# Adenosine Receptors and the Central Nervous System

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## Contents



**Abstract** The adenosine receptors (ARs) in the nervous system act as a kind of "go-between" to regulate the release of neurotransmitters (this includes all known neurotransmitters) and the action of neuromodulators (e.g., neuropeptides, neurotrophic factors). Receptor–receptor interactions and AR–transporter interplay

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occur as part of the adenosine's attempt to control synaptic transmission. $A_{2A}$ARs are more abundant in the striatum and $A_1$ARs in the hippocampus, but both receptors interfere with the efficiency and plasticity-regulated synaptic transmission in most brain areas. The omnipresence of adenosine and $A_{2A}$ and $A_1$ ARs in all nervous system cells (neurons and glia), together with the intensive release of adenosine following insults, makes adenosine a kind of "maestro" of the tripartite synapse in the homeostatic coordination of the brain function. Under physiological conditions, both $A_{2A}$ and $A_1$ ARs play an important role in sleep and arousal, cognition, memory and learning, whereas under pathological conditions (e.g., Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, stroke, epilepsy, drug addiction, pain, schizophrenia, depression), ARs operate a time/circumstance window where in some circumstances $A_1$AR agonists may predominate as early neuroprotectors, and in other circumstances $A_{2A}$AR antagonists may alter the outcomes of some of the pathological deficiencies. In some circumstances, and depending on the therapeutic window, the use of $A_{2A}$AR agonists may be initially beneficial; however, at later time points, the use of $A_{2A}$AR antagonists proved beneficial in several pathologies. Since selective ligands for $A_1$ and $A_{2A}$ ARs are now entering clinical trials, the time has come to determine the role of these receptors in neurological and psychiatric diseases and identify therapies that will alter the outcomes of these diseases, therefore providing a hopeful future for the patients who suffer from these diseases.

**Keywords** Adenosine receptors · A1 adenosine receptor · A2A adenosine receptor · Central nervous system · Receptor cross-talk · G protein coupled receptors · Neurotrophic factor receptors · Ionotropic receptors · Receptor dimmers · Caffeine · Drug addiction · Neurodegenerative diseases · Pain · Ischemia · Hypoxia · Adenosine levels

## Abbreviations

| | |
|---|---|
| AC | Adenylate cyclase |
| ACh | Acetylcholine |
| ADO | Adenosine |
| ADP | Adenosine 5′-diphosphate |
| AK | Adenosine kinase |
| AMP | Adenosine 5′-monophosphate |
| AMPA | α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| AOPCP | α, β-Methylene ADP |
| Ap5A | Diadenosine pentaphosphate |
| AR | Adenosine receptor |
| ATP | Adenosine 5′-triphosphate |
| BDNF | Brain-derived neurotrophic factor |
| BRET | Bioluminescence resonance energy transfer |
| CamK | Calmodulin-dependent kinase |

| | |
|---|---|
| cAMP | Cyclic adenosine 5′-monophosphate |
| CB | Cannabinoid |
| CGRP | Calcitonin gene-related peptide |
| DA | Dopamine |
| DARPP | Dopamine- and cAMP-regulated phosphoprotein |
| DPCPX | 1,3-Dipropyl-8-cyclopentylxanthine |
| ENT | Equilibrative nucleoside transporter |
| ERK | Extracellular signal-regulated kinase |
| GABA | γ-Aminobutyric acid |
| GAT | GABA transporter |
| GLU | Glutamate |
| GDNF | Glial cell line-derived neurotrophic factor |
| GPCRs | G-protein-coupled receptors |
| HEK cells | Human embryonic kidney cells |
| HFS | High-frequency stimulation |
| IL-6 | Interleukin 6 |
| IP3 | Inositol triphosphate |
| i.v. | Intravenous |
| KO | Knockout |
| LFS | Low-frequency stimulation |
| LTD | Long-term depression |
| LTP | Long-term potentiation |
| MAPK | Mitogen-activated protein kinase |
| mGluR | Metabotropic glutamate receptor (mGlu1–8 refer to mGluR subtypes) |
| NAc | Nucleus accumbens |
| nAChR | Nicotinic acetylcholine receptor |
| NBTI | Nitrobenzylthioinosine |
| NGF | Nerve growth factor |
| NMDA | *N*-Methyl-D-aspartate |
| NT | Neurotransmitter |
| NTR | Neurotransmitter receptor |
| NPY | Neuropeptide Y |
| NR | NMDA receptor subunit |
| NT-3 | Neurotrophin 3 |
| NTDase | Ecto-5′-nucleotidase |
| NTPDase | Ectonucleoside triphosphate diphosphohydrolase |
| PDE | Phosphodiesterase |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PLC | Phospholipase C |
| PTX | Pertussis toxin |
| REM | Rapid eye movement |
| Trk receptors | Tropomyosin-related kinase receptors |
| VIP | Vasoactive intestinal peptide |

