances (e.g., in PD and in schizophrenia). Studies in animal models of PD, restless legs syndrome, and epilepsy have indicated a link between upregulation of A<sub>2A</sub>Rs and motor problems. Gilles de la Tourette syndrome, a disorder in which dopaminergic hyperactivity leads to the appearance of tics, is significantly associated with polymorphism rs5751876 of the A<sub>2A</sub>R gene in the Polish population. 

_Medicinal Research Reviews_ DOI 10.1002/med
### Table III. Human Diseases Associated with Changes of A<sub>2A</sub>R Expression

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Where observed, maximal change</th>
<th>Reference</th>
<th>Details or conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulation of A&lt;sub&gt;2A&lt;/sub&gt;Rs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyotrophic late-ral sclerosis (ALS)</td>
<td>Lymphocytes (6.8-fold)</td>
<td>[83]</td>
<td>Correlates with score on ALS functional rating scale—revised</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>Platelets (+44%)</td>
<td>[84]</td>
<td>Chronic (but not acute) treatment with typical antipsychotics upregulates A&lt;sub&gt;2A&lt;/sub&gt;Rs</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>Heart (+56%), peripheral blood mononuclear cells (PBMC, +73%)</td>
<td>[64]</td>
<td>Upregulated in the failing heart and PBMC of patients with heart failure; normalized after cardiac transplantation; related to immune activation</td>
</tr>
<tr>
<td>Experimental endotoxemia</td>
<td>PBMC (+80%)</td>
<td>[85]</td>
<td>Upregulated after infusion of bacterial lipopolysaccharide</td>
</tr>
<tr>
<td>HD</td>
<td>Platelets, lymphocytes, neutrophils (1.6- to 3.5-fold)</td>
<td>[86–88]</td>
<td>Upregulated in the presymptomatic stage of the disease; more upregulated at younger age of disease onset</td>
</tr>
<tr>
<td>Mild cognitive impairment (MCI)</td>
<td>PBMC (+75%)</td>
<td>[89]</td>
<td>A&lt;sub&gt;2A&lt;/sub&gt;R density increases linearly with increased cognitive impairment</td>
</tr>
<tr>
<td>Multiple sclerosis (MS)</td>
<td>Lymphocytes (7.6-fold)</td>
<td>[90]</td>
<td>A&lt;sub&gt;2A&lt;/sub&gt;R stimulation could be beneficial in MS</td>
</tr>
<tr>
<td>Parkinson’s disease (PD)</td>
<td>Striatum (2.2- to 2.9-fold)</td>
<td>[91, 92]</td>
<td>Early event in the development of PD; A&lt;sub&gt;2A&lt;/sub&gt;R increase is correlated to severity of motor and cognitive problems</td>
</tr>
<tr>
<td>Pick’s disease</td>
<td>Lymphocytes (2.3- to 3.3-fold)</td>
<td>[91, 93]</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Frontal cortex (+91%)</td>
<td>[94]</td>
<td>Related to memory problems?</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>Lymphocytes (2.3- to 3.3-fold)</td>
<td>[95, 96]</td>
<td>Increase of A&lt;sub&gt;2A&lt;/sub&gt;R inversely correlated with disease activity score and abolished after successful treatment</td>
</tr>
<tr>
<td></td>
<td>Striatum (+74%)</td>
<td>[97, 98]</td>
<td>Probably related to clozapine treatment; upregulated after treatment with atypical antipsychotics (6 weeks); amount of increase may be related to sensory gating before treatment</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>[99]</td>
<td>May be related to an abnormal immune/inflammation response</td>
</tr>
</tbody>
</table>

Continued
(ii) Dopaminergic reward pathways. Drugs that affect the dopaminergic system (antipsychotics) affect the expression or function of A₂A Rs.

3. Cognitive impairment. Changes of cerebral A₂A R expression may be linked to cognitive impairment (e.g., in MCI, PD, and Pick’s disease). Binding assays in aged rodents, rodent models of attention-deficit hyperactivity disorder (ADHD), and of dementia have confirmed that cognitive impairment is associated with A₂A R upregulation.

4. Anxiety. A₂A R-mediated signaling may be altered in pathological anxiety. In monoamine oxidase A knockout mice, striatal A₂A R are significantly upregulated. Many studies have suggested that polymorphisms of the human A₂A R gene are associated with an increased risk of developing panic disorder, an anxious personality, agoraphobia, and blood injury phobia, although this observation may only be valid in Western and not in Asian populations. Chronic diseases associated with activation of the immune system have been linked to anxiety and this link may involve A₂A Rs. Peripherally administered adenosine increases caspase-1 activity and interleukin-β levels in the brain of wild-type mice via a pathway that involves A₂A Rs, protein kinase A, and ATP-sensitive potassium channels. These changes are associated with increased anxiety-like behaviors of the animals in the open field test and the elevated zero-maze. Fear acquisition by mice is accompanied by upregulation of A₂A R expression and function in the amygdala, and selective downregulation of A₂A Rs in this brain region by administration of a lentivirus with a silencing shRNA impairs both fear acquisition and fear retrieval. Treatment of mice with A₂A R antagonists (SCH 58261, caffeine) attenuates the retrieval of fear (both after 1–2 and 7–8 days).

Although these experiments suggest that A₂A R antagonists could be useful in the management of pathological fear, experiments in which A₂A Rs were region-selectively deleted have indicated that the relationship between A₂A R expression and fear is complex. Selective deletion of A₂A R in the mouse striatum increases fear conditioning, but when A₂A R are deleted in the entire forebrain (striatum, hippocampus, and cortex), the animals develop an anxiolytic phenotype, and global knockout of A₂A Rs in all organs of the body increases anxiety-like behavior in comparison to wild-type mice.

Medicinal Research Reviews DOI 10.1002/med
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Where observed, maximal change</th>
<th>Reference</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulation of A&lt;sub&gt;2A&lt;/sub&gt;Rs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aging</td>
<td>Hippocampus (2.4-fold, rat)</td>
<td>[104, 105]</td>
<td>Upregulated in aged animals; this upregulation is counteracted by treadmill running</td>
</tr>
<tr>
<td></td>
<td>Limbic cortex (+75%, rat)</td>
<td>[106]</td>
<td>Upregulated in limbic cortex (but not striatum) of aged rats</td>
</tr>
<tr>
<td>Attention-deficit hyperactivity disorder</td>
<td>Dorsal striatum (threefold, rat)</td>
<td>[107]</td>
<td>Upregulated in animal model of this disorder (Wig rat)</td>
</tr>
<tr>
<td>Dementia/diabetes</td>
<td>Hippocampus (+83%, rat)</td>
<td>[61]</td>
<td>Persistent upregulation in animal model of dementia (streptozotocin-treated rats)</td>
</tr>
<tr>
<td>Endotoxemia, bacterial infection</td>
<td>Monocytes (5.5-fold, horse)</td>
<td>[108]</td>
<td>Upregulated after stimulation with bacterial lipopolysaccharide or TNF-α or IL-1β</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>PC12 cells (+64%)</td>
<td>[109]</td>
<td>Upregulation in amygdala kindling and kainate models; also in 3-mercaptopropionic acid model</td>
</tr>
<tr>
<td></td>
<td>Cerebral cortex (3.9-fold, rat)</td>
<td>[62]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Striatum (+45%, rat)</td>
<td>[110]</td>
<td></td>
</tr>
<tr>
<td>Glutamate exposure</td>
<td>Neonate brain (2.7- to 5.5-fold, rat)</td>
<td>[111]</td>
<td>Exposure during lactation upregulates A&lt;sub&gt;2A&lt;/sub&gt;Rs in the neonate brain</td>
</tr>
<tr>
<td>Huntington’s disease (HD)</td>
<td>Striatum (+27%, mouse)</td>
<td>[112]</td>
<td>Transient upregulation in early disease phase in mouse model of HD</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>C6, PC12 cells (2.6- and 5.5-fold)</td>
<td>[71, 113]</td>
<td>Upregulated in rat pheochromocytoma and rat glioma cells by hypoxia</td>
</tr>
<tr>
<td>Monoamine oxidase A (MAO-A) deficiency</td>
<td>Basal ganglia (+29%, mouse)</td>
<td>[114]</td>
<td>Upregulated in MAO-A knockout mice; both MAO-A and A&lt;sub&gt;2A&lt;/sub&gt;R may play a role in anxiety</td>
</tr>
<tr>
<td>Multiple sclerosis (MS)</td>
<td>No receptor assays performed</td>
<td>[115, 116]</td>
<td>In experimental allergic encephalomyelitis (animal model of MS) A&lt;sub&gt;2A&lt;/sub&gt;R knockout worsens and treatment with CGS 21680 improves the symptoms</td>
</tr>
<tr>
<td>Neuroleptic treatment</td>
<td>Striatum (+33%, rat)</td>
<td>[117]</td>
<td>A&lt;sub&gt;2A&lt;/sub&gt;R antagonists may counteract negative side effects of antipsychotics (tardive dyskinesia)</td>
</tr>
</tbody>
</table>

Continued
Table IV.  Continued

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Where observed, maximal change</th>
<th>Reference</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson’s disease (PD)</td>
<td>Striatum (only mRNA data, rat)</td>
<td>[118]</td>
<td>In the 6-hydroxydopamine model of PD, L-DOPA treatment causes A2A Rs upregulation in the lesioned striatum</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>Peritoneum (+86%, mouse)</td>
<td>[119]</td>
<td>Upregulation in acute peritonitis (after 24 hr)</td>
</tr>
<tr>
<td>Pulsed electro-magnetic fields</td>
<td>Neutrophils (+71%, human)</td>
<td>[120]</td>
<td>Upregulated after exposure of cells (or animals) to this form of anti-inflammatory therapy</td>
</tr>
<tr>
<td>(low frequency)</td>
<td>Chondro-, synoviocytes (+89%, +138%, cow)</td>
<td>[121]</td>
<td>Cerebral cortex (twofold, rat)</td>
</tr>
<tr>
<td>Restless legs syndrome</td>
<td>Striatum (+83%, rat)</td>
<td>[123]</td>
<td>Upregulation in iron deficiency (animal model of restless legs syndrome)</td>
</tr>
<tr>
<td>Downregulation of A2A Rs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aging</td>
<td>Heart (rat, −55%)</td>
<td>[124]</td>
<td>Loss of A2A R in myocardium</td>
</tr>
<tr>
<td>Chronic caffeine treatment</td>
<td>Rostral striatum (rat, −11%)</td>
<td>[125]</td>
<td>Downregulated after 14 days of caffeine in drinking water, or exposure to caffeine during pregnancy</td>
</tr>
<tr>
<td>Chronic sleep restriction</td>
<td>Heart (rat, −54%)</td>
<td>[126]</td>
<td>Decrease of A2A R in sleep restricted animals</td>
</tr>
<tr>
<td>Deficiency of 5-HT transporter</td>
<td>Olfactory tubercle (rat, −26 to −31%)</td>
<td>[127]</td>
<td>Downregulated in striatum of 5-HT transporter knockout mice</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>Nucleus accumbens (mouse, −32%)</td>
<td>[114]</td>
<td>Downregulated in striatum of 5-HT transporter knockout mice</td>
</tr>
<tr>
<td>Hypoxic ischemia</td>
<td>Brain (rat, −30%)</td>
<td>[128]</td>
<td>Downregulated after exposure of animals to hyperoxia</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Neonatal brain (rat, only mRNA data)</td>
<td>[129]</td>
<td>Loss of A2A R in ligated hemisphere</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>Spleen (−67%), lymph nodes (−54%) (rat)</td>
<td>[130]</td>
<td>A2A Rs in cells of the immune system downregulated in animal model of myasthenia gravis</td>
</tr>
<tr>
<td></td>
<td>Brain (rat, only mRNA data)</td>
<td>[131]</td>
<td>Downregulated 3 months after ovariectomy</td>
</tr>
</tbody>
</table>

Binding assays for A2A Rs (and other receptors) are normally performed in biopsy material that is collected during surgery, or acquired postmortem. If such material is not available, blood samples are drawn and radioligand binding is studied in membranes isolated from various classes of blood cells. Such in vitro methods have several drawbacks. Since isolated receptors have lost their relationships with tissue components that were not isolated, their properties may be altered. Any difference between receptors on the cell surface and internalized...
receptors has disappeared, and the isolated receptors may reflect a selective fraction of the total receptor population. Biopsies provide an indication of receptor numbers in the specific locations where such biopsies were taken, but the regional distribution of receptors in the entire target organ remains elusive. For obvious reasons, tissue samples are collected only in certain classes of patients but not in all patients or in healthy volunteers. Finally, changes of receptor populations in blood cells may not always reflect corresponding changes in a target organ, such as the brain.

