Adenosine A<sub>1</sub> receptor: Functional receptor-receptor interactions in the brain

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Received: 23 July 2007 / Accepted: 24 July 2007 / Published online: 5 September 2007

Abstract Over the past decade, many lines of investigation have shown that receptor-mediated signaling exhibits greater diversity than previously appreciated. Signal diversity arises from numerous factors, which include the formation of receptor dimers and interplay between different receptors. Using adenosine A<sub>1</sub> receptors as a paradigm of G protein-coupled receptors, this review focuses on how receptor-receptor interactions may contribute to regulation of the synaptic transmission within the central nervous system. The interactions with metabotropic dopamine, adenosine A<sub>2A</sub>, A<sub>3</sub>, neuropeptide Y, and purinergic P2Y<sub>1</sub> receptors will be described in the first part. The second part deals with interactions between A<sub>1</sub>Rs and ionotropic receptors, especially GABA<sub>A</sub>, NMDA, and P2X receptors as well as ATP-sensitive K<sup>+</sup> channels. Finally, the review will discuss new approaches towards treating neurological disorders.

Keywords Adenosine · G protein-coupled receptors · Receptor interactions · Neurotransmission · Adenosine receptors · Ionotropic receptors

Abbreviations

| Abbreviation | Full Form |
|--------------|-----------|
| A<sub>1</sub>R | adenosine A<sub>1</sub> receptor |
| A<sub>2A</sub>R | adenosine A<sub>2A</sub> receptor |
| A<sub>2B</sub>R | adenosine A<sub>2B</sub> receptor |
| A<sub>3</sub>R | adenosine A<sub>3</sub> receptor |
| 2-CA | 2-chloro-adenosine |
| CHA | N<sup>6</sup>-cyclohexyladenosine |
| CI-IB- | N<sup>6</sup>-(3-iodobenzyl)adenosine-5′-N<sup>6</sup>-methyluronamide |
| MECA | N<sup>6</sup> cyclopentyladenosine |
| CPA | 8-cyclopentyl-1,3-dimethyl-xanthine |
| D<sub>1</sub>R | dopamine D<sub>1</sub> receptor |
| D<sub>2</sub>R | dopamine D<sub>2</sub> receptor |
| DIDS | chloride channel blocker |
| EPSPs | excitatory postsynaptic potentials |
| GIRK<sub>1-4</sub> | inward rectifying K<sup>+</sup> channels |
| GPCR | G protein-coupled receptor |
| GRAFS | glutamate, rhodopsin, adhesion, fizzled/taste and secretin-like GPCRs |
| IP3 | inositol 1,4,5-triphosphate |
| IPSPs | inhibitory postsynaptic potentials |
| K<sub>ATP</sub> | ATP-sensitive K<sup>+</sup> channels |
| LTP | long-term potentiation |
| MPTP | methylphenyltetrahydropyridine |
| MRS-1191 | [3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(-/+-)-dihydropyridine-3,5-dicarboxylate] |
| NHE1 | Na<sup>+</sup>/H<sup>+</sup> exchange |
| NPY | neuropeptide Y |
| PLA | phospholipase A |
| PLC | phospholipase C |
| PSSPs | postsynaptic potentials |
| R-PIA | (R)-N6-(2-phenylisopropyl)adenosine |
| SKF 38939 | (+/−)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide |
| VDCC | voltage-dependent Ca<sup>2+</sup> channels |
| Y<sub>1-5</sub>R | neuropeptide Y<sub>1-5</sub> receptor |

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Introduction

The vertebrate central nervous system (CNS) is characterized by a dynamic interplay between signal transduction molecules and their cellular targets. Modulation of synaptic transmission by metabotropic or ionotropic receptors is an important source of control and dynamical adjustment in synaptic activity.

Recent studies have provided new insights into the role of ligand-gated ion channels in modifying synaptic transmission. Along with a growing list of different types of pre- and postsynaptic ionotropic receptors and the cell types that express them, there have also been advances in characterizing the modulatory mechanisms of the receptors that link to receptor activation. This is important due to the convergence of data from biochemical, molecular, and electrophysiological studies, implicating ionotropic receptors in the effects of psychoactive and addictive drugs.

G protein-coupled receptors (GPCRs) make up the largest and most diverse family of membrane receptors in the human genome, relaying information on the presence of diverse extracellular stimuli to the cell interior. An estimated 1% of the mammalian genome encodes for GPCRs, and about 450 of the approximately 950 predicted human GPCRs are thought to be receptors for endogenous ligands [1]. The manipulation of transmembrane signaling by GPCRs may constitute the most important therapeutic target in medicine. Nearly 40% of all current therapeutic drugs target GPCRs [2].