## 1 Introduction

Before we go into the scope of this review, we would like to stress that we are starting with some areas of high general complexity: (a) the *nervous system*, the most complex biological system in the human body; (b) *adenosine*, which is ubiquitously present in all cells, with receptors distributed throughout all brain areas; any imbalance of such a widespread system is expected to lead to neurological diseases; (c) *caffeine*, an antagonist of all subtypes of ARs and the most widely consumed psychostimulant drug; moreover, chronic or acute intake of caffeine may affect ARs in different and even opposite ways; (d) finally, ARs or adenosine-related molecules are potential therapeutic targets for neurologic diseases, but this role can be *multifactorial*, with different receptors involved, different time windows of action, age-related changes, etc.

Many publications are now appearing that are devoted to research in animal models or humans that is directed towards many nervous system pathologies and towards novel therapeutic approaches based on adenosine and ARs. We have therefore chosen to focus the present review on the insights gained from recent studies related to the subtle way that ARs regulate other receptors and transporters for neurotransmitters and neuromodulators, and on the pathophysiological implications of this regulation. We believe that further advances in the therapeutic potential of adenosine-related drugs require a deeper understanding of the above mentioned complexities in the context of fine-tuning modulation by adenosine. This might be accomplished at the receptor–receptor level or through several receptors in sequence and/or in parallel and/or via the transducing system cascade. What occurs inside the cells with the transducing system's variability and crosstalk may also occur at the extracellular membrane level via receptor–receptor interaction and formation of heteromers.

The AR field in neuroscience started with an apparent paradox reported by Sattin and Rall (1970): the ability of adenosine to increase cyclic adenosine 5′-monophosphate (cAMP) in the brain was prevented by theophylline, which at the time was known only as a phosphodiesterase (PDE) inhibitor. This was the starting point for the hypothesis that adenosine was acting through a membrane receptor antagonized by theophylline, but several years elapsed before the birth of the first nomenclature of purinergic receptors, proposed by Burnstock (1976). The field for the identification of different subtypes of ARs was then opened, and a further breakthrough was attained by the end of the 1970s by van Calker et al. (1979), who first proposed ARs in brain cells as $A_1$ (inhibitory) and $A_2$ (stimulatory). At that time, as for most receptors, the AR classification relied upon pharmacological criteria and transducing pathways and the ability to stimulate or inhibit adenylate cyclase. AR cloning possibilities had to wait until the beginning of the 1990s. The first AR to be cloned was the $A_1$AR from brain tissue (Mahan et al. 1991). All four G-protein-coupled ARs ($A_1$, $A_{2A}$ and $A_{2B}$, and $A_3$) have been cloned. Major advances have been made in the pharmacological tools available for all of them, as reviewed in great detail in the first five chapters of this book.

Animal research in the last two to three decades firmly established that ARs are involved in several pathophysiological conditions, and that manipulation of their degree of activation might prove therapeutically useful. Therefore, the time is now ripe for studies in humans. In fact, the number of adenosine-related research reports in humans is increasing. From the summary in Table 1, it is clear that the highest incidence of adenosine-related research in humans is related to sleep and Parkinson's disease. This is certainly due to the great advances made in basic research in these fields, which have allowed the clear identification of the role of $A_{2A}$ARs in Parkinson's disease as well as that of adenosine in sleep and epilepsy. The identification of caffeine and theophylline as AR antagonists, together with the empirical knowledge at the time that xanthine-rich beverages such as coffee and tea affect sleep also boosted the interest in adenosine-related research into human sleep. Objective-oriented adenosine-related research in epileptic humans is still scarce, but one retrospective (Miura and Kimura 2000) and one case report study (Bahls et al. 1991) clearly identified an increased risk of seizures in patients taking theophylline as a bronchodilator. Interestingly, and highlighting the frequent gap between basic and clinical research, neither of those two reports mentioned the putative scientific grounds for the increased risk of seizures induced by theophylline: its ability to antagonize ARs. At the time, adenosine had already been recognized as an anticonvulsant, with the pioneering report being published as early as 1984 (Barraco et al. 1984) and the first review highlighting the subject appearing in the 1980s (Chin 1989).