A nondestructive and quantitative imaging technique such as PET makes it possible to measure receptor binding potentials (BPs) in living animals or human volunteers. Receptors can be studied in situ in the target organ. The regional distribution of tracer binding can be visualized with a linear resolution of about 1 mm (in rodents) and a few millimeters (in the case of human volunteers). Different stages of a disease, or different classes of patients can be examined and an age-matched control group can be included. Because of the low radiation burden of most positron-emitting radionuclides, multiple studies can be performed in a single subject and longitudinal studies are possible, each subject serving as its own control. The application of PET imaging may thus result in improved understanding of the involvement of A$_{2A}$Rs in the pathophysiology and pathogenesis of brain diseases.

5. RADIOLIGANDS FOR PET IMAGING OF A$_{2A}$Rs

Several radioligands for PET imaging of cerebral A$_{2A}$Rs have been developed since the 1990s. An overview of the available ligands and of their preclinical imaging results was presented in a recent review. Here, we will only mention ligands that are applicable for studies in humans. These include [7-methyl-11$^C$]-((E))-8-(3,4,5-trimethoxy styryl)-1,3,7-trimethylxanthine ([11$^C$]TMSX), 5-amino-7-((3-([11$^C$]methoxy-phenyl)propyl)-2-(2-furyl) pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine ([11$^C$]SCH442416), 7-(2-(4-(2-[18$^F$] fluoroethoxy)phenyl)piperazin-1-yl)ethyl)-2-(furan-2-yl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine ([18$^F$]ethyl-pyrimidine ([18$^F$]MNI-444), and 2-(2-furanyl)-7-[2-[4-([2-11$^C$]methoxyethoxy)phenyl]-1-piperazinyl] ethyl]7H-pyrazolo [4,3-e] [1,2,4] triazolo[1,5-c]-pyrimidine-5-amine ([11$^C$]preladenant). The chemical structures of these probes are shown in Figure 3 and their binding characteristics are summarized in Table V. An additional radioligand with xanthine structure, [11$^C$]KW-6002 (Fig. 2), has been applied in humans, but was not further developed since it demonstrated inadequate specificity, as it bound not only to A$_{2A}$R but also to unidentified additional sites in rat and human brain.

Of the listed probes, TMSX (also known as KF18446) is the ligand with the longest history. [11$^C$]TMSX has been extensively characterized in several mammalian species (mouse, rat, nonhuman primate, man). The tracer is susceptible to photoisomerization and human PET scans with [11$^C$]TMSX display rather poor signal-to-noise ratios, but the injected probe is resistant to metabolic degradation. More than 90% of plasma radioactivity in humans and more than 50% of plasma radioactivity in mice represents parent [11$^C$]TMSX at 60 min after injection. [11$^C$]TMSX kinetics in the human brain can be quantified with Logan graphical analysis and compartment models (a two-tissue compartment fit in A$_{2A}$R-rich regions and a one-tissue compartment fit in regions devoid of A$_{2A}$Rs). Nondisplaceable BP (BP$_{ND}$) of this ligand can also be derived by reference tissue Logan graphical analysis without the need for blood sampling, using the centrum semiovale as reference region.

In contrast to some nonxanthine PET tracers for A$_{2A}$Rs, which demonstrate superior target-to-nontarget ratios in the brain, [11$^C$]TMSX has been reported to show some specific binding in peripheral organs (i.e., tissues outside the brain). Uptake of the probe in the heart, skeletal muscle, and kidneys was reduced (by 61, 38, and 56% in mice, and by 44, 27,
Table V. In Vitro Binding Characteristics of A\textsubscript{2A}R Ligands for PET Imaging

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TMSX</th>
<th>SCH 442416</th>
<th>MNI-444</th>
<th>Preladenant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_i$ (nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human A\textsubscript{1}</td>
<td>n.d.</td>
<td>1111</td>
<td>n.d.</td>
<td>1474</td>
</tr>
<tr>
<td>Human A\textsubscript{2A}</td>
<td>n.d.</td>
<td>0.048</td>
<td>2.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Human A\textsubscript{2B}</td>
<td>n.d.</td>
<td>&gt;10,000</td>
<td>n.d.</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Human A\textsubscript{3}</td>
<td>n.d.</td>
<td>&gt;10,000</td>
<td>n.d.</td>
<td>&gt;1700</td>
</tr>
<tr>
<td>Rat A\textsubscript{1}</td>
<td>1600</td>
<td>1815</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Rat A\textsubscript{2A}</td>
<td>5.9</td>
<td>0.50</td>
<td>n.d.</td>
<td>2.5</td>
</tr>
<tr>
<td>Rat A\textsubscript{2B}</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Rat A\textsubscript{3}</td>
<td>n.d.</td>
<td>&gt;10,000</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Selectivity ($\times$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A\textsubscript{1}/A\textsubscript{2A} human</td>
<td>n.d.</td>
<td>23,146</td>
<td>n.d.</td>
<td>1340</td>
</tr>
<tr>
<td>A\textsubscript{1}/A\textsubscript{2A} rat</td>
<td>271</td>
<td>3630</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Reference</td>
<td>[147, 148]</td>
<td>[149]</td>
<td>[150]</td>
<td>[68, 151]</td>
</tr>
</tbody>
</table>

n.d. = not determined.

and 36% in rats, respectively) after pretreatment of animals with the A\textsubscript{2A}R antagonist 8-(3-chlorostyryl)-caffeine (CSC) (Fig. 2), the nonsubtype selective AR antagonist theophylline or an excess of nonradioactive TMSX, but was not affected after pretreatment with the A\textsubscript{1}R antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX).\textsuperscript{152, 155} A pilot study in two human volunteers suggested that specific binding of $[^{11}\text{C}]$TMSX to A\textsubscript{2A}Rs in human heart and triceps muscle may be detectable with PET, since the distribution volume of the tracer in these tissues was slightly reduced (by 20 and 10%, respectively) after treatment of the subjects with theophylline.\textsuperscript{155} When mice were forced to swim immediately after $[^{11}\text{C}]$TMSX injection, the
tissue-to-plasma concentration ratios of radioactivity after 15 min were significantly reduced in heart, forelimb muscle, and hindlimb muscle (by 32, 34, and 22%, respectively), but unchanged in the striatum compared to the values observed in nonswimming controls. These data were interpreted as evidence for exercise-induced reductions of A<sub>2A</sub>R density in mammalian heart and skeletal muscle, but they may also reflect exercise-induced increases of extracellular adenosine.

6. PET FINDINGS IN HUMANS AND MODELS OF HUMAN DISEASE

In a rat model of HD (intrastriatal injection of quinolinic acid resulting in loss of striatopallidal GABAAergic enkephalin neurons), the BP<sub>ND</sub> of [<sup>11</sup>C]TMSX in the striatum and globus pallidus (GP) was reduced (by 25%). The observed reduction of A<sub>2A</sub>R binding was similar to the loss of dopamine D<sub>2</sub> ([<sup>11</sup>C]raclopride), but smaller than the loss of dopamine D<sub>1</sub> receptor ([<sup>11</sup>C]SCH 23390) binding that was about 36%.<sup>156,157</sup>

Significant increases of the distribution volume of [<sup>11</sup>C]TMSX were reported in the myocardium (from 3.1 ± 0.4 to 3.6 ± 0.3) and triceps brachii muscles of Asian subjects (from 1.2 ± 0.2 to 1.7 ± 0.3) as a consequence of endurance training. These increases were supposed to reflect an adaptive upregulation of A<sub>2A</sub>R in these organs. However, the finding could not be reproduced in a later study on Caucasian subjects, where no difference in myocardial A<sub>2A</sub>R density between untrained men and endurance athletes was observed.<sup>159</sup> Since the left ventricle of the heart is enlarged as a consequence of endurance training, a reduced partial volume effect may have resulted in an apparent increase of tracer distribution volume in the earlier study, but it is also possible that A<sub>2A</sub>Rs of Asian and Caucasian subjects respond differently to endurance training, or that training results in reduced levels of extracellular adenosine in heart and skeletal muscle of Asian men.

When young and aged humans were scanned with the PET tracers [<sup>11</sup>C]MPDX (A<sub>1</sub>R) and [<sup>11</sup>C]TMSX (A<sub>2A</sub>R), an age-related loss of striatal adenosine A<sub>1</sub>R binding was observed, but A<sub>2A</sub>R binding in this brain region was rather well-preserved.<sup>160</sup>

In a rat model of PD (unilateral injection of 6-hydroxydopamine in the substantia nigra resulting in loss of dopaminergic nerve endings in the striatum), the uptake (% ID/g) of a radiofluorinated analog of SCH 442416 ([<sup>18</sup>F]MRS5425) at the lesioned side was significantly increased (on average by 9–12%, at some time points >20%) compared to the intact side.<sup>161</sup> The authors concluded that an upregulation of A<sub>2A</sub>R may contribute to the symptoms of PD. When dopamine D<sub>2</sub> receptors were stimulated by the D<sub>2</sub> agonist quinpirole, a striking 25–30% decrease of the uptake of [<sup>18</sup>F]MRS5425 was noted both in the lesioned and intact striatum. In contrast to quinpirole, the D<sub>2</sub> antagonist raclopride did not affect the striatal binding of [<sup>18</sup>F]MRS5425.<sup>161</sup> The decrease after administration of quinpirole may reflect an agonist-induced internalization or down regulation of dopamine D<sub>2</sub>/A<sub>2A</sub>R heteromers.