All known GPCRs share a common architecture of seven membrane-spanning helices connected by intracellular and extracellular loops. Drugs acting on GPCRs have been classified as agonists, partial agonists, or antagonists based on a “two-state model of receptor function.” Since experimental evidence pointed out the impossibility of explaining the operation of GPCRs without considering dimers as the minimum structure for many GPCRs the “two-state dimer receptor model” was developed based on the communication between the two subunits of the receptor dimmer [1, 3, 4]. This model is an extension of the “two-state model of receptor function” but considers dimeric structures able to bind one molecule to the orthosteric center in each monomer.

GPCR signaling is subject to extensive negative regulation through receptor desensitization, sequestration, and downregulation, termination of G protein activation by GTPase-activating proteins, and enzymatic degradation of second messengers. Additional protein-protein interactions positively modulate GPCR signaling by influencing ligand binding and specificity.

Multiprotein complexes mediate most cellular functions. In neurons, these complexes are directly involved in the neuronal transmission, which is responsible for learning, memory, and developments. The first publication in this direction came from Hökfelt’s group in 1983. The publication describes how substance P may modulate the high-affinity serotonin (5-HT) binding site in a spinal cord membrane preparation [5]. Over the past decade, the number and outcomes of interactions between receptors have increased continuously [6]. Recent studies have demonstrated close physical interactions where activation of one receptor affects the function of the other.

Adenosine is an endogenous purine nucleoside that has evolved to modulate many physiological processes. Extracellular adenosine mostly originates from release of intracellular adenosine and from release and extracellular breakdown of cAMP and ATP by ecto-5′-nucleotidase and phosphodiesterase [7]. Cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A1Rs, A2ARs, A2BRs, and A3Rs), all of which are seven-transmembrane-spanning GPCRs. Of the four known adenosine receptors, A1Rs and A2ARs are primarily responsible for the central effects of adenosine, especially in modulating synaptic transmission [8]. Adenosine can act on A1Rs to depress transmitter release and neuronal sensitivity to the transmitter [9, 10]. As a result, the A1Rs are important in the regulation of synaptic plasticity, playing a role in determining the amplitude of long-term potentiation or long-term depression [11].

There are numerous reviews that describe regulation of brain adenosine levels, adenosine receptors, their cellular and subcellular localization, signaling pathways, and function in the brain under physiological and pathophysiological conditions as well as selective receptor agonists and antagonists. Using A1Rs as a paradigm of GPCRs, this review focuses on how receptor-receptor interactions contribute to regulatory processes within the central nervous system.

Considering the various types of receptors, one may expect to find three principle paths of receptor interaction: (1) interactions between ionotropic receptors, (2) interactions between a metabotropic receptor and an ionotropic receptor, and (3) interactions between metabotropic receptors. The examples mentioned below stem from the second and third type of interaction. Interactions with metabotropic dopamine receptors as well as A2A, A3, NPY, and P2Y1 receptors will be described in the first part. The second part deals with interactions between A1Rs and ionotropic receptors, especially the GABA A, NMDA, and P2X receptors as well as ATP-sensitive K+ channels. Finally, new approaches for neurological disorders will be discussed.

Functional interactions with metabotropic receptors

Two forms of GPCR classification exist. There is the historical division into three main families: (1) rhodopsin-like family which includes adenosine receptors, (2) secretin-like
family, and (3) metabotropic glutamate receptor-like family. The families share some basic similarities—the seven-transmembrane-spanning domains, intracellularly located C terminus, and extracellularly residing N terminus. Differences between the families arise in the length of the intracellular and extracellular termini and amino acid sequences, disulfide bridge linking, and conserved domains. Five different groups can be classified by applying phylogenetic analyses. The GRAFS system distinguishes between glutamate, rhodopsin, adhesion, fizzled/taste, and secretin-like GPCRs [12].

The agonist binding on the receptor results in coupling to heterotrimeric G proteins and regulates a variety of cell responses. In brief, an exchange of G protein-bound GDP to GTP occurs, and the heterotrimer dissociates into the α subunit and the βγ dimer. The resulting products activate or inhibit effectors independently from each other. Currently, 16 different genes encode G protein α subunits, five genes encode β subunits, while 14 genes encode χ subunits [13]. The α subunits can be categorized into four basic groups: the stimulatory G
αi family couples to adenylate cyclase and increases cAMP levels, whereas the inhibitory Gαo/i0 family acts in the opposite way. Moreover, the Gα11/12 family activates the phospholipase Cβ (PLCβ), and lastly, the Gγ12/13 family which regulates Rho proteins. Gβδ dimers are capable of triggering effects on inward rectifier K+ channels (GIRK1-4), voltage-dependent Ca2+ channels (VDCC), and phospholipase A2 (PLA2), PLCγ, and the Na+/H+ exchanger (NHE1). Thus, it is not surprising that GPCRs are such interesting candidates in current drug research with their amazing potential in affecting signaling events. A single GPCR possesses the potential to activate more than just one signaling pathway [12]; for example, activation of A1Rs includes coupling to Gαi/o and increasing of IP3 level [14, 15]. Furthermore, homodimerization and heterodimerization are common paths of interaction and have been described in A1Rs and the A2Rs several times [16–18]. In addition, functional interactions on the A1R without receptor assembling have already been revealed [19, 20], or are currently being elucidated. The next paragraphs will deal with a few selected examples of this limitless wide topic.