In this review, we will pay particular attention to the implications of AR function in neuropathophysiological conditions, but before we do this, we will briefly provide an overview of the state of the art on how adenosine acts as a neuromodulator, the distribution of ARs in the brain, and their ability to interact with other receptors to harmoniously fine-tune neuronal activity.

## 2 Adenosine as a Ubiquitous Neuromodulator

While ATP may function as a neurotransmitter in some brain areas (Burnstock 2007; Edwards et al. 1992), adenosine is neither stored nor released as a classical neurotransmitter since it does not accumulate in synaptic vesicles, and is released from the cytoplasm into the extracellular space through a nucleoside transporter. The adenosine transporters also mediate adenosine reuptake, the direction of the transport being dependent upon the concentration gradient on both sides of the membrane (Gu et al. 1995). Since it is not exocytotically released, adenosine behaves as an extracellular signaling molecule that influences synaptic transmission without itself being a neurotransmitter. Using G-protein-coupled mechanisms, that not only lead to changes in second-messenger levels but also to the modulation of ion channels (such as calcium and potassium channels), adenosine modulates neuronal activity—presynaptically by inhibiting or facilitating transmitter release, postsynaptically by affecting the actions of other neurotransmitters, and nonsynaptically by

**Table 1** AR research relating to the human central nervous system

| Target | Comment/reference |
|---|---|
| Cognition | Caffeine facilitates information processing and motor output in healthy subjects. Dixit et al. (2006) |
| | Caffeine appears to reduce cognitive decline in women without dementia. Ritchie et al. (2007) |
| Sleep | Decrease in sleep efficiency and of total sleep in healthy subjects by preingestion (16 h) of caffeine. Landolt et al. (1995a, b) |
| | Attenuation of sleep propensity in healthy subjects. Landolt et al. (2004) |
| | Insomnia patients with greater sensitivity to awakening caffeine actions. Salín-Pascual et al. (2006) |
| | Involvement of adenosine in individual variations in sleep deprivation sensitivity. Rétey et al. (2006) |
| | Variations in $A_{2A}$ receptor gene associated with objective and subjective responses to caffeine in relation to sleep. Rétey et al. (2007) |
| | Prolonged wakefulness induces $A_1$ receptor upregulation in cortical and subcortical brain regions. Elmenhorst et al. (2007) |
| Epilepsy | An increased risk of seizures after theophylline or caffeine intake. Bahls et al. (1991), Kaufman and Sachdeo (2003), Miura and Kimura (2000), Mortelmans et al. (2008) |
| | *Increase* (Angelatou et al. 1993) or *decrease* (Glass et al. 1996) in $A_1$ receptor density in different post-mortem brain areas of epileptic subjects |
| Parkinson's disease | Decrease in $A_{2A}$ mRNA levels in post-mortem caudate and putamen, and increase in $A_{2A}$ mRNA in the substantia nigra in Parkinson's disease patients. Hurley et al. (2000) |
| | Polymorphism of $A_{2A}$ receptors did not confer susceptibility to Parkinson's disease in a Chinese population sample. Hong et al. (2005) |
| | Caffeine improved the "total akinesia" type of gait freezing in Parkinson's disease patients. Kitagawa et al. (2007) |
| | Caffeine administered before levodopa may improve its pharmacokinetics in some parkinsonian patients. Deleu et al. (2006) |
| | Significant association between higher caffeine intake and lower incidence of Parkinson's disease. Ross et al. (2000) |
| Alzheimer's disease | Caffeine intake inversely associated with risk of Alzheimer's disease. Maia and de Mendonça (2002), Ritchie et al. (2007) |
| | Co-localization of $A_1$ receptor and β-amyloid or tau and increase in $A_{2A}$ receptor expression in post-mortem cerebral cortex and hippocampus of Alzheimer's disease patients. Angulo et al. (2003) |
| | Upregulation of $A_1$ and $A_{2A}$ receptor expression and function in post-mortem cerebral cortex of Alzheimer's disease patients. Albasanz et al. (2008) |
| Pick's disease | Upregulation of $A_1$ and $A_{2A}$ receptor expression and function in post-mortem frontal lobe of Pick's disease patients. Albasanz et al. (2006, 2007). |
| Pain | Beneficial effects of adenosine (i.v.) in 2 patients with neuropathic pain. Sollevi et al. (1995) |
| | Intrathecal adenosine reduces allodynia in patients with neuropathic pain, but has a side effect of backache. Eisenach et al. (2003) |

(continued)