Two PET studies of A<sub>2A</sub>R expression in PD were published in 2011. A slight (7.5%) but significant increase of the distribution volume of [<sup>11</sup>C]TMSX was noted in the putamen of Parkinson patients with dyskinesias compared to healthy controls, but tracer binding in the putamen of drug-naïve patients was not increased.<sup>162</sup> Much greater changes were reported for the BP<sub>ND</sub> of [<sup>11</sup>C]SCH 442416. This was increased in the putamen (+69%) and caudate nucleus (+81%) of Parkinson patients with levodopa-induced dyskinesias, but unchanged in patients without dyskinesias.<sup>163</sup> Two independent studies have thus reported an upregulation of striatal A<sub>2A</sub>Rs in PD patients with dyskinesias.

PET studies with [<sup>11</sup>C]TMSX have also been performed in patients with secondary progressive MS. Various techniques were tested to acquire an input function (arterial blood sampling...
with metabolite correction, a population-based arterial input function, and a non-invasive approach to extract reference voxels using supervised cluster analysis. The distribution volume of $[^{11}\text{C}]$TMSX in normal-appearing white matter of the patients was significantly ($+22\%$) increased in comparison to the value observed in healthy controls and this increase was associated with higher scores on the expanded disability status scale.$^{164,165}$ The results of these studies were interpreted as evidence for increases of A$_{2A}$R density in white matter of MS patients, related to inflammatory processes.

PET can not only be used to examine changes of receptor populations in disease, but also as an imaging tool in drug development. For this reason, the following sections of this review are focused on therapeutic application of A$_{2A}$R ligands.

7. THERAPEUTIC APPLICATIONS OF A$_{2A}$R ANTAGONISTS

A. Parkinson Disease

Smooth, well-controlled movements of the body require a close cooperation of cortical areas with deeper-laying areas of the brain, particularly the striatum, GP, and thalamus. An important aspect of movement control is the communication between the striatum and the internal segment of the GP (GP$_{i}$). These parts of the brain are connected via two different pathways (Fig. 4): (i) a direct (one-step) connection via GABAergic/substance P neurons, and (ii) an indirect (three-step) connection. The indirect pathway consists of GABAergic/enkephalin neurons projecting from the striatum to the external segment of the GP (GP$_{e}$), GABAergic neurons projecting from the GP$_{e}$ to the subthalamic nucleus (STN) and finally, glutamatergic neurons running from the STN to the GP$_{i}$. From the GP$_{i}$, GABAergic cells project to the ventral lateral (VL) and ventral anterior (VA) nuclei of the thalamus, and, finally, glutamatergic neurons in the thalamus project to the motor cortex (Fig. 4).

Since glutamatergic neurons have excitatory but GABAergic neurons have inhibitory actions, activation of either the direct or indirect pathway has opposite effects. Activation of the direct pathway results in inhibition of the GP$_{i}$, followed by disinhibition (i.e., less inhibition) of the thalamic nuclei and finally, by increased excitation of the motor cortex (Fig. 4).
Thus, the direct pathway turns up motor activity. Activation of the indirect pathway results in inhibition of the GP\textsubscript{e}, followed by disinhibition of the STN and increased excitation of the GP\textsubscript{i}. This causes inhibition of the thalamic nuclei and, finally, decreased excitation of the motor cortex (Fig. 4). Thus, the indirect pathway turns down motor activity. Movement is controlled by a delicate balance between the direct and indirect pathways, that is, by parallel processing.\textsuperscript{166}

The activity in the direct and indirect pathways is modulated by various neurotransmitters, particularly dopamine.\textsuperscript{167} Dopaminergic neurons in the substantia nigra pars compacta (SNc) project to the striatum. These neurons stimulate the direct pathway via dopamine D\textsubscript{1} receptors and they inhibit the indirect pathway via dopamine D\textsubscript{2} receptors (Fig. 4). Both actions of dopamine result in a facilitation of motor activity. PD is characterized by a progressive loss of dopaminergic neurons from the substantia nigra, a corresponding decrease of the cerebral levels of dopamine, and less dopaminergic control of the direct and indirect pathways. This results in various motor symptoms such as body tremors, gait problems, postural instability, slowness of movement, and muscle stiffness. Activity in the indirect pathway is also modulated by extracellular adenosine via (excitatory) A\textsubscript{2A}Rs.\textsuperscript{168} Thus, adenosine increases activity of the indirect pathway, which results in motor inhibition. For this reason, A\textsubscript{2A}R antagonists have been proposed as therapeutic drugs for the treatment of motor complications in PD (see [169, 170] for some recent reviews). Such compounds reduce the activity of the indirect pathway that may result in a shifted balance between the direct and indirect pathways and in improved motor control. The A\textsubscript{2A}R antagonist istradefylline (KW-6002, Fig. 2) has been approved in Japan as an adjuvant in treatment of PD patients with L-DOPA (where DOPA is dihydroxyphenylalanine).\textsuperscript{171–174} It can reduce motor fluctuations (off-time) during dopamine replacement therapy. However, efficacy of another A\textsubscript{2A}R antagonist (preladenant) could not be proven in two randomized clinical trials, which resulted in termination of the development of preladenant as a therapeutic drug.\textsuperscript{175}

The value of these trials may be questioned, since the positive control in the studies also failed to show a beneficial effect.

### B. Epilepsy

Since A\textsubscript{2A}Rs are upregulated in rodent models of epilepsy (amygdala kindling, intraperitoneal injection of kainate) and since A\textsubscript{2A}R stimulation has excitatory actions, A\textsubscript{2A}R antagonists have been proposed as anticonvulsive drugs.\textsuperscript{62} In a piriform cortex kindling model of epilepsy, the A\textsubscript{2A}R agonist CGS 21680 increased the after-discharge duration, whereas this effect was counteracted by pretreatment of animals with the A\textsubscript{2A}R antagonist ZM241385.\textsuperscript{176, 177} In a more recent study, intracerebroventricular administration of ZM241385 resulted in a dose-dependent decrease of the after-discharge duration in amygdala-kindled rats.\textsuperscript{178} In the WAG/Rij rat strain, an animal model of human absence epilepsy, A\textsubscript{2A}Rs in somatosensory cortex, reticular and ventrobasal thalamic nuclei are upregulated when animals become epileptic and injection of a specific A\textsubscript{2A}R agonist in these brain areas increases, whereas an A\textsubscript{2A}R antagonist decreases the number and duration of recorded absence seizures.\textsuperscript{179} In childhood rats exposed to cerebral hyperthermia, A\textsubscript{2A}R agonists decrease and A\textsubscript{2A}R antagonists increase the threshold temperature that leads to seizure onset. Thus, the blocking of A\textsubscript{2A}R-mediated signaling makes animals less susceptible to hyperthermia-induced seizures.\textsuperscript{180} In an animal model of temporal lobe epilepsy (pilocarpine injection), pretreatment of animals with the A\textsubscript{2A}R antagonist SCH 58261 reduces the occurrence of seizures.\textsuperscript{181} A\textsubscript{2A}R-deficient mice are partially resistant to limbic seizures.\textsuperscript{182} Such animal data suggest that stimulation of A\textsubscript{2A}Rs has a proconvulsive effect, and A\textsubscript{2A}R antagonists may be useful in the treatment of epilepsy. However, tests in patients have not yet been reported.
C. Cerebral Ischemia and Stroke

\(A_{2A}\)Rs may contribute to the development of postischemic damage, because these receptors are upregulated in neurons and microglia after an ischemic event\(^{183}\) and their activation stimulates the release of excitatory neurotransmitters (glutamate, aspartate). Animal experiments have suggested that administration of \(A_{2A}\)R antagonists (SCH 58261, ZM 241385) within a short time frame after an ischemic event may have a beneficial (neuroprotective) effect. Positive findings were reported for a hippocampal slice model (oxygen and glucose deprivation),\(^{184}\) permanent middle cerebral artery,\(^{185–188}\) and transient bilateral carotid artery\(^{189}\) occlusion models in rats, and a hypoxia-ischemia model in neonatal piglets.\(^{190}\) However, when an \(A_{2A}\)R antagonist (SCH 58261) is administered more than 24 hr after the ischemic event, it has no longer any beneficial effect since the excitotoxic damage has then already taken place.\(^{191}\) These findings suggest that \(A_{2A}\)R antagonists may be beneficial if they are administered early after stroke or cerebral ischemia (i.e., within 24 hr), but tests in humans have not yet been conducted.

D. Depression

Unpredictable chronic mild stress in rats leads to an increase of \(A_{2A}\)R availability in the striatum and an increase of depression-like behaviors.\(^{192}\) On the other hand, \(A_{2A}\)R-deficient mice display less depression-like behaviors than wild-type mice after a “depressant” challenge, and \(A_{2A}\)R antagonists inhibit such behaviors in rodent models of depression, namely the tail suspension and forced swim tests\(^{193–195}\) (review in\(^{196–198}\) ). \(A_{2A}\)R antagonists may be useful in the treatment of depression-associated psychomotor slowing and anergia, since such compounds enhance the operant response rates of rats in behavioral reinforcement paradigms.\(^{199–201}\) Such animal findings have raised the hope that specific \(A_{2A}\)R antagonists could be developed as rapidly acting antidepressants. Since \(A_{2A}\)Rs are involved in dopaminergic reward circuits, \(A_{2A}\)R antagonists could restore weakened signaling in such circuits and could thus improve mood.\(^{202, 203}\)

E. Cognitive Impairment

Animal experiments have indicated that cognitive impairment is associated with upregulation of \(A_{2A}\)Rs, whereas pharmacological blockade or genetic deletion of \(A_{2A}\)Rs may reverse existing memory deficits. Acute treatment of rats with the nonsubtype selective AR antagonist caffeine reverses age-related deficits in odor discrimination and improves the ability of old animals to recognize a young individual from the same species (or a novel object) within a short period of time. This cognition-enhancing effect of caffeine appears to be mediated via \(A_{2A}\)R, since it can be mimicked by treating animals with the specific \(A_{2A}\)R antagonist ZM 241385 but not by the specific \(A_{1}\)R antagonist DPCPX.\(^{204, 205}\) The improvement of selective attention by caffeine in various rat strains\(^{206, 207}\) and the improvement of social memory in spontaneously hypertensive rats (SHR), an animal model of ADHD,\(^{208}\) are also \(A_{2A}\)R-mediated, in contrast to the improvement of short-term object recognition in SHR rats that involves both \(A_{2A}\)Rs and \(A_{1}\)Rs.\(^{209}\) Hyperactive wiggling (Wig) rats, another animal model of ADHD with impaired working memory, show an increased \(A_{2A}\)R expression in the dorsal striatum.\(^{107}\) Transgenic rats with overexpression of the \(A_{2A}\)R suffer from working memory deficits.\(^{210}\) On the other hand, \(A_{2A}\)R knockout mice display an improved spatial recognition memory.\(^{211, 212}\) Genetic deletion of the \(A_{2A}\)R or treatment of animals with a specific \(A_{2A}\)R antagonist (MSX-2) protects mice against the deficits of spatial learning and memory induced by tauopathy\(^{213}\) or the neurodegeneration induced by \(\alpha\)-synuclein.\(^{214}\) Memory dysfunction after intracerebroventricular administration of \(\beta\)-amyloid peptide can be prevented by treating rats or mice with \(A_{2A}\)R.
antagonists (SCH 58261, KW-6002, or caffeine), or by genetic inactivation of A2A Rs. In APP/PS1 (transgenic mice overexpressing mutated forms of the genes for human amyloid precursor protein and presenilin 1) mice, another animal model of Alzheimer disease, A2A Rs in hippocampal neurons are upregulated at an early stage of disease development and treatment with A2A R antagonists reverts the disease-associated memory deficits. In a rat model of sporadic dementia (intracerebroventricular administration of streptozotocin), hippocampal A2A Rs are upregulated. Chronic caffeine consumption in this model is neuroprotective and prevents both the memory impairment and the upregulation of A2A Rs.