Relationship between A1Rs and A3Rs

The A3R was the latest receptor subtype of the adenosine receptor family to be identified [21], and its functional role is still controversially discussed. Several findings indicate neuroprotective as well as neurotoxic action depending on experimental approach [22–29]. A1Rs couple to inhibition of adenyl cyclase as well as to activation of PLC, and to elevation of inositol triphosphate levels [30, 31]. Furthermore, an increase in intracellular Ca2+ levels due to release from intracellular stores and Ca2+ influx has been described [32, 33]. One interesting example of A1Rs’ functional role is their involvement in acute neurotoxic situations and interplay with A1Rs. Dunwiddie et al. [34] reported on the potential of A2Rs to modify responses via A1Rs in the hippocampus. The activation of hippocampal A2Rs induced a desensitization of A1Rs on combined superfusion of Cl-IB-MECA and adenosine. This phenomenon was thought to reduce the protective effects of endogenous adenosine caused by the lack of sensitivity of A1Rs. Further investigations on pyramidal cells of the rat cingulate cortex did not confirm Dunwiddie et al.’s assumption [35]. In this brain area, A1Rs and A2Rs did not show any interaction. The receptor subtypes were unable to affect each other. The discrepancy was taken to be a genetic phenomenon, such as alternative splicing of the rat A3R transcript causing distinguished pharmacological and functional properties in the brain. Furthermore, Hentschel et al. [36] demonstrated the involvement of A2Rs in inhibition of excitatory neurotransmission during hypoxic conditions, indicating a neuroprotective action of endogenously released adenosine on A2Rs in addition to A1Rs. Lastly, Lopes et al. [37] attempted to define the possible role of A1Rs in the rat hippocampus using experiments similar to those of Dunwiddie et al. in non-stressful and stressful situations, with particular attention to whether A3Rs control A1Rs. These data suggested that no interaction between the two receptor subtypes exist, but confirm that A2Rs do not affect synaptic transmission on superfusion with A1R agonist CI-IB-MECA or A3R antagonist MRS 1191. The authors pointed out that CI-IB-MECA binds to A1Rs even at low nanomolar concentrations. Thus, the existence of an interaction between A1Rs and A3Rs has to wait for reliable ligands.

Antagonistic interaction between A1Rs and A2ARs

A2ARs are widely distributed in the CNS, but local and subcellular differences in allocation exist. They show high levels in all subregions of the striatum and in the globus pallidus. A2ARs are also expressed in neurons in the neocortex and limbic cortex, but at a density a twentieth of that found in basal ganglia [38]. Colocalization of A1Rs and A2ARs was approved for glutamatergic nerve terminals in the hippocampus [39]. In the striatum, A1R/A2AR heteromers were found on synapses with spines of medium spiny neurons and integrated in the presynaptic membrane of glutamatergic terminals that represent the cortical-limbic-thalamic input [18]. A1Rs and A2ARs modulate excitatory synaptic transmission, albeit in an opposite manner. A1R activation inhibited glutamatergic synaptic transmission mainly through presynaptic inhibition of glutamate release, while A2ARs have been shown to facilitate glutamatergic synaptic transmission [40–42]. At first sight, stimulating
Adenosine receptors (A1Rs) and inhibiting A2A receptors (A2A Rs) may have a neuroprotective influence on the mature CNS. However, problems arise due to long-term desensitization of A1Rs. A2A Rs do not up-regulate after antagonist administration, but have a low abundance in hippocampal and cortical areas compared with A1Rs [40, 43, 44]. A1Rs and A2A Rs cannot be regarded in isolation from one another since cross talk between the subtypes has been described several times [16, 17, 45–47]. A2A R activation by agonists caused A1 R desensitization resulting in decreased binding affinity for CPA in the hippocampus in young adult rats. Controlling A1Rs by A2A Rs was mediated by protein kinase C in a cAMP-independent manner. A2A R activation was seen to play a role in fine-tuning A1Rs by attenuating the tonic effect of presynaptic A1Rs located on glutamatergic nerve terminals [46, 47]. In the striatal system, A1R/A2AR heteromers became prominent to show an antagonistic reciprocal interaction [18]. As in the hippocampus, A2A R stimulation decreased the affinity of A1Rs for agonists. The A1R/A2AR heteromer allows adenosine to perform a detailed modulation of glutamate release [16, 48]. Regarding A1Rs and A2A Rs, basal conditions generate a low tone of endogenous adenosine and cause A1R activation, in contrast to situations of increased adenosine where A2A R activation becomes dominant. When adenosine concentrations rise, as during anoxia, likely also time appears to be important in regulating A2A receptor activity, which means A2A receptors are “active” under prolonged stimulation [49]. Finally, activation of the A1R/A2AR heteromer contributes to A2A R signaling when adenosine levels are elevated and may provide a mechanism to facilitate plastic changes in the excitatory synapse [18].