**Table 1** (continued)

| Target | Comment/reference |
|---|---|
| | Reduction of secondary hyperalgesia by adenosine in human models of cutaneous inflammatory pain. Sjölund et al. (1999) |
| | Local opioid receptor stimulation in the spinal cord of humans induces the release of adenosine. Eisenach et al. (2004) |
| | Theophylline improves esophageal chest pain (a randomized, placebo-controlled study), possibly by altering adenosine-mediated nociception. Rao et al. (2007) |
| Anxiety | $A_{2A}$ receptor gene polymorphism associated with increases in anxiety in healthy volunteers. Alsene et al. (2003) |
| | $A_{2A}$ receptor gene polymorphism associated with increases in anxiety response to amphetamine in healthy volunteers. Hohoff et al. (2005) |
| Panic disorder | Panic disorder patients with increased sensitivity to one cup of coffee. Boulenger et al. (1984) |
| | Anxiogenic and panic-inducing effects of caffeine in a double-blind study. Klein et al. (1991) |
| | Adenosine $A_1$ receptor supersensitivity, a probable compensatory process. DeMet et al. (1989) |
| Panic disorder and anxiety | $A_{2A}$ receptor gene polymorphism associated with anxiety or panic disorder in Occidental but not Asiatic populations. Alsene et al. (2003), Deckert et al. (1998), Hamilton et al. (2004), Lam et al. (2005) |
| Schizophrenia | Increase in $A_{2A}$ receptor density in post-mortem striatum of schizophrenic patients, a consequence of typical antipsychotic medication. Deckert et al. (2003) |
| | Polymorphism of $A_{2A}$ receptors is not related to the pathogenesis of schizophrenia in a Chinese population sample. Hong et al. (2005) |
| Bipolar disorders | Typical, but not atypical, antipsychotics induce an upregulation of $A_{2A}$ receptors assessed in platelets of patients with bipolar disorders. Martini et al. (2006) |
| Dependence behavior | Polymorphism of the $A_{2A}$ receptor gene related to caffeine consumption in healthy volunteers. Cornelis et al. (2007) |
| Ventilation dyspnea and apnea | Adenosine produces hyperventilation and dyspnea in humans resulting from a direct activation of the carotid body. Uematsu et al. (2000), Watt et al. (1987) |
| | Caffeine and theophylline are effective in the treatment of apnea in premature and newborn infants. Aranda and Turmen (1979), Bairam et al. (1987), Uauy et al. (1975) |
| Miscellaneous ($A_2/D_2$ interaction) | $A_2/D_2$ interaction in post-mortem human striatal brain sections. Díaz-Cabiale et al. (2001) |
| | $A_2/D_2$ dimers in human platelets. Martini et al. (2006) |
| Miscellaneous (receptor localization) | Distribution of $A_1$ and $A_{2A}$ receptors in post-mortem human brain. James et al. (1992), Svenningsson et al. (1997) |
| | Mapping $A_{2A}$ receptors in the human brain by PET. Ishiwata et al. (2005), Mishina et al. (2007) |
| | Mapping $A_1$ receptors in the human brain by PET. Elmenhorst et al. (2007), Fukumitsu et al. (2003, 2005) |

hyperpolarizing or depolarizing neurones. Curiously, the discovery of the presynaptic inhibitory action of adenosine, which we now know occurs through $A_1$AR, had the same paradoxical starting point as the identification of theophylline as a putative AR antagonist; that is, adenosine was used at the neuromuscular junction in an attempt to increase cAMP in motor nerve terminals and, in contrast with the expected excitatory effect, adenosine markedly inhibited neurotransmitter release (Ginsborg and Hirst 1971). ATP, released together with acetylcholine (ACh) (Silinsky 1975), mimicked the adenosine effect (Ribeiro and Walker 1973), decreasing both the synchronous and the asynchronous release of acetylcholine, with the maximum effect being about 50%. This presynaptic inhibitory action of ATP results from its extracellular hydrolysis into adenosine (Ribeiro and Sebastião 1987). Interestingly, high-affinity ARs positively coupled to adenylate cyclase do enhance neurotransmitter release, and this was also first identified in cholinergic synapses almost simultaneously in the central nervous system (Brown et al. 1990) and at the cholinergic nerve terminals of the neuromuscular junction (Correia-de-Sá et al. 1991). In the latter case, due to the reduced complexity of the model, it was possible to clearly demonstrate for the first time that both $A_1$ and $A_{2A}$ARs are present at the same nerve terminal (Correia-de-Sá et al. 1991). Adenosine research at cholinergic motor nerve endings to some degree anticipated and inspired the studies at central excitatory glutamatergic synapses, where adenosine decreases both synchronous and asynchronous transmitter release (Lupica et al. 1992; Prince and Stevens 1992).