Chronic caffeine consumption (1 g/L in the drinking water) protects mice also against the impact of chronic unpredictable stress, by preventing decreases of memory performance and increases of helpless-like behavior. Similar protection could be obtained by treating animals with the A2A R antagonist KW-6002, by global deletion of the A2A R or by selective deletion of A2A R in forebrain neurons. Chronic blockade of A2A Rs with SCH 58261 can not only prevent but can also cure the effects of stress on memory function. In helpless mice, an animal model of depression, chronic caffeine consumption (0.3 g/L in the drinking water) could revert the memory deficits but not the increases of helpless and anxiety behavior compared to wild-type mice.

Studies in mouse models of HD have suggested that hyperactivation of A2A Rs contributes to cognitive dysfunction, whereas treatment with an A2A R antagonist (KW-6002) can reverse the working memory deficits in early stages of the disease. Knockout of A2A Rs or chronic caffeine consumption improves memory and cognitive function in mice with mild to moderate blast-induced brain injury, and A2A R antagonists improve goal-directed behavioral control in methamphetamine-exposed rats.

Based on such animal data, A2A R antagonists have been proposed as cognition-enhancing drugs and caffeine consumption as a prophylactic strategy to prevent memory decline.

F. Immunotherapy of Cancer

The microenvironment of solid carcinomas is characterized by elevated levels of extracellular adenosine (0.2–2.4 μM vs. 0.03 μM in normal tissue). This increase is due to inefficient production of ATP in tumor tissue. Glycolysis in tumor cells results in the formation of two molecules of ATP and lactic acid as end product, whereas in normal tissue the breakdown of glucose leads to formation of pyruvate that (after oxidation in the tricarboxylic acid cycle) yields 38 molecules of ATP. The low phosphorylation potential of lactate glycolysis (compared to oxidative phosphorylation) results in a low value of the adenylate energy charge and increased levels of adenosine 5'-monophosphate (AMP) in tumor cells. Many tumors also express ecto-5'-nucleotidase (CD73), the enzyme that catalyzes the breakdown of extracellular AMP to adenosine. High levels of CD73 expression are usually associated with poor prognosis.

The use of lactate glycolysis for energy production should not be considered as a wasteful and primitive feature of tumor metabolism, but as a characteristic that promotes the survival of neoplastic cells in tissues of the host. Elevated levels of extracellular adenosine and increased signaling via A2A Rs play an important role in tumor development and diffusion and constitute a mechanism by which tumor cells avoid attack and removal by the immune system. Adenosine exerts immunosuppressive and anti-inflammatory effects via stimulation of A2A Rs. Moreover, elevated levels of adenosine stimulate angiogenesis. Such actions of adenosine promote the survival and proliferation of tumor cells. Adenosine may not only be released by the tumor cells themselves, but also by regulatory T cells in a hypoxic tumor microenvironment. The functions of adenosine in tumor biology can be categorized in the following way:
1. Evasion of the Immune System

CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and CD73 are overexpressed in >80 and > 90% of human ovarium carcinomas, in ovarium carcinoma cell lines (SK-OV-3, OaW42), and in primary cultures of human ovarian carcinoma. When small interfering RNAs (siRNAs) or specific inhibitors of CD39 and CD73 were added to a combined culture of human immune and ovarium carcinoma cells, adenosine generated by the tumor cells was found to inhibit CD4\(^+\) T-cell proliferation, cytotoxic T-cell priming, and natural killer (NK) cell cytotoxicity via stimulation of A\(_{2A}\)Rs. The authors concluded that the adenosine signaling pathway is an interesting target for the development of novel treatments in ovarian carcinoma.

When CL8-1 (mouse melanoma cell line) melanoma or RMA T lymphoma cells were inoculated in immune-competent CL57BL/6 (inbred strain of laboratory mouse [Black 6]; wild-type) mice, all animals died within a few weeks because of the presence of tumors. However, if the same cells were inoculated in A\(_{2A}\) R knockout (A\(_{2A}\) R\(^{-/-}\)) mice, the tumors were completely rejected in 60% of the cases and 60% of the animals survived. Elimination of A\(_{2A}\) R signaling in T cells did not prevent the initial growth of inoculated tumors, but led in many cases to destruction of tumors after these had reached a certain size. Treatment of wild-type mice with A\(_{2A}\) R antagonists (ZM241385 or caffeine) delayed the growth of CL8-1 tumors and increased the lifespan of tumor-bearing animals.

In an immunotherapy model, tumor growth was significantly decreased and the life span of the animals was increased if the therapeutic T cells were pretreated with siRNA for the A\(_{2A}\) R. The authors performed various control experiments and proved that stimulation of A\(_{2A}\)Rs in CD8\(^+\) T cells prevents that such cells attack the tumor cells. A\(_{2A}\) R signaling during T-cell activation does not reduce the proliferation of these cells, but strongly inhibits their cytotoxic and cytokine-producing activity. Since the effector functions remain impaired even after removal of the A\(_{2A}\) R agonist, the immunoregulatory effect of adenosine appears to be stored in a T-cell memory. A\(_{2A}\) R stimulation does not only directly inhibit T-cell activation, but produces also a long-lasting inhibition by increasing the number of regulatory T cells (Treg) and by enhancing their immunosuppressive actions. A\(_{2A}\)Rs are not only expressed on T-cells, but also on tumor-associated macrophages, dendritic cells, and myeloid-derived suppressor cells. By selective deletion of A\(_{2A}\) R in myeloid cells, receptors on these cell types were shown to also contribute to the suppression of cytotoxic immune responses in an adenosine-rich tumor microenvironment.

Mice lacking A\(_{2A}\)Rs showed a delayed growth of inoculated lymphoma (EL4) cells and stronger antitumor responses than wild-type mice when immunotherapy was applied. Anti-tumor responses (against EL4 lymphoma or B16 melanoma in wild-type mice) were increased when immunotherapy in these animals was combined with daily injections of the A\(_{2A}\) R antagonist ZM241385. In two mice models of CD73-positive tumors, blockade of A\(_{2A}\)Rs with tozadenant (Fig. 2) strongly increased the efficacy of immunotherapy with anti-PD-1 (where PD-1 is programmed cell death protein 1) antibodies, by enhancing anti-tumor T cell responses.

These marked enhancements of antitumor responses when A\(_{2A}\) R are blocked (or absent) suggest that adenosine and A\(_{2A}\) R play an important role in mediating tumor evasion of the immune system. Blockade of adenosine signaling via A\(_{2A}\)Rs may thus be applied as adjuvant treatment in immunotherapy to prevent inhibition of antitumor responses in the tumor microenvironment. This “checkpoint blockade” approach has been discussed in several reviews. Such treatment has the additional benefit that it inhibits the adenosine-mediated vessel formation that promotes tumor growth. Adenosine signaling via A\(_{2A}\)Rs is beneficial in acute inflammation since it protects healthy tissue from damage by overactive immune cells.

Medicinal Research Reviews DOI 10.1002/med
but the same process is harmful in malignancies because it protects tumor cells from immune attack and stimulates permissive angiogenesis.253–255

2. Stimulation of Proliferation

Some human malignancies (e.g., non-small cell lung cancer,256 hormone-dependent breast cancer,257 and neuroendocrine tumors76) express functional A2ARs that stimulate tumor cell proliferation when adenosine is present. A2AR antagonists (ZM 241385, SCH 58261) induce apoptotic cell death in nonsmall cell lung cancer cells, suppress the growth of cancer-associated fibroblasts and tumor cells in vitro, and inhibit the growth of PC9 xenografts in tumor-bearing mice.256 However, a report on human colonic cancer cells (Caco-2) has suggested that very high concentrations of adenosine (1–20 mM) may induce apoptosis rather than stimulate proliferation.258 The effects of adenosine may thus be cell type dependent, or high concentrations of the nucleoside may have different actions than concentrations in the physiological range.

Mice that regularly consumed caffeine (0.1% in drinking water) or A2AR knockout mice developed tumors much less frequently than normal mice after injection of the carcinogen 3-methylcholanthrene (MCA) or after inoculation of B16 melanoma or MCA-induced transformed cells.259 Rodents in which the formation of adenosine was inhibited (CD73-deficient mice or animals that were treated with anti-CD73 monoclonal antibodies) also developed less fibrosarcomas after injection of MCA, and less TRAMP-C1 (mouse transgenic prostate cancer cell line) prostate tumors and lung metastases.260

Apparently, blockade of A2AR signaling does not only boost T-cell-mediated tumor rejection and reduce tumor-related angiogenesis, but can also directly inhibit the growth of cancer-associated immunosuppressive fibroblasts and the growth of the tumor cells themselves.

3. Promotion of Metastasis

Genetic deletion of A2ARs or the administration of A2AR antagonists result in significant protection of mice from tumor metastasis (4T1.2 breast cancer, B16-F10 melanoma).261 An even more striking reduction of the metastatic burden was achieved by treating these mouse models of breast cancer and melanoma with a combination of an A2AR antagonist (SCH 58261) and an anti-PD-1 monoclonal antibody.262 The combination therapy was only effective if the treated tumors were CD73-positive, and it depended on NK cells and interferon-γ. Thus, the combination of A2AR blockade with immunotherapy holds promise not only for the treatment of primary tumors, but also for the suppression of residual and metastatic disease.

Although treatment with A2AR antagonists has shown very promising results in several animal models of cancer and cancer immunotherapy, studies in experimental animals have also indicated that elimination of A2AR signaling does not inhibit the growth of all tumor types. The growth of ectopic melanomas (B16-F10) and urothelial carcinomas (MB49) in A2AR-deficient mice was enhanced rather than decreased compared to wild-type mice. In the particular microenvironment of these murine tumors, the positive effect of A2AR blockade on T-cell cytotoxicity appears to be offset by impaired T-cell survival and differentiation.263 The positive findings in tumor-bearing animals can thus not be generalized to all types of tumors, and careful dosing of A2AR antagonists may be required in clinical studies in order to prevent activation-induced T-cell death.

G. Opportunities for PET in A2AR Antagonist Drug Development

PET studies of receptor occupancy are an important tool in drug development, since such studies can answer many questions, namely (i) does the candidate drug reach its intended target? (ii) Which dose is required for half-maximal target occupancy? (iii) How is target
occupancy related to plasma concentration or plasma kinetics of the drug? (iv) Which fraction of the receptor population should be occupied in order to acquire the desired beneficial effect and to avoid unwanted side effects? (v) What is the optimal dose regimen for drug treatment? (vi) Is receptor occupancy different in drug responders and non-responders? and (vii) Can metabolites of the drug enter the brain and occupy the target receptor?