Interactions between adenosine and dopaminergic system

Dopamine is an important transmitter in basal ganglia and is noted for influencing motor activity, playing an important role in Parkinson’s disease. Adenosine-dopamine interactions are complex and cannot be limited on functional considerations of A1Rs. Intramembrane heteromeric receptor-receptor interactions and the involvement of A2A Rs in influencing dopaminergic signaling have to be mentioned due to the implications in the treatment of Parkinson’s disease. Ginés et al. [50] described the formation of functionally interacting heteromeric complexes between dopamine D1 receptors (D1 Rs) and A1Rs in mouse fibroblast Ltk− cells cotransfected with respective cDNAs. Coaggregation occurred when cells were pretreated with R-PIA as A1 R agonist, but was decreased by combined pretreatment with R-PIA and SKF-38393, a D1R agonist. Furthermore, the D1R agonist-induced cAMP accumulation was reduced by combined pretreatment of D1R- and A1R agonist, but remained unaffected when given alone, respectively. The results confirmed an antagonistic interaction between A1Rs and D1Rs that had already been observed by Ferré et al. [51] in behavioral studies using reserpinized mice and rabbits. In vivo and in vitro data on adenosine-dopamine interactions were mostly obtained from investigations in the basal ganglia and limbic regions [52, 53] due to the high abundance of A1Rs, A2 ARs, D1Rs, and D2Rs in these areas and their involvement in the pathology of Parkinson’s disease. The antagonistic interaction of combined receptor activation seems to distinguish between adenosine and dopamine receptor subtypes. While A1Rs communicate mainly with the D1R subtype in strionigral-striopallidopontuncular neurons, A2AR and D2R interaction occurs in striopallidal neurons. Studies on mice and monkeys pretreated with MPTP suggest that some degree of dopaminergic activity is needed to obtain adenosine antagonistic-induced motor activity. Furthermore, blockade of dopaminergic neurotransmission counteracts the antagonistic effect induced by adenosine [54]. Sufficient endogenous adenosine is present interstitially in the substantia nigra pars reticulata to control dopaminergic effects. The effects of adenosine are absent when dopaminergic influence is suppressed [53]. Thus, it seems that monotherapy with A2A R antagonists may only be useful in the early stages of Parkinson’s disease, but could support a therapeutic treatment with dopamine agonists in advanced stages. A promising approach using these therapeutic strategies can be seen in istradefylline, an A2AR antagonist that has since successfully passed clinical trials [55]. However, A1R blockade may also contribute to an increased dopamine release but this effect seems without clinical relevance.

Relationship between A1Rs and NPY

Neuropeptide Y (NPY) is one of the most abundant neuropeptides and exerts various functions on at least six GPCR subtypes (Y1Rs-Y5Rs, Y6Rs). Immunohistochemical investigations revealed the appearance of the Y1R and Y3R subtypes in the rat frontal cortex [56-58]. Activation of NPY receptors results in an inhibition of excitatory synaptic transmission, while a presynaptic influence on cortical neurons has been postulated [59]. NPY receptors affect pertussis toxin-sensitive G proteins, which inhibit adenyl cyclase and decrease cAMP levels. Inhibitory and facilitating effects on K+ and Ca2+ mobilization have also been observed [60]. Receptor-receptor interactions between Y1Rs have already been described, such as the antagonistic interaction with galanin receptors in the hypothalamus of the rat and their functional relevance for food intake. In contrast, a facilitatory interaction between the two receptors exists in the amygdala which may be of relevance for fear-related behavior [61].

In the CNS, A1Rs and NPY receptors share some similarities in distribution. Both A1Rs and Y1Rs are located...
on neurons of the prefrontal cortex, and their activation inhibits glutamatergic neurotransmission [62]. This is evidence for potential interaction between A1Rs and Y1Rs that may modulate long-term desensitization of A1Rs during pathophysiological situations. To investigate possible functional interactions, postsynaptic potentials (PSPs) were generated by electrical field stimulation on pyramidal neurons of layer V in the rat cingulate cortex as described by Brand et al. [35] and Hentschel et al. [36]. The Y1R agonist [F7,P34]pNPY inhibited the amplitude of PSPs. The inhibitory effect was reversible and reproducible, indicating that no desensitization appeared (Fig. 1a). An additional decrease in PSP amplitude was observed when NPY was superfused in combination with the A1R agonist CPA (Fig. 1b).