The past few years have brought new insights into our understanding of the role of the tripartite synapse and gliotransmission in neurological diseases (Halassa et al. 2007). Adenosine and ATP are also among the most relevant players in neuron–glia communication (Fields and Burnstock 2006). ATP has a dual role since it acts upon its own receptors, mostly of the P2Y subtype, which are abundant in astrocytes and are relevant to calcium signaling; ATP is also a substrate of ectonucleotidases leading to adenosine formation, which then operates its own receptors. The adenosine system is critically involved in modulating glial cell functions, namely glycogen metabolism, glutamate transporters, astrogliosis and astrocyte swelling (Daré et al. 2007). ARs on oligodendrocytes regulate white matter development and myelinization (Daré et al. 2007; Fields and Burnstock 2006).

## 3 Manipulation of Endogenous Levels of Adenosine and its Neuromodulation

By using highly selective $A_1$AR antagonists such as 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), it is possible to unmask a tonic $A_1$AR-mediated adenosinergic tonus. A similar strategy can be used towards endogenous $A_{2A}$AR activation, using selective $A_{2A}$AR antagonists such as SCH-58621 or ZM-241385. Simultaneous removal of tonic adenosinergic influences over all receptor types has been achieved through the use of adenosine deaminase, an enzyme used in isolated preparations that does not penetrate cell membranes and that deaminates adenosine into inosine,

usually an inactive ligand for ARs. In studies with this enzyme, an appropriate control with an adenosine deaminase inhibitor together with adenosine deaminase (Sebastião and Ribeiro 1985) will allow actions of the enzyme not related to its enzymatic activity to be determined. The ability of inosine to influence the biological signal in the assay has to be directly tested since inosine can activate ARs, namely those of the $A_3$AR subtype, in some circumstances (Jin et al. 1997). Whenever aiming to evaluate endogenous adenosine actions in a mirror-like way (i.e., by using selective AR antagonists or extracellular adenosine removal), one must also bear in mind that irreversible AR-mediated actions will not be apparent. In these cases, further receptor activation by exogenously added ligands may be the only way to demonstrate a role for adenosine, providing that the system is not already fully saturated with endogenous adenosine actions.

Inhibition of the cascade of ectoenzymes that metabolize ATP to adenosine can be used whenever attempting to discriminate between ATP- and AR-mediated actions. Due to the multiplicity and redundancy of the ectoenzymes involved in the process (Zimmermann 2006), it has been difficult or almost impossible to fully inhibit extracellular ATP breakdown into adenosine. Ectonucleoside triphosphate diphosphohydrolase (NTPDase; EC 3.6.1.5), previously identified as ecto-ATPase, ecto-ATPDase or CD39, is the ectonucleotidase mainly responsible for the sequential hydrolysis of β- and γ-phosphates of tri- and diphosphonucleosides. Its inhibition by ARL-67156 has proven useful to enhance extracellular ATP actions and/or to avoid its breakdown into adenosine (Sperlágh et al. 2007). For a discussion on the therapeutic potential of NTPDase inhibition, see Gendron et al. (2002). Inhibition of adenosine formation from released adenine nucleotides can also be partially achieved through the use of an ecto-5′-nucleotidase inhibitor, α, β-methylene ADP (AOPCP). By comparing the action of adenosine deaminase with that of AOPCP, it was possible to estimate that the formation of adenosine from adenine nucleotides and the release of adenosine as such contribute in nearly equal amounts to the pool of endogenous adenosine that presynaptically inhibits acetylcholine release from motor nerve terminals at the neuromuscular junction (Ribeiro and Sebastião 1987), as well as in the hippocampus (Cunha et al. 1994c). However, this relationship may differ in other brain areas and according to neuronal activity and the regional distribution of ecto-5′-nucleotidase. For example, in cholinergic nerve terminals of the hippocampus, there is significant activity from ecto-5′-nucleotidase, whereas this is not the case in cerebral cortical cholinergic nerve terminals (Cunha et al. 1992). High-frequency neuronal firing favors ATP release (Cunha et al. 1996a) as well as activation of adenosine $A_{2A}$AR, and this has been shown to occur both in a peripheral nervous system preparation, the neuromuscular junction (Correia-de-Sá et al. 1996), and in a central nervous system preparation, the hippocampus (Almeida et al. 2003). At the neuromuscular junction, however, ecto-AMP deaminase shunts the pathway for adenosine formation, thus reducing its ability to activate $A_{2A}$AR (Magalhães-Cardoso et al. 2003).