Several studies in humans and nonhuman primates have proven that the dose-dependent occupancy of the striatal A$_{2A}$R population by antagonist drugs can be measured using $[^{11}\text{C}]$SCH441416 or $[^{18}\text{F}]$MNI-444 and PET. They involved the nonsubtype-selective antagonist ASP5854, and the A$_{2A}$R antagonists vipadenant (Fig. 2), tozadenant, and preladenant. These studies showed that more than 85% of the A$_{2A}$R population should be occupied by ASP5854 to inhibit haloperidol-induced catalepsy, that a single daily dose of vipadenant is possible as a treatment regimen in PD, a dose of 2.5 mg resulting in 74–94%, and a dose of 100 mg in 100% A$_{2A}$R occupancy, and that receptor occupancy in the primate brain after a single clinical dose of tozadenant is more sustained than after administration of preladenant.

In future research, measurements of A$_{2A}$R occupancy in the brain may be combined with physiological or behavioral tests in experimental animals to acquire greater understanding of the mechanisms of action of A$_{2A}$R antagonists in epilepsy, stroke, depression, and cognitive impairment. If A$_{2A}$R expression in activated immune cells can be detected with suitable radioligands and PET (see Section 9 of this review), dose-finding studies with A$_{2A}$R antagonists may also be performed in immunotherapy of cancer.

8. THERAPEUTIC APPLICATIONS OF A$_{2A}$R AGONISTS

A. Transplantation

Since A$_{2A}$Rs on neutrophils have anti-inflammatory actions and since inflammatory reactions are a major cause of transplant failure, A$_{2A}$R agonists have been proposed as therapeutic drugs that could be applied to increase the success of transplantation and to prevent the occurrence of graft-versus-host disease. Positive results of agonist treatment have been reported in various animal models of lung and trachea transplantation, liver transplantation, pancreatic islet cell transplantation, allogenic hematopoietic stem cell transplantation, skin transplantation, arthroplasty, and bone regeneration after surgery.

General findings in such models were the following:

1. Treatment with A$_{2A}$R agonists suppressed the formation of proinflammatory cytokines, decreased neutrophil infiltration, inhibited apoptosis, improved the function or survival of the transplanted tissue (or the implanted prosthesis), and increased survival of the transplant recipients;
2. these beneficial effects were no longer observed if a selective A$_{2A}$R antagonist was coadministered with the therapeutic drug; and
3. A$_{2A}$R knockout mice, or animals in which the formation of adenosine had been reduced by knockout of the gene for CD73 showed increased inflammation and more severe complications after transplantation than wild-type mice.

These positive results have suggested that A$_{2A}$R agonists may be useful in the management of patients after transplantation or after a joint intervention, for example, total hip arthroplasty. However, not all experiments with A$_{2A}$R agonists in transplantation models...
have had a successful outcome. In a rat model of hindlimb transplantation, CGS 21680 did not prolong but rather shortened allograft survival.

**B. Joint Inflammation**

A$_2$A Rs are upregulated in lymphocytes from rheumatoid arthritis patients. The magnitude of the upregulation is inversely related to the disease activity score and stimulation of these receptors with agonists results in inhibition of the NF-$\kappa$B (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway and reduced formation of inflammatory cytokines. The receptor numbers are normalized after successful anti-inflammatory therapy. Based on these observations and on positive results of animal experiments, A$_2$A R agonists have been proposed as therapeutic drugs for the treatment of various forms of joint inflammation.

In a rabbit model of septic arthrosis, treatment with the A$_2$A R agonist ATL146e resulted in a strong decrease of inflammation and in prevention of articular cartilage destruction without compromising the clearance of bacteria from the inflamed joint. Suppression of inflammation and an improvement of the status of the joints were also observed after administration of CGS 21680 to mice with collagen-induced arthritis. Treatment efficacy in the collagen model could be increased by combining an intraperitoneally administered A$_2$A R agonist (CV-1808, Fig. 1) with an inhibitor of small hyaluran fragments (Pep-1). Such combination treatment resulted in a greater suppression of inflammation and apoptosis and better protection of the cartilage than either of the treatments administered alone.

A problem with the use of A$_2$A R agonists as anti-inflammatory agents is the strong vasodilation associated with therapeutic doses of such drugs. This problem may be circumvented by applying inactive prodrugs that are locally activated by enzymes at the target site. The feasibility of this approach has been demonstrated in the collagen model of arthritis. An injected phosphorylated prodrug, 2-(cyclohexylethylthio)-adenosine-5$' $-monophosphate, was locally activated at the site of inflammation by CD73 and reduced both inflammation and damage to the joints. The beneficial effect of the prodrug was strongly diminished when it was combined with a selective A$_2$A R antagonist or when CD73 was inhibited.

**C. Pulmonary Inflammation**

In several animal studies, A$_2$A R agonists were applied to suppress pulmonary inflammation, for example, in allergic asthma or chronic obstructive pulmonary disease. Potent anti-inflammatory effects were observed, and the presence of A$_2$A Rs on cells derived from bone marrow was found to be required for agonist-induced immunosuppression. Consistent with the hypothesis that A$_2$A R stimulation limits pulmonary inflammation, A$_2$A R knockout mice displayed enhanced airway reactivity and inflammation after an allergen challenge. Although the results in animal models seemed promising, several findings indicated that treatment of pulmonary disease with A$_2$A R agonists may have important drawbacks:

1. Blood pressure was decreased at the same dose of CGS21680 as was required for its anti-inflammatory activity. Systemic side effects may thus complicate the use of A$_2$A R agonists in the treatment of asthma.

2. In some asthma models, CGS 21680 showed a smaller spectrum of beneficial effects than the corticosteroid budesonide. The influx of inflammatory cells was reduced but allergen-induced bronchoconstriction or airway hyperreactivity were not affected.

3. The positive results with A$_2$A R agonists in preclinical experiments could not be reproduced in clinical studies. Treatment failure in humans may have been caused by interactions of the test drugs with other subtypes of ARs that counteracted their A$_2$A R-mediated
actions, or by systemic side effects of the drugs that precluded their administration at sufficiently high doses. 292

Although several attempts were made to develop A2AR agonists with extended lung retention after inhalation, it has not yet proven possible to acquire a compound that combines a high selectivity for the human A2AR subtype with adequate dose separation between the desired anti-inflammatory and undesired systemic side effects. 293–295 This problem of agonist therapy may perhaps be circumvented by the application of inactive prodrugs (see above) or positive allosteric modulators of the A2AR rather than A2AR agonists. 296, 297 Such compounds will increase A2AR signaling if CD73 and extracellular adenosine are present. They may thus show selectivity for inflammatory tissue and may display less side effects than A2AR agonists at therapeutic dosing. A strong anti-inflammatory effect of the modulator AEA061 has been demonstrated in a mouse model of endotoxemia (intraperitoneal (i.p.) injection of LPS) and in LPS-stimulated macrophages and splenocytes. 297 Highly polar, highly water-soluble, perorally nonabsorbable A2AR agonists have also been proposed as a potential strategy to avoid systemic side effects. Such compounds (which can bear sulfonic acid groups) are deprotonated under physiological conditions and will therefore be locally acting. 298

D. Gastrointestinal, Hepatic, and Renal Inflammation

Selective stimulation of A2ARs has also been tested for the treatment of inflammation in abdominal organs. A2AR agonists (ATL-146e, ATL313, ATL370, ATL1222, Fig. 1) were applied in rabbit and mouse models of inflammatory bowel disease. 299–302 The candidate drugs were also tested in mouse and rat models of liver injury 303, 304 and gastritis. 305–307 Treatment with the A2AR agonist CGS 21680 has also been tested in a rat model of glomerulonephritis that normally progresses to lethal kidney failure. 308 Findings in these models were the following:

1. Significant anti-inflammatory effects of the administered A2AR agonists were observed, for example, a reduction of acute inflammation and tissue necrosis, 299, 301 a suppression of inflammatory cell infiltration and villus distortion in the intestinal mucosa 299, 301 (or ulcer formation in the gastric mucosa, 305, 306 or glomerular lesions in the kidney 308), and a diminished release of proinflammatory cytokines. 299, 301, 303–308 Mice infected with Clostridium difficile showed less weight loss and diarrhea if they were treated with vancomycin. However, when treatment with the antibiotic was discontinued, virtually all animals died because of recurrent disease. If vancomycin was combined with an A2AR agonist, weight loss was minimized and long-term survival was improved. 302 The treatment efficacy of an A2AR agonist in toxin A induced ileitis and cecitis models could be improved by combining the test drug with administration of alanyl glutamine. This stable derivative of glutamine stimulates repair of the intestinal mucosa and decreases apoptosis of enterocytes. 300

2. Hepatic function after liver injury was strongly improved, as indicated by measurements of enzyme levels in serum. 303, 304 Kidney function after chronic inflammation was also improved as demonstrated by decreased proteinuria, and progressive fibrosis in the kidney was prevented. 308 A strong reduction or a complete prevention of mortality was observed in the chronic colitis and acute liver injury models. 299, 303

3. The protective effects of the test drugs were shown to be A2AR-mediated, since they were abolished after coadministration of the A2AR antagonist ZM 241385. 301, 306, 307

4. A2AR-deficient mice showed more severe symptoms of gastritis than wild-type animals after Helicobacter infection. 307 A2AR knockout mice showed also a greater production
Thus, selective activation of A2ARs seems to hold promise as a treatment strategy for gastrointestinal, hepatic, and renal inflammation, but trials in humans have not yet been reported.

E. Wound Healing

Since wound healing comprises inflammation, formation of new tissue, and tissue remodeling, and since A2ARs are involved in the control of inflammation and angiogenesis, American investigators hypothesized that stimulation of A2ARs might affect the rate of wound healing. In an early study, CGS 21680 was topically applied to wounds in normal healthy rats and mice, and to wounds in rats with streptozotocin-induced diabetes. Treatment with this A2AR agonist significantly increased the rate of wound healing in all groups of animals, and wounds in CGS 21680 treated diabetic rats healed equally rapid, or even more rapid, than wounds in untreated healthy rats. In subsequent experiments in mice, CGS 21680 was shown to stimulate both the recruitment of endothelial progenitor cells (vasculogenesis) and local sprouting of blood vessels (angiogenesis) at an early stage (≤ 3 days) of wound repair.

In an in vitro model of airway repair, both adenosine and the A2AR agonist CPCA (5′-(N-cyclopropyl)carboxamidoadenosine; Fig. 1) dose-dependently stimulated repair. This effect was counteracted by the A2AR antagonist ZM 241385 and the antagonist impeded wound closure when it was administered alone.

Based on such animal and in vitro data, A2AR agonists have been proposed as therapeutic drugs that could be applied to improve wound healing after skin injury. A clinical trial with the selective A2AR agonist polydeoxyribonucleotide (PDRN) has been performed in patients with diabetes. The conclusion of the trial was that agonist treatment significantly facilitates the healing of diabetic foot ulcers. It should be noted that although A2AR agonists promote wound healing, they may also stimulate the production of collagen and scar tissue. In mouse models of human scar formation and irradiation-induced dermal injury, the A2AR antagonist ZM 241385 diminished scarring.