The additional inhibition induced by CPA was in the same range as that found with CPA alone (48.1±5% vs 55±3%). NPY inhibited PSPs after blockading CPA-mediated inhibitory effects by DPCPX. No significant changes existed before and after blockading A1Rs (Fig. 2). The results suggest that no interaction between A1Rs and Y1Rs exist. Each neuromodulator contributes to inhibitory regulation of excitatory neurotransmission. Regarding the desensitization of A1Rs but not of Y1Rs, this may be important under pathophysiological conditions with increased adenosine concentration in the synaptic cleft.

Interaction of A1Rs and P2Y1Rs

P2Y1Rs have been cloned and characterized in several species including human and rat, whereas mRNA was detected in various regions of the brain. The receptor subtype can be activated by ATP, but ADP as a degradation product of ATP is a more potent endogenous agonist. Cellular signaling differs between A1Rs and P2Y1Rs since A1Rs couple to Gi/o and P2Y1Rs to Gq/11. In fact, P2Y1Rs can be assumed to exert stimulatory effects in cells. P2Y1R signaling occurs in non-neuronal and non-muscular cell types, as well as on neurons in the CNS [63] where a colocalization of A1Rs and P2Y1Rs was demonstrated immunohistochemically in rat brain cortex, hippocampus,
and cerebellum [64]. In 1996, Ikeuchi et al. reported the activation of an undefined P2YR by adenosine in patch clamp and calcium imaging experiments on hippocampal neurons [65]. Furthermore, extensive heteromerization experiments have been conducted on cotransfected HEK293 cells using immunoprecipitation, Western blotting, and bioluminescence resonance energy transfer (BRET). Receptor binding experiments in combination with cAMP assays have also been described [64, 66–70]. These respective studies confirmed heteromerization associated with changes in the agonist binding and signaling compared to monomer properties. The binding for selective A1R agonists was decreased while the A1R antagonist binding remained unaffected. Interestingly, ADP binding was blocked by DPCPX but not by the P2Y1R antagonist, suggesting an altered binding pocket on the A1R/P2Y1R complex. The G protein-coupling was sensitive to pertussis toxin and revealed a G_{i,0} status for the heteromer. Although colocalization of A1Rs and P2Y1Rs in several brain areas has been demonstrated, there is still a lack of functional investigations. Nevertheless, the physiological relevance of this interaction has been postulated as follows: costorage and release of ATP with neurotransmitters, such as glutamate or noradrenaline, occurs in the CNS [71–73]. ADP, the degradation product of ATP, acts as an A1R agonist due to the activation of the A1R/P2Y1R complex and contributes to the inhibitory modulation of excitatory synaptic transmission via adenosine acting on A1Rs. This interaction can be assumed as an additional mechanism for influencing and fine-tuning synaptic neurotransmission.

### Functional interaction with ionotropic receptors

Neuronal excitability is regulated by voltage and ligand-gated ion channels. Ionotropic receptors also referred to as ligand-gated ion channels (LGICs) are a group of intrinsic transmembrane ion channels that open and close in response to binding of a chemical messenger, as opposed to voltage-gated ion channels or stretch-activated ion channels. Ion channels are regulated by a ligand and are usually very selective to one or more ions such as Na^+, K^+, Ca^{2+}, or Cl^−. These receptors located at synapses convert the chemical signal of presynaptically released neurotransmitter directly and very quickly into a postsynaptic electrical signal. Many LGICs are additionally modulated by allosteric ligands by channel blockers, ions, or membrane potential.

Nicotinic acetylcholine receptor serves as the prototypical LGIC [74] and consists of a pentamer of protein subunits with two binding sites, which, when bound, alter the receptor configuration and cause an internal pore to open. This pore, permeable to Na^+ ions, allows them to flow down their electrochemical gradient into the cell. With a sufficient number of channels opening at once, the intracellular Na^+ concentration rises to the point at which the positive charge within the cell is sufficient to depolarize the membrane, and an action potential is initiated [75]. Many important ion channels are ligand-gated, and they show a great degree of homology at the genetic level. The LGICs are classified into three superfamilies; the first—the Cys-loop receptor family—is subdivided into the anionic GABA_\alpha_ and glycine receptors on the one hand, and cationic 5-HT_3 serotonin and nicotinic acetylcholine receptors on the other. The second group—ionotropic glutamate receptors—consists of NMDA, kainate, and AMPA receptors. The third group covers the ATP-gated channels—the P2X receptors [76].

Adenosine is known to inhibit glutamatergic neurotransmission by activation of presynaptic A1Rs [35]. This is probably due to reduction of the calcium influx, possibly by modulating both P/Q- and N-type presynaptic voltage-dependent calcium channels, which in turn controls transmitter release [77]. Furthermore, A1Rs have long been known to mediate neuroprotection by reduction of excitatory effects at the postsynaptic level [10, 78, 79]. In addition to its direct presynaptic and postsynaptic actions on neurons, A1R interaction with NMDA [80–82, 84], GABA_\alpha_ [85–88], and P2X receptors [80, 89–91] contributes to fine-tuning neuromodulation via adenosine.