An enhancement of extracellular adenosine levels can be achieved by inhibiting intracellular enzymes that are responsible for keeping intracellular adenosine concentrations low, such as the adenosine kinase (AK) that phosphorylates adenosine

into AMP. Inhibition of this enzyme selectively amplifies extracellular adenosine concentrations at cell and tissue sites where adenosine release occurs. AK can be inhibited with iodotubercidin, which markedly enhances extracellular adenosine levels and causes an inhibition of synaptic transmission (Diógenes et al. 2004) at sites where $A_1$ARs are operative (e.g., hippocampus). The therapeutic antiepileptic potential of AK inhibition or of its underexpression in implanted cells has been highlighted recently (Li et al. 2007).

Manipulation of adenosine transporter activity with inhibitors such as dipyridamole or nitrobenzylthioinosine (NBTI) has also proven to be a useful approach, but one has to keep in mind that, due to the equilibrative nature of adenosine transporters in the brain cells, adenosine transport inhibition can either enhance or reduce extracellular adenosine levels according to the gradient of concentration of adenosine across the cell membrane, as well as according to the proportion of extracellular adenosine that is formed from the catabolism of released adenine nucleotides.

A still less explored way to increase extracellular adenosine levels is deep brain stimulation, and a very interesting report on this subject appeared recently (Bekar et al. 2008). Deep brain stimulation is used empirically to treat tremor and other movement disorders (Yu and Neimat 2008) as well as psychiatric diseases, including obsessive–compulsive disorders and depression (Larson 2008). As Bekar et al. (2008) clearly showed, deep brain stimulation is associated with a marked increase in the release of ATP from thalamic nuclei, resulting in accumulation of its catabolic product, adenosine. ATP, which is released in a nonexocytotic way, probably from astrocytes or other glial cells, is therefore crucial in adenosine accumulation following deep brain stimulation, leading to $A_1$AR-mediated inhibition of synaptic transmission in the thalamus (Bekar et al. 2008), in a way that is probably similar to the inhibition of synaptic transmission induced by ATP in the hippocampus, which requires localized extracellular catabolism by ectonucleotidases and channeling to $A_1$ARs (Cunha et al. 1998). Infusion of $A_1$AR agonists directly into the thalamus reduces tremor, whereas $A_1$AR-null mice show involuntary movements and seizures at stimulation intensities below the therapeutic level, suggesting that depression of synaptic transmission in the thalamus controls the spread of excitability and reduces side effects of deep brain stimulation (Bekar et al. 2008). Depression of synaptic transmission due to deep brain stimulation mimics in several aspects the depression of synaptic transmission caused by hypoxia, which is neuroprotective and can also be reversed by $A_1$AR antagonists (Sebastião et al. 2001). Hypoxia is, indeed, another highly efficient way to increase extracellular adenosine levels (Fowler 1993; Frenguelli et al. 2003), but in this case adenosine is mostly released as such (Latini and Pedata 2001), while deep brain stimulation appears to predominantly induce ATP release (Bekar et al. 2008).

A schematic representation of the different pathways involved in the control of extracellular adenosine concentrations, as well as the relevance of neuronal firing frequency to $A_1$AR vs. $A_2$AR activation by extracellularly formed adenosine, is depicted in Fig. 1.