F. Obesity

A few studies in experimental animals have indicated that A2AR agonists may be useful for the treatment of obesity. The agonist CGS 21680 suppresses food-reinforced lever pressing and the consumption of laboratory chow in rats. On the other hand, the A2AR antagonist KW-6002 increases food-reinforced lever pressing and the A2AR antagonist MSX-3 increases food intake. In a later study, two A2AR agonists were shown to inhibit both the intake of high-palatable food in a rat model of binge eating and the intake of low-palatable food in food-deprived rats. These effects of A2AR ligands on food intake may be related to adenosinergic–dopaminergic interactions, since dopamine plays an important role in the initiation of feeding and the reinforcement of food-seeking behavior.

A publication in the journal Nature indicated that in mice and humans (as opposed to rats) A2AR agonists have significant additional effects that would be beneficial in the treatment of obesity. Stimulation of A2ARs in brown adipose tissue of these species causes physiological activation of adipocytes and stimulates energy expenditure. Moreover, stimulation or increased expression of A2ARs in white fat causes “browning” of these cells, that is, their transformation in beige adipocytes. In mice which received a high-fat diet, treatment with an A2AR agonist (PSB-077) resulted in increased energy expenditure, a lower development of body fat, and
an improved glucose tolerance. Thus, stimulation of \( A_{2A} \)Rs protected the animals from diet-induced obesity.\(^{320}\)

Such animal data suggest that selective stimulation of \( A_{2A} \)R in adipocytes, or gene therapy (transfer of the \( A_{2A} \)R gene to white adipose tissue in humans) may be a strategy to combat obesity.

**G. Transient Opening of the BBB**

The blood–brain barrier (BBB) employs different strategies to protect the mammalian central nervous system (CNS): (i) Tight junctions between endothelial cells limit paracellular transport and result in an extremely low permeability of the capillary endothelium, and (ii) brain capillary endothelial cells express several active efflux transporters, such as \( P \)-glycoprotein (\( P \)-gp), breast cancer resistance protein 1 (BCRP-1), and multidrug resistance protein 1. Substrates for these proteins are pumped back from endothelium to blood before they can enter the brain, and transcellular transport of such compounds is effectively prevented.

An early study examined the expression of various AR subtypes in an in vitro model of the BBB (coculture of bovine capillary endothelial cells and rat astrocytes). Adenosine \( A_1 \)Rs were not detected, but \( A_{2A} \)Rs and \( A_3 \)Rs were present in the endothelial cells. Selective agonists for these receptors (cyclopentyladenosine, CGS 21680, and adenosine-5’-N-ethylcarboxamide) did not induce significant changes in trans-endothelial electrical resistance or in the transport of the paracellular markers sodium fluorescein and dextran. The study concluded that “the functional role of adenosine receptor subtypes in regulating the paracellular permeability of the BBB is probably small.”\(^{321}\)

Later studies in intact animals suggested a more important role for adenosine and \( A_{2A} \)R. Experimental autoimmune encephalomyelitis (EAE) in mice is frequently used as an animal model of MS. CD73 knockout mice are resistant to EAE and have less infiltrating lymphocytes in their brains compared to wild-type mice. However, when CD73-positive T cells from wild-type animals are transferred to these knock-out mice, the knock-out animals become susceptible to the induction of EAE. On the other hand, when wild-type animals are treated with the \( A_{2A} \)R-specific antagonist, SCH 58261, they become resistant and are protected from EAE induction. Thus, CD73 expression and \( A_{2A} \)R signaling appear to be required for efficient entry of lymphocytes into the CNS and for the induction of EAE.\(^{322,323}\)

Endothelial cells from human brain (hCMEC/D3) and mouse brain express functional \( A_{2A} \)Rs and \( A_1 \)Rs.\(^{324,325}\) These data suggest that the endothelium of brain capillaries is capable of responding to increases of extracellular adenosine.

A very interesting study in mice\(^{325}\) reported that activation of ARs by systemic administration of agonists results in the entry of macromolecules (10 and 70 kD dextrans, anti-\( \beta \)-amyloid antibodies) from the blood into the CNS. \( A_1 \)R or \( A_{2A} \)R knockout mice showed less striking increases of dextran entry after 5’-N-ethylcarboxamidoadenosine (NECA) administration, and in \( A_1 \)R knockout mice that were pretreated with the selective \( A_{2A} \)R antagonist SCH 58261, dextran entry was not increased at all. Thus, the increases of BBB permeability were AR-mediated. Experiments in which wild-type mice were treated with subtype-selective \( A_1 \)R and \( A_{2A} \)R agonists indicated that activation of either the \( A_1 \)R or \( A_{2A} \)R facilitates the entry of macromolecules into the CNS, whereas simultaneous activation of both ARs has an additive effect. Increased entry of dextrans into the brain of mice and rats was also observed after treatment of these animals with the FDA-approved \( A_{2A} \)R agonist regadenoson (“Lexiscan,” Fig. 1). When a transgenic mouse model of Alzheimer disease was treated with NECA, an intravenously administered anti-\( \beta \)-amyloid antibody (monoclonal 6E10) entered the CNS and bound to \( \beta \)-amyloid plaques in the brain. In vitro experiments with the mouse endothelial cell line Bend.3 indicated that stimulation with NECA or regadenoson resulted in transient
decreases of trans-endothelial electrical resistance, increased actinomyosin stress fiber formation, and altered tight junction protein expression, but not in increased transcytosis. Apparently, AR stimulation induces remodeling of the actin cytoskeleton and causes changes of the shape or size of endothelial cells with resulting increases of the intercellular space and greater macromolecular diffusion. Similar observations as in these intact mice were also made in an in vitro model of the human BBB. NECA or Lexiscan increased the passage of 10 kDa dextrans or the anticancer drug gemcitabine across the barrier.

Although stimulation of endothelial A2AR seems an attractive strategy for facilitating drug delivery to the brain, the feasibility of this approach is limited by the fact that existing subtype-selective A2AR agonists have very short half-lives in the mammalian circulation (ranging from 5 sec to 5 min). In an attempt to improve the circulation lifetime and receptor signaling efficacy of an A2AR agonist, Chinese investigators prepared a series of nanoagonists by coupling different numbers of regadenoson molecules to a dendrimer. When it was administered to Bend.3 monolayers in vitro, Den-Reg16 induced a greater decrease of trans-endothelial electrical resistance than parent regadenoson (68% as opposed to 30%) and a greatly prolonged increase of monolayer permeability (2.8 hr vs. less than 20 min). The applicability of Den-Reg16 was tested in nude mice. PEGylated dextran (45 kDa, dual labeled with 99mTc-DTPA [where DTPA is diethylenetriaminepentaacetic acid] and a rhodamine fluorophore) was used as a model drug, whereas the A2AR nanoagonist was labeled with the near-infrared fluorophore IR783. The brain uptake of the model drug could be monitored in the living animal with SPECT-CT and the regional distributions of Den-Reg16 and model drug were analyzed post-mortem with optical imaging (by simultaneous detection of the fluorescence signals of A2AR agonist and model drug at different wavelengths). Den-Reg16 caused a 3.1-fold higher uptake of the model drug than parent regadenoson and BBB opening after administration of the nanoagonist was extended from 10 min to > 2 hr, whereas after administration of regadenoson it lasted from 30 to 50 min. The authors suggest that by choosing a nanoagonist with proper ligand stoichiometry (1–16 copies per dendrimer), the time-window of BBB opening can be matched to the pharmacokinetics of a therapeutic drug. Thus, drug delivery to the brain can be maximized and unwanted BBB leakage minimized.

Two animal studies have suggested that AR stimulation is indeed an efficient strategy that may be applied to overcome drug resistance. The first study used an epilepsy model in adult male Wistar rats (daily electrical stimulation of the amygdala). Animals were either untreated, treated with the anti-epileptic drug phenytoin, or treated with a combination of phenytoin and NECA. The addition of NECA resulted in a significant increase of the levels of phenytoin in the brain and a dramatic decrease of seizure frequency, duration, and severity. The second study employed healthy F344 rats. Animals received temozolomide, a chemotherapeutic drug that is frequently applied for treatment of patients with glioblastoma, either alone (p.o.) or in combination with intravenous regadenoson. Two hours after the oral administration of temozolomide, the concentration of the test drug was 60% higher in the brain of regadenoson-treated animals than in rats that had not received regadenoson, whereas the plasma concentrations of temozolomide were not significantly altered. Thus, A2AR stimulation may be applied to improve the efficacy of chemotherapy in patients with brain tumors.

Interestingly, stimulation of ARs on brain capillary endothelial cells causes not only transient opening of the tight junctions but also a transient decrease of the efflux function of P-gp and BCRP-1. Both in primary human endothelial cells and in a human brain endothelial cell line (hCMEC/D3), P-gp expression and function were rapidly decreased after treatment of the cells with regadenoson, and less rapidly decreased after treatment with NECA. In these experiments, P-gp expression was determined by western blotting and P-gp function by measuring the cellular uptake of rhodamine-123 that provides a fluorescent signal. The passage of rhodamine-123 through an in vitro model of the BBB was increased after AR stimulation.
Various mechanisms appear to be involved in the observed downregulation of P-gp: increased association of P-gp with the cytoskeleton, upregulation of matrix metalloprotease-9 leading to cleavage of P-gp and release of transporter fragments in the extracellular space, and rapid ubiquitylation of P-gp. Transient decreases of P-gp expression and function were also observed in the cerebral endothelium of intact mice, and the brain uptake of the antitumor drug (and P-gp substrate) epirubicin was increased under these conditions.

These data suggest that extracellular adenosine produced after damage of brain tissue opens the BBB so that nutrients, regulatory substances, and cells of the immune system can enter the brain. Modulation of ABC transporter function in the BBB with an adenosine A\textsubscript{2A}R agonist may be an effective and safe method of delivering transported drugs into the CNS.

H. PET in the Development of A\textsubscript{2A}R Agonists as Therapeutic Drugs

A\textsubscript{2A}R agonists (or agonist prodrugs) can be labeled with positron emitters and administered to living animals or humans to determine their pharmacokinetics and biodistribution. At the time of this writing, it is not yet clear whether PET imaging with the currently available antagonist radioligands (Table V) can be used to measure the occupancy of the A\textsubscript{2A}R population in brain or other target tissues by (nonradioactive) agonist drugs. Published in vivo competition studies concerned drug candidates that were A\textsubscript{2A}R antagonists, not agonists. It remains to be established whether the in vivo binding of the existing radioligands is reduced after administration of an exogenous agonist or after experimental manipulations aimed at increasing the levels of extracellular adenosine. The question whether any change of probe binding can be detected after treatment of animals with a positive allosteric modulator for A\textsubscript{2A}Rs should also still be answered. However, the immunosuppressive effect of A\textsubscript{2A} agonists in inflammatory conditions can be assessed by PET imaging using existing probes that bind to specific targets on activated immune cells, such as the TSPO (translocator protein) on macrophages, or the interleukin-2 receptor on T cells.