Interaction between A1Rs and NMDA receptors

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. In most brain areas, glutamate mediates fast synaptic transmission by activating ionotropic receptors of the AMPA, kainate, and NMDA type. Additionally, NMDA receptors play a critical role in synaptic plasticity, synaptic development, and neurotoxicity. Recent studies suggest that some NMDA-mediated actions are altered or mediated by adenosine. Synaptic currents mediated by glutamate in rat substantia nigra pas reticulata neurons were reduced by adenosine acting via A1Rs. The inhibitory action was not mediated by a postsynaptic site since adenosine did not block currents evoked by local application of glutamate [86].

NMDA is known to increase the extracellular level of adenosine via bidirectional adenosine transporters or from released adenosine nucleotides degraded by a chain of ectonucleotidases [92, 93]. On the other hand, endogenous adenosine present in the extracellular fluid of hippocampal slices tonically inhibits NMDA receptor-mediated dendritic spikes as well as AMPA/kainate receptor-mediated synchronized EPSPs by activation of A1Rs in CA1 pyramidal cells [81]. In line with these results, it has been shown that the tonic activation of A1Rs by ambient adenosine depressed field potentials in the striatum. The effect of adenosine in
the striatum [84] or hippocampus [94] has not been found in A1R knockout mice and clearly demonstrates the involvement of A1Rs. The involvement of A1Rs was also supported by experiments using the selective receptor ligand 2-CA. In isolated rat hippocampal pyramidal cells [95] and in bipolar cells of the retina [96], 2-CA decreased inward currents induced by iontophoretic application of NMDA.

Another interesting interaction concerns NMDA preconditioning to protect against glutamate neurotoxicity. The A1R antagonist 8-CPT has been shown to prevent neuroprotection evoked by NMDA preconditioning against glutamate-induced cellular damage in cerebellar granule cells [83]. In this study, the functionality of A1Rs was not affected by NMDA preconditioning, but this treatment promoted A2AR desensitization in concert with A1R activation [83]. These results are in line with other studies indicating that adenosine downregulates excitatory and inhibitory synaptic transmission in several brain areas through activation of A1Rs and A2ARs [97, 98]. Furthermore, activation of A1Rs mediates reversal of long-term potentiation (LTP) produced by brief application of NMDA in hippocampal CA1 neurons [99].
Taken together, there are several ways in which adenosine may interact with NMDA-induced cellular events. Adenosine can affect glutamatergic transmission via both presynaptic and postsynaptic mechanisms by activating A1Rs [78]. NMDA receptors and A1Rs interact to downregulate glutamate release presynaptically in pyramidal cells of the cingulate cortex [35], neurons of the hippocampus [100], and striatal neurons [84]. Another putative mechanism is related to postsynaptic A1Rs; adenosine elevates the threshold to open NMDA receptor-operated channels by antagonizing membrane depolarization [101]. Interaction between A1Rs and GABA<sub>A</sub> receptors

Fast synaptic inhibition in the brain and spinal cord is largely mediated by GABA<sub>A</sub> receptors that are also targeted by drugs such as benzodiazepines, barbiturates, neurosteroids, and some anesthetics. The modulation of their function will have important consequences for neuronal excitation [102]. One accepted means of modifying the efficacy is a functional interaction with adenosine. Adenosine may have an effect on either presynaptic GABA release in interneurons and/or on postsynaptic GABA<sub>A</sub> receptors in projection neurons. The site of action may be studied electrophysiologically by inducing fast inhibitory postsynaptic potentials (IPSPs) or application of GABA directly onto the cell. Adenosine and selective A1R agonist CHA reduced the amplitude of the fast IPSP in lateral amygdala slice preparations. The effect of CHA was blocked by DPCPX, indicating the involvement of A1Rs. Additionally, adenosine did not block currents evoked by local application of GABA [85]. Thus, the modulatory effect of adenosine on the GABAergic neurotransmission appears to take place on a presynaptic site by inhibiting GABA release from nerve terminals [85, 86]. The assumption that the activation of A1Rs can presynaptically modulate inhibitory postsynaptic responses agrees with findings in several brain areas, such as the thalamus [87], suprachiasmatic and arcuate nucleus [88], and substantia nigra pars compacta [86].

There is some evidence that activation of A1Rs is also involved in GABA<sub>A</sub> receptor downregulation, implying a facilitation of the neurotransmission on a postsynaptic site. GABA but not adenosine evoked an inward current in rat sacral dorsal commissural neurons (SDCN). The GABA-induced current was significantly reduced by adenosine. CHA and DPCPX, but not selective ligands for A2A Rs, mimicked or blocked the inhibitory effect of adenosine, respectively [103]. Adenosine and muscimol induced a concentration-dependent reduction in the amplitude of population potentials in hippocampal slices. Additionally, adenosine potentiated the ability of muscimol to inhibit evoked potentials, which were blocked by the A1R-selective antagonist 8-CPT. The effects of adenosine as well as muscimol were reduced by the chloride channel blocker DIDS, indicating the ability of adenosine to regulate the GABA<sub>A</sub> chloride channel by activation of A1Rs [104].