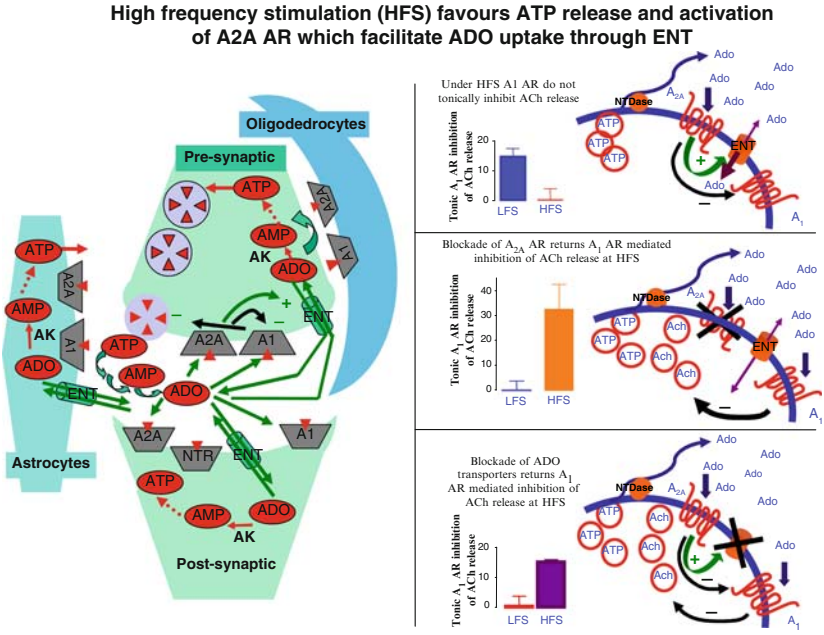


**Fig. 1** Main pathways that control extracellular adenosine (*ADO*) concentrations, and their relationships to the activation of $A_1$ or $A_{2A}$ adenosine receptors (*ARs*) under low or high neuronal firing rates induced experimentally by low- or high-frequency stimulation (*LFS* or *HFS*). Adenosine can be formed from extracellular catabolism of ATP by a cascade of ectoenzymes, the ecto-5′-nucleotidases (*NTDase*), or can be released as such through an equilibrative nucleoside transporter (*ENT*). Intracellularly the key enzyme influencing ADO concentration is adenosine kinase (*AK*), which is present in most cell types, including neurons and glia. The intracellular pathways for ADO metabolism into ATP are not depicted in oligodendrocytes for the sake of clarity. $A_1$ and $A_{2A}$ receptors are present pre- and postsynaptically as well as in astrocytes and glia. At nerve terminals, $A_1$ARs decrease neurotransmitter (*NT*) release, thus reducing the availability to activate postsynaptic NT receptors (*NTR*). $A_{2A}$ receptors have been shown to inhibit (*minus symbol*) $A_1$ receptor functiong in nerve terminals. $A_1/A_{2A}$ receptor interactions might also occur in other cell types, namely in astrocytes (*see text*), but they are not represented for the sake of clarity. $A_{2A}$ARs are preferentially activated at high-frequency neuronal firing, which favors ATP release and adenosine formed from extracellular ATP catabolism. $A_{2A}$ARs enhance (*plus symbol*) adenosine transport through ENT, which in the case of HFS is in the inward direction, decreasing the availability of ADO for $A_1$ARs, the main consequence of which is a lower tonic inhibition of neurotransmitter release. Data shown in the *left panels* are adapted from data published by Pinto-Duarte et al. (2005), who reported the influence of the firing rate upon the tonic inhibition of acetylcholine (*ACh*) release from the CA3 area of hippocampal slices, and how it is related to the ability of $A_{2A}$ receptors to enhance ENT activity at hippocampal nerve terminals. See text for further references

## 4 Distribution of ARs in the Central Nervous System and the Effect of Aging

Neuromodulation by adenosine is exerted through the activation of high-affinity $A_1$ and $A_{2A}$ ARs, which are probably of physiological importance, and of low-affinity $A_{2B}$ARs, which may be relevant in pathological conditions. The $A_3$AR is

a high-affinity receptor in humans, but has a low density in most tissues. These four ARs are also known as P1 purinoceptors, from the P1 (adenosine-sensitive)/P2 (ATP-sensitive) nomenclature (Burnstock 1976). They belong to the G-protein-coupled receptor (GPCR) family and all have been cloned and characterized from several mammalian species including humans (Fredholm et al. 2001).

The adenosine $A_1$AR is highly expressed in brain cortex, cerebellum, hippocampus, and dorsal horn of spinal cord (Ribeiro et al. 2003). The $A_{2A}$AR is highly expressed in the striatopallidal γ-aminobutyric acid (GABA)ergic neurones and olfactory bulb, and for a long time it was assumed that this receptor was circumscribed to these brain areas. The first evidence that the $A_{2A}$AR could influence neuronal communication outside the striatum or olfactory bulb was reported in 1992 using hippocampal slices (Sebastião and Ribeiro 1992). This was followed by evidence that $A_{2A}$AR mRNA and protein are expressed in the hippocampus (Cunha et al. 1994a). The initial scepticism was broken (Sebastião and Ribeiro 1996), and it is now widely recognized that $A_{2A}$ARs are expressed in several brain regions albeit in lower levels than in the striatum. $A_{2B}$ARs are expressed in low levels in the brain (Dixon et al. 1996), and the level of expression for the $A_3$AR is apparently moderate in the human cerebellum and hippocampus and low in most other areas of the brain (Fredholm et al. 2001) (Fig. 2).