The application of PET imaging may accelerate the development of strategies for transient opening of the BBB and the translation of such strategies from experimental animals to humans:

1. **Assessment of the impact of A\textsubscript{2A}R agonists on P-gp function in the BBB.** Since noninvasive measurements of P-gp function in the human BBB are possible using a radiolabeled P-gp substrate (e.g., \((R)-[\textsuperscript{11}C]verapamil) and PET, and since the adenosine A\textsubscript{2A}R agonist regadenoson (Lexiscan) has been approved by the FDA for pharmacological stress testing of the human heart, PET studies may be performed in healthy volunteers to prove that administration of regadenoson results in a transient decrease of P-gp-mediated drug efflux in the human brain. Since biomolecules can be labeled with the positron emitter \textsuperscript{11}C without changing their chemical structures, therapeutic drugs that are P-gp substrates (such as phenytoin or temozolomide) can also be radiolabeled and their entry in the human brain be continuously monitored with PET, both at baseline and after AR stimulation. The labeling of phenytoin with \textsuperscript{11}C has been reported in several publications, and the synthesis of \textsuperscript{11}C-temozolomide has been described.

2. **Assessment of the impact of A\textsubscript{2A}R agonists on paracellular transport.** Macromolecules (e.g., antibodies) or cells of the immune system may be labeled with a positron emitter of longer physical half-life than \textsuperscript{11}C or \textsuperscript{18}F (e.g., \textsuperscript{89}Zr). Their entry into the rodent CNS can then be monitored using small animal PET, both at baseline and after experimental manipulation of the BBB.

3. **Assessment of the kinetics of AR agonists.** After injection of a mixture of a radiolabeled AR agonist and the nonradioactive parent compound, the cerebral kinetics of
this endothelium-stimulating agent may be monitored and be correlated to the kinetics of BBB opening (which has been determined in the experiments described above). The synthesis and pharmacological properties of an $^{18}$F-labeled analog of NECA have been reported.\textsuperscript{344,345}

9. LIMITATIONS OF PET IMAGING

A. Insufficient Specificity of the Probe

Published data on PET imaging of A$_{2A}$Rs have indicated differences between the xanthine ligand $[^{11}$C]TMSX and nonxanthine ligands.

1. The affinity of $[^{11}$C]TMSX for A$_{2A}$Rs and the signal-to-noise ratios of PET images made with this radioligand are lower than the affinities and signal-to-noise ratios of the nonxanthine radioligands (see Table V and compare the data reported in [163, 346]).

2. Yet, $[^{11}$C]TMSX shows specific binding in peripheral tissues (skeletal muscle and heart\textsuperscript{152,155}), whereas such binding is not detected with the nonxanthine radioligands $[^{11}$C]SCH 442416\textsuperscript{149} or $[^{11}$C]preladenant.\textsuperscript{151}

The evidence for specific binding of $[^{11}$C]TMSX in peripheral tissues is based on experiments in which subjects were scanned at baseline and after pretreatment with nonradioactive antagonists. Tracer uptake in heart and skeletal muscle was not altered after administration of DPCPX (which binds selectively to the A$_1$R subtype), but was significantly reduced after pretreatment with the A$_1$/A$_{2A}$R ligand theophylline, the A$_{2A}$R ligand CSC, or nonradioactive TMSX.\textsuperscript{152,155} All blockers that decreased the myocardial and muscular uptake of $[^{11}$C]TMSX in these studies (theophylline, CSC, and nonradioactive TMSX) had xanthine structures such as the radiotracer itself. The observed decline of $[^{11}$C]TMSX uptake in myocardium and skeletal muscle may thus be not related to specific binding of the tracer to A$_{2A}$Rs but may reflect a saturable binding of $[^{11}$C]TMSX to an unidentified site that binds xanthines but does not bind nonxanthine A$_{2A}$R ligands.

PET studies in experimental animals have indeed suggested that xanthine radioligands for A$_{2A}$Rs may interact with sites that are blocked by nonradioactive xanthines but not by A$_{2A}$R ligands that lack the xanthine structure. Myocardial binding of the xanthine $[^{11}$C]KF17837 in intact rabbits was completely blocked by nonradioactive KF17837 or by CSC, but much less reduced after administration of the nonxanthine A$_{2A}$R antagonists ZM 241385 or SCH 58261.\textsuperscript{347} Yet, ZM 241385 and SCH 58261 have a considerably higher affinity to A$_{2A}$R than CSC. Uptake of $[^{11}$C]KF17837 in the mouse striatum was decreased by coinjection of nonradioactive KF17837 or CSC, but not by coinjection of ZM 241385 or SCH 58261.\textsuperscript{348}

The data on specific binding of $[^{11}$C]TMSX in peripheral organs (or extrastriatal regions of the brain) should thus be interpreted with caution. Such binding may reflect an interaction of the probe with sites that are not A$_{2A}$Rs.

B. Insufficient Sensitivity of the Probe

When regional uptake of the PET tracer $[^{11}$C]preladenant was compared in rats pretreated with saline or the nonradioactive A$_{2A}$R antagonist KW-6002, significant differences were observed in the striatum, but not in other brain areas, myocardium, skeletal muscle, blood, or spleen.\textsuperscript{151} Receptor-mediated uptake of this potent nonxanthine A$_{2A}$R antagonist (K$_d$ 1.1 nM) was only detected in tissues where A$_{2A}$R are expressed at high levels (in vitro $B_{\text{max}}$/in vitro $K_d > 25$, see Table I). In vitro binding assays with tritiated ligands have indicated that A$_{2A}$R
expression in splenocytes or hippocampus is $<10\%$ of the value in striatum. At such low levels of receptor expression, specific in vivo binding of $[^{11}\text{C}]$preladenant could not be detected. Similar observations were made for other nonxanthine radioligands ($[^{11}\text{C}]	ext{SCH 442416}$, $[^{18}\text{F}]	ext{MNI-444}$).

For various reasons, the apparent in vivo affinity of a radioligand in PET studies can be much lower than its affinity measured by in vitro binding assays:

1. Free radioligand may be not homogeneously distributed in living tissue. A lipophilic probe may accumulate more in lipophilic than in hydrophilic tissue areas, and may acquire much higher concentrations in biomembranes than in the body fluid as free ligand. Such phenomena will result in a small $f_{\text{ND}}$ (free fraction of the ligand in tissue). As the specific in vivo binding of the ligand ($BP_{\text{ND}}$) can be approximated as:

$$BP_{\text{ND}} = f_{\text{ND}} \times \left( \frac{B_{\text{max}}}{\alpha} \times K_d \right),$$

where $B_{\text{max}}$ and $K_d$ are derived from in vitro binding assays, and $\alpha$ is the conversion factor from 1 mg protein to 1 mg tissue, the amount of radioligand bound to receptors in target tissue can be considerably lower than the quotient of $B_{\text{max}}$ and in vitro $K_d$. The concentration of bound ligand can be up to four orders of magnitude lower in the case of very small $f_{\text{ND}}$. 349–351

2. Intravenously injected radioligands may bind to proteins in blood plasma. The free fraction of radioligand in plasma is usually much lower than the total plasma concentration. If the ligand bound to protein is slowly dissociating and if only free ligand can cross the capillary endothelium, protein binding will result in a reduced availability of the ligand for binding to receptors in tissue. 351,352

3. Specific pharmacokinetic features of the radioligand, that is, rapid degradation to metabolites with negligible affinity for the target receptor, diffusion barriers separating the target sites from the blood, and rapid clearance or excretion of the radioligand may also decrease the in vivo availability of the probe and may result in limited probe binding to receptors in target tissue. Although these factors do not affect $BP_{\text{ND}}$, they can result in increased

*Figure 5.* Parametric PET images showing the distribution volume ($V_T$) and binding potential ($BP_{\text{ND}}$) of $[^{11}\text{C}]$preladenant in rhesus monkey brain. Regional $V_T$ values were determined by Logan graphical analysis and arterial blood sampling (A); $BP_{\text{ND}}$ was determined by the reference tissue-based Logan plot (B). Specific binding of the probe was detected only in the striatum.
statistical noise, a decreased signal-to-noise ratio and difficulty to detect specific binding of the radioligand in areas with low values of BP_{ND}.

Apparently, mechanisms such as these result in a high detection limit for A_{2A}Rs in PET imaging and detection of a specific signal only in the striatum (Fig. 5). It may be of interest to evaluate PET tracers for A_{2A}Rs in future research not only in healthy rodents, but also in tumor-bearing animals and animal models of inflammation. In macrophage-like cells, A_{2A}R expression has been shown to increase from a low and almost undetectable level at baseline to 40% of the level in striatum upon activation (Table II). Such levels of A_{2A}R expression may be above the detection limit of [{[^{11}C]}]preladenant (or [{[^{11}C]}]SCH 442416 or [{[^{18}F]}]MNI-444) and PET. If A_{2A}Rs in inflammatory cells can be detected with PET imaging, PET may be used in the evaluation of A_{2A}R antagonists as immunity-boosting and A_{2A}R agonists as immunosuppressive drugs.

10. CONCLUSION

The expression and function of A_{2A}Rs is altered in various diseases and many therapeutic applications of A_{2A}R agonists and antagonists have been proposed. A_{2A}R agonists may suppress transplant rejection and graft-versus-host disease, be used to treat various inflammatory disorders (asthma, inflammatory bowel disease, rheumatoid arthritis), be locally applied to promote wound healing, and be employed in a strategy for transient opening of the BBB, so that therapeutic drugs and monoclonal antibodies can enter the brain. Increasing A_{2A}R signaling in adipose tissue is also a potential strategy to combat obesity. A_{2A}R antagonists may be applied to treat motor fluctuations, epilepsy, postischemic brain damage or cognitive impairment, and to control an immune checkpoint during immunotherapy of cancer. Imaging could play an important role in the development and evaluation of such compounds. Several PET probes for imaging of A_{2A}Rs have been developed since the 1990s. Particularly radioligands that lack the xanthine structure appear to offer excellent specificity for the A_{2A}R subtype. Currently available radioligands allow quantitative imaging of A_{2A}R in the mammalian striatum, but not in other areas of the brain. Whether these ligands are capable of detecting A_{2A}R expression in inflammatory cells and quantifying dose-dependent occupancy of the A_{2A}R population by nonradioactive agonists should still be examined. Radiochemical efforts may be aimed at the synthesis of positron-emitting radioligands with affinities in the picomolar range, which could allow imaging of A_{2A}R in extrastriatal areas of the brain, such as the cerebral cortex and the hippocampus. The existing probes can already be applied in PET studies of the involvement of A_{2A}Rs in various cerebral disorders and in the development of A_{2A}R antagonists as therapeutic drugs. PET imaging with currently available tracers may also be used to optimize A_{2A}R-based strategies for transient opening of the BBB.