Sebastiao’s group studied the mechanisms by which GABA modulates adenosine-mediated effects and found that endogenous GABA exerts an inhibitory effect through GABA<sub>A</sub> receptors via a predominant adenosine-mediated action in the hippocampus. The authors concluded that there is an A1R-mediated ability to inhibit synaptic transmission [19]. Further, this study showed that the blockade of GABAergic inhibition induced the release of NO, which was able to potentiate the inhibitory action of adenosine. They therefore suggested that the modulation of the A1R-mediated response by activation of GABA<sub>A</sub> receptors occurs indirectly via NO [19].

Activation of GABA<sub>A</sub> receptors is effective in limiting neuronal ischemic damage [105] and endogenous adenosine that arises during hypoxia, and acts neuroprotectively partly by activating A1Rs [36]. Therefore, the contribution and potential interactions of GABA and adenosine as modulators of synaptic transmission during hypoxia has been investigated. Activation of A1Rs inhibits the release of GABA from the ischemic cerebral cortex in vivo [106]. In contrast, the administration of an A1R agonist in the hippocampus failed to affect the release of GABA during ischemia [107]. In the light of these controversial results,
the role of the two neuromodulators during hypoxia was investigated in the CA1 area of rat hippocampal slices using selective A1R antagonists [108]. Indeed, activation of A1R and GABA_A receptors is partly involved in the inhibition of synaptic transmission during hypoxia. The action of GABA becomes evident when A1Rs are blocked. Regarding the desensitization of A1Rs during hypoxia [109, 110], it may be assumed that GABA_A-mediated inhibition of the synaptic transmission is evident when the A1R is desensitized or downregulated [108].

Comodulation by A1Rs and GABA_A receptors was also suggested in acute cerebellar ethanol-induced ataxia. Using GABA_A and A1R agonists and antagonists, respectively, a functional similarity between GABA_A receptors and A1Rs has been shown even though both receptor types are known to couple to different signaling systems [111]. This provides conclusive evidence that A1Rs and GABA_A receptors both play a comodulatory role in ethanol-induced cerebellar ataxia without any direct interaction.

Functional interaction between A1Rs and P2X receptors

P2X receptors are ligand-gated ion channel receptors; seven subunits (P2X1-P2X7) have been identified [63]. The P2X receptor subunits show many differences in localization, pharmacology, kinetics, and signaling pathways [112, 113]. The P2X1 to P2X5 receptors have 379–472 amino acids, with a predicted tertiary structure of transmembrane segments, a large extracellular loop and intracellular C and N termini. The P2X2, P2X4, and P2X4/P2X6 receptors appear to be the predominant neuronal types [91]. These subunits may occur as homooligomers or as heterooligomeric assemblies of more than one subunit. The P2X7 receptor has a similar structure, but with a much larger intracellular C terminus. This contrasts strikingly with any of the other known ligand-gated ionotropic receptors [114]. P2X7 subunits do not form heterooligomeric assemblies, but are involved in mediating apoptosis and necrosis in glial cells and possibly neurons.

Interaction between adenosine receptor-mediated and P2 receptor-mediated effects have been shown to occur in neuronal and non-neuronal cells [80]. Both adenosine and ATP induce astroglial cell proliferation and formation of reactive astrocytes [89]. In hippocampus, adenosine and ATP are released on stimulation and are potent neuronal transmission inhibitors [115, 116]. It should be pointed out that the interpretation of effects induced by both is difficult since ATP is degraded enzymatically to adenosine [38]. Adenosine is formed by extracellular catabolism of released ATP via the ectonucleotidase pathway [90, 117]. The role of the ectonucleotidases in forming adenosine is difficult to study since this system is extremely efficient, and it is difficult to block an enzyme system. The experimental paradigm used by Cunha et al. [118] demonstrates that ATP has to be converted outside the cell into adenosine to exert its inhibitory effects on hippocampal synaptic transmission. The inhibitory effect of ATP was not modified by the P2 receptor antagonist suramin, but was attenuated by the ecto-5′-nucleotidase inhibitor and was nearly prevented by the adenosine A1R antagonist DPCPX, whereas dipyridamole, an inhibitor of adenosine uptake, potentiated the inhibitory effect of ATP [118]. These results offer evidence for localized catabolism of adenine nucleotides followed by substrate channeling to A1Rs. This localized catabolism may mask the adenosine-mediated ATP effect [119]. Recently it was demonstrated that the exogenous application of ATP or ATP X5S reduced the hippocampal neurotransmission. The inhibitory effect was blocked by the selective A1R antagonist DPCPX and was potentiated by different ecto-ATPase inhibitors [120]. These results suggest that the synaptic inhibition may consist of an inhibitory purinergic component of ATP itself in addition to degradation to adenosine.