ABBREVIATIONS

\[
\begin{align*}
A_1R &= \text{adenosine A}_1 \text{ receptor} \\
A_{2A}R &= \text{adenosine A}_{2A} \text{ receptor} \\
A_{2B}R &= \text{adenosine A}_{2B} \text{ receptor} \\
A_3R &= \text{adenosine A}_3 \text{ receptor} \\
ADHD &= \text{attention-deficit hyperactivity disorder} \\
AMP &= \text{adenosine 5'-monophosphate} \\
ATP &= \text{adenosine 5'-triphosphate}
\end{align*}
\]
B16 = mouse spontaneous melanoma cell line
B16-F10 = mouse spontaneous melanoma cell line (highly metastatic)
BBB = blood–brain barrier
BCRP-1 = breast cancer resistance protein 1
Bend.3 = transformed mouse endothelial cell line
C6 = rat glioma cell line
CD39 = ecto-nucleoside triphosphate diphosphohydrolase 1
CD73 = ecto-5'-nucleotidase
CL8-1 = mouse melanoma cell line
CL57BL/6 = inbred strain of laboratory mouse (“Black 6”)
CNS = central nervous system
CSC = 8-(3-chlorostyryl)-caffeine
EAE = experimental autoimmune encephalomyelitis (animal model of multiple sclerosis)
GP = globus pallidus
hCMEC/D3 = immortalized human brain endothelial cell line (model of human blood brain barrier)
HD = Huntington’s disease
MCA = 3-methylcholanthrene
MCI = mild cognitive impairment
MS = multiple sclerosis
MSX-2 = 3-hydroxypropyl-7-methyl-8-(3-methoxystyryl)-1-propargylxanthine
NECA = 5'-N-ethylcarboxamidoadenosine
NK = natural killer
OaW42 = human ovarian tumor epithelial cell line
P-gp = P-glycoprotein
PCR = polymerase chain reaction
PD = Parkinson’s disease
PD-1 = programmed cell death protein 1
PET = positron emission tomography
RNA = ribonucleic acid
RT-PCR = reverse transcriptase-polymerase chain reaction
SHR = spontaneously hypertensive rats
siRNA = small interfering RNA

REFERENCES


Medicinal Research Reviews DOI 10.1002/med

*Medicinal Research Reviews* DOI 10.1002/med


ADENOSINE A\textsubscript{2A} RECEPTOR LIGANDS

50. Lindström K, Ongini E, Fredholm BB. The selective adenosine A\textsubscript{2A} receptor antagonist SCH 58261 discriminates between two different binding sites for [\textsuperscript{3}H]-CGS 21680 in the rat brain. Naunyn Schmiedebergs Arch Pharmacol 1996;354:539–541.


53. Fredholm BB, Lindström K, Dionisotti S, Ongini E. [\textsuperscript{3}H]SCH 58261, a selective adenosine A\textsubscript{2A} receptor antagonist, is a useful ligand in autoradiographic studies. J Neurochem 1998;70:1210–1216.


61. Duarte JM, Oliveira CR, Ambrosio AF, Cunha RA. Modification of adenosine A\textsubscript{1} and A\textsubscript{2A} receptor density in the hippocampus of streptozotocin-induced diabetic rats. Neurochem Int 2006;48:144–150.


69. Pinna A. Adenosine A$_{2A}$ receptor antagonists in Parkinson’s disease: Progress in clinical trials from the newly approved istradefylline to drugs in early development and those already discontinued. CNS Drugs 2014;28:455–474.


77. By Y, Jacquin L, Franceschi F, Durand-Gorde JM, Condo J, Michelet P, Guieu R, Ruf J. Fall in oxygen tension of culture medium stimulates the adenosinergic signalling of a human T cell line. Purinergic Signal 2012;8:661–667.


84. Martini C, Tuscano D, Trincavelli ML, Cerrai E, Bianchi M, Ciapparelli A, Alessio L, Novelli L, Catena M, Lucacchini A, Cassano GB, Dell’Osso L. Upregulation of A$_{2A}$ adenosine receptors in
ADENOSINE A\textsubscript{2A} RECEPTOR LIGANDS


88. Maglione V, Cannella M, Martino T, De BA, Frati L, Squitieri F. The platelet maximum number of A\textsubscript{2A}-receptor binding sites (B\textsubscript{max}) linearly correlates with age at onset and CAG repeat expansion in Huntington’s disease patients with predominant chorea. Neurosci Lett 2006;393:27–30.


92. Villar-Menendez I, Porta S, Buira SP, Pereira-Veiga T, az-Sanchez S, Albazanz JL, Ferrer I, Martin M, Barrachina M. Increased striatal adenosine A\textsubscript{2A} receptor levels is an early event in Parkinson’s disease-related pathology and it is potentially regulated by miR-34b. Neurobiol Dis 2014;69:206–214.


Medicinal Research Reviews DOI 10.1002/med


aspirin through activation of A\textsubscript{2A} adenosine receptor in rats. World J Gastroenterol 2006;12:568–573.


**Aren van Waarde** studied animal physiology and received a Ph.D. degree from Leiden University in the Netherlands. He worked as a postdoctoral research associate in the Department of Molecular Biophysics and Biochemistry at Yale University (1986–1988). After his return from the United States, he was appointed in Leiden as a Fellow of the Royal Dutch Academy of Sciences and worked on in vivo NMR spectroscopy of aquatic animals. For this research he received an award (C. J. Kok prize, Leiden University). Since 1991 he is a member of the permanent staff of the Department of Nuclear Medicine and Molecular Imaging (formerly: PET Center) at the University Medical Center Groningen (Netherlands), and is involved in the preclinical evaluation of novel radiopharmaceuticals.
Rudi Dierckx studied medicine and neuropsychiatry at the Free University of Brussels. He was trained as a nuclear medicine physician at the University of Antwerp (1988–1994). Rudi has headed the Department of Nuclear Medicine at the University Hospital of Ghent (Belgium) from 1994 to 2004, and has acquired a Master of Business Administration (MBA) at the Vlerick School of Management in Leuven (2004, cum laude). Since 2005 he is Head of the Department of Nuclear Medicine and Molecular Imaging at University Medical Center Groningen (UMCG) in The Netherlands. Recently (2013) he was also appointed as Head of the Medical Imaging Center, and as Chairman ad interim of the Department of Radiology at UMCG. His primary research interests are PET studies of the human brain.

Xiaoyun Zhou acquired a bachelor in Chemistry at the College of Chemistry of Sichuan University (Sichuan, China) in 2007 and a master of Medicine degree at Beijing Normal University, Beijing, China in 2010. From 2011 to 2015, she performed Ph.D. research at the Department of Nuclear Medicine and Molecular Imaging (University Medical Center Groningen, Netherlands). Her Ph.D. project concerned the synthesis and preclinical evaluation of novel radiopharmaceuticals for adenosine A2A receptor imaging with PET. She now works as a radiopharmaceutical chemist (postdoc) at the University of Cambridge (United Kingdom).

Shivashankar Khanapur acquired a Master of Pharmacy degree at Rajiv Gandhi University of Health Sciences in Bangalore (India). He performed Ph.D. research at the Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, The Netherlands (2010–2014). His Ph.D. thesis was entitled “Development and Preclinical Evaluation of Radioligands for PET Studies of Cerebral Adenosine A1 and A2A Receptors” (2014). He was a post-doctoral research fellow at the Department of Chemistry, University of Oslo (Norway) and Radboud Translational Medicine B.V., Nijmegen (The Netherlands) from 2015 to 2016.

Hideo Tsukada received a Ph.D. degree from Shizuoka College of Pharmacy, Japan. He was a visiting researcher at the Uppsala University PET Center, directed by Professor Bengt Längström, from 1990 to 1991. At present, he is the senior manager of the PET Center at the Central Research Laboratory of Hamamatsu Photonics in Japan, and conducts both preclinical and clinical studies that involve PET imaging. He has published more than 250 papers, and has received an award from the Society for Nuclear Medicine (2009), and the Japan Molecular Imaging Award (2010). He is serving as a visiting Professor at the Hamamatsu University School of Medicine, and the University of Shizuoka School of Pharmaceutical Sciences.

Kiichi Ishiwata studied chemistry at Ibaraki University and biochemistry at the Graduate School of Tohoku University in Japan. From 1981 to 1991 he worked as an Assistant Professor (later Associate Professor) at the Cyclotron and Radioisotope Center of Tohoku University and as a visiting scientist in the Isotope Laboratory at Groningen University, the Netherlands under the supervision of Professor Willem Vaalburg (1984–1985). In June 1991, he moved to the Postitron Medical Center in the Tokyo Metropolitan Institute of Gerontology as a Senior Research Scientist, and has headed the Positron Medical Center (later Research Team for Neuroimaging) from 2001 to 2015. In October 2015, he was appointed as Director of the Institute of Cyclotron and Drug Discovery Research, Southern TOHOKU Research Institute for Neuroscience (Koriyama, Japan) and Professor at the Department of Biofunctional Imaging, Fukushima Medical University (Fukushima, Japan). His research is focused on the development of radiopharmaceuticals for diagnosis of disorders of the human brain and of cancer. Kiichi has published more than 350 papers.
Gert Luurtsema was trained as a radiopharmaceutical chemist in Groningen and performed Ph.D. research at the VU University Medical Centre in Amsterdam (The Netherlands). His Ph.D. project aimed to develop an in vivo assay for noninvasive measurement of P-glycoprotein function with positron emission tomography (PET). After the acquisition of his Ph.D. degree, Gert worked as a research scientist at the VU University Medical Centre (2006–2009). By the end of 2009, he returned to Groningen where he is employed as supervisor of tracer productions and coordinator of the radiopharmaceutical facilities in the Department of Nuclear Medicine and Molecular Imaging. His primary research interest is the development of specific radiopharmaceuticals for quantification of the function of ABC transporters in the blood–brain barrier.

Erik de Vries graduated in chemistry (cum laude) at the University of Leiden (The Netherlands) in 1991 and obtained his Ph.D. in natural sciences at the same university in 1995. After a short project as a chemist at the pharmaceutical company Gist-Brocades in Delft, the Netherlands, he moved to the University Medical Center Groningen in 1996, where he is still working as an Associate Professor at the Department of Nuclear Medicine and Molecular Imaging. Since 2007, he is the research coordinator of this department. He was also guest lecturer in molecular and diagnostic imaging at the University Sapienza in Rome, Italy from 2007 to 2013. Erik has a special interest in the development and application of radiopharmaceuticals for PET imaging of inflammation and tumor drug targets.

Philip Elsinga was trained in radiochemistry at the Departments of Organic Chemistry and Nuclear Medicine at Groningen University (The Netherlands). Since 1995 he is the chief radiochemist of the Department of Nuclear Medicine and Molecular Imaging at the same university. Philip was visiting scientist at the Imaging Research Lab (University of Washington, Seattle, USA) in 1998 and at the Tokyo Metropolitan Institute of Gerontology (Japan) in 2000. Since 2007, he is visiting Professor at the University of Ghent in Belgium and in 2012 he was appointed as Full Professor of Radiopharmaceutical Chemistry at the University of Groningen. Philip is President and cofounder of the Dutch Society for Clinical Radiochemistry, member of the Board of Directors of the Society of Radiopharmaceutical Sciences, and Editor-in-Chief of EJNMMI Radiopharmacy and Chemistry.