Interaction with neuronal ATP-sensitive K⁺ channels

ATP-sensitive K⁺ channels (K_ATP) are widely expressed in the cytoplasmic membrane of neurons and couple cell metabolism to excitability [121]. These channels are regulated by the intracellular ATP/ADP ratio [122] and modulated by many endogenous mediators, including adenosine, via A1Rs. Activation of A1Rs inhibited the activity of inspiratory neurons in the brainstem by opening K_ATP in neonatal mice [123]. A1R stimulation promotes K_ATP activity in principal dopamine neurons in the substantia nigra pars compacta [124] and hippocampus [125]. In contrast, one recent study has demonstrated that adenosine induces internalization of K_ATP, resulting in a decrease in K_ATP-mediated response in the hippocampus [126]. The discrepancy might be due to the additional activation of A2A Rs by adenosine located in hippocampus, but not in the substantia nigra pars compacta [127]. In addition to the inhibitory effect on the presynaptic site, the activation of A1Rs acts as an inhibitory modulator to electrical activity on the postsynaptic site, and this effect has been attributed to enhancement of K_ATP activity. The modulating effect on the membrane potential may differ depending on the brain regions, as neuronal K_ATP is heterogeneous in different neurons.

A1R interactions—new approaches for neurological disorders

By activation of its receptors, adenosine regulates many pathophysiological processes, particularly in excitable
tissues of the brain (Fig. 3). Its widespread functions in the body include regulation of seizure susceptibility [128, 129], neuroprotection [40], regulation of pain perception [130], sleep induction [131], and involvement in Parkinson’s disease [132]. There is increasing evidence that the functional interaction of A1Rs with other neuronal receptors may contribute to fine tuning in synaptic transmission, and A1R agonists may represent a useful therapeutic approach for the treatment of some neurological disorders by regulating homeostasis in transmitter systems. However, the use of A1R agonists has not proved clinically useful due to mainly cardiovascular side effects as well as low brain permeability. Pioneering experimental approaches have been evaluated using focal drug delivery in epilepsy models. One experimental study has used intraventricular implantation of an adenosine-releasing synthetic polymer [128]. In a later study, Guttinger et al. [133] used encapsulated C2C12 myoblasts that were engineered to release adenosine by disruption of their adenosine kinase gene [133]. The local delivery of adenosine by implanted cells appears to be a promising strategy for the control not only for affecting seizure activity but also other neurodegenerative diseases with dysregulated synaptic neurotransmission.

Concluding remarks

As a consequence of its ubiquitous distribution and because of its linkage to the energy pool, adenosine has evolved as an important messenger in extracellular signaling. Modifications in extracellular adenosine levels with subsequent alterations in the activation of its receptors interferes with the action of other receptor systems. Figure 4 summarizes possible interactions of A1Rs with metabotropic receptors, Fig. 5 shows the interactions between A1Rs and ionotropic receptors as well as with KATP, and Fig. 3 shows the neurological disorders where A1R interactions may play a role.

There is evidence that various regulatory mechanisms exist as well as multiple mechanisms that act independently of each other on the same cell depending on the brain region and cell type. The overaction and redundancy principle ensures transmitter homeostasis under pathophysiological conditions in a special time window. The function of adenosine receptors in the regulation of the synaptic transmission is complex.

The key receptor in regulation of the neuronal transmission may be the A2AR, whereas the interaction of the A1R with metabotropic and ionotropic receptors as well as with KATP serves as fine-tuning to inhibit synaptic transmission, as mentioned by Sebastiao and Ribeiro [80]. A1Rs may play a nonessential role in normal physiology as demonstrated in mice lacking the A1Rs [134]. However, they play an important protective role under pathophysiological conditions especially during hypoxia. The activation initiates a fast inhibition of the glutamatergic neurotransmission and the receptor interactions may contribute to its maintenance or can support the A1R-mediated effects.

Most of our knowledge on receptor-receptor interactions involving the A1R results from experiments on cell cultures, slice preparation or, to a lesser extent, from in vivo experiments where regulation can be studied in principle or new drug targets can be characterized. These findings may contribute to a better understanding of disturbances in transmitter homeostasis. As our understanding of the complexity of receptor signaling and interaction develops, we may well gain new perspectives in new drug development. The clinical relevance of the testing models has often been questioned, however. Discordance between studies on cells and animal and human studies may be due to bias or failure of models to mimic clinical disease to an adequate degree. There are new techniques such as neuroimaging, nanotechnology, siPCR, and new selective receptor ligands that will help to overcome some of these aspects in the near future.

Acknowledgement The authors thank Dr. Annette G. Beck-Sickinger, University of Leipzig for the generous gift of the Y1 receptor agonist [F7, P34]pNPY.
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