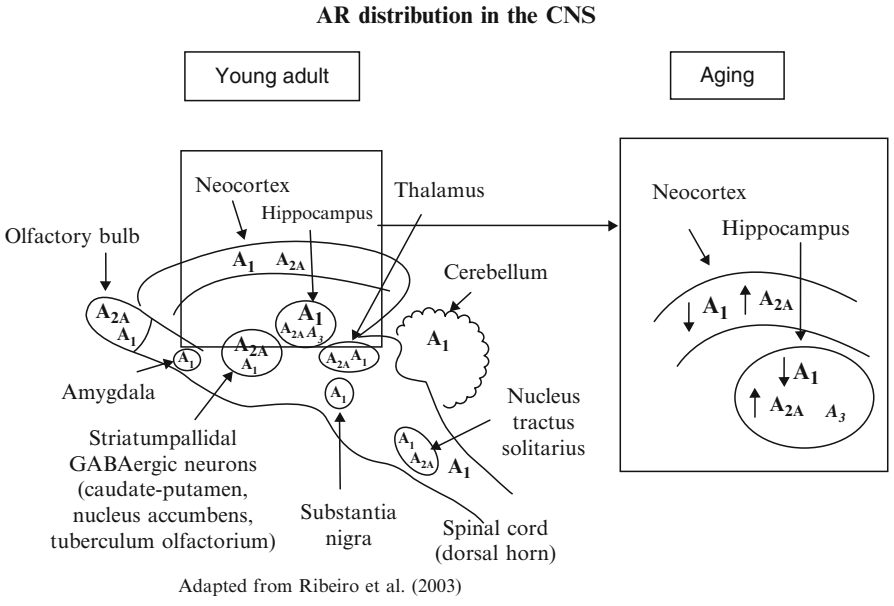


**Fig. 2** Schematic representation of the distribution of adenosine receptors (*ARs*) in the different brain areas. The *inset* illustrates the reported changes in AR density in the forebrain (hippocampus and cortex). In aged rats, the density and functioning of $A_{2A}$ARs is increased (*upward arrow*) in the hippocampus and cortex, whereas the density and functioning of $A_1$ARs is decreased (*downward arrow*). No information, so far, is available for age-related changes in $A_3$AR density upon aging. See text for references

The relative densities of $A_1$ and $A_{2A}$ ARs in subregions of the same brain area may differ. For instance, with respect to the modulation of acetylcholine in the hippocampus, there is a preponderance of $A_1$AR-mediated modulation by endogenous adenosine in both the CA1 and CA3 areas, but the CA3 has a relatively higher influence of $A_{2A}$ARs than the CA1 (Cunha et al. 1994b). Whenever two receptors coexist, one may ask about their relative importance (i.e., the hierarchy of one receptor with respect, to the other). This may change with neuronal activity, age, and even with other molecules that are in the vicinity of the site of action and that may be relevant for the production or inactivation of the ligand. High-frequency neuronal firing favours ATP release (Cunha et al. 1996a), and adenosine formed from released adenine nucleotides seems to prefer $A_{2A}$AR activation (Cunha et al. 1996b), which may be due to the geographical distribution of ecto-5-nucleotidadases and $A_{2A}$ARs. $A_{2A}$AR activation activates adenosine transport, which in the case of high neuronal activity and ATP release is in the inward direction (Fig. 1). This induces a decrease in extracellular adenosine levels and a reduced ability of $A_1$AR s to be activated by endogenous extracellular adenosine (Pinto-Duarte et al. 2005). By themselves, $A_{2A}$ARs are able to attenuate $A_1$AR activation (Cunha et al. 1994a), which may further contribute to a decreased activity of $A_1$AR s under high-frequency neuronal firing. The ability of $A_1$ARs to inhibit synaptic transmission is attenuated by protein kinase C (PKC) activation (Sebastião and Ribeiro 1990), and a similar mechanism appears to be involved in the $A_{2A}$AR-mediated attenuation of $A_1$AR responses (Lopes et al. 1999a).

Aging also decreases the ability of $A_1$ARs to inhibit neuronal activity (Sebastão et al. 2000a). This may be a function of an age-related decrease in the density of $A_1$ARs in the brain, which has been shown in both mice (Pagonopoulou and Angelatou 1992) and humans (Meyer et al. 2007). Low $A_1$AR receptor density and function, however, can be compensated for by higher levels of extracellular adenosine, which keep tonic inhibition high in aged animals (Bauman et al. 1992). While comparing changes in $A_1$AR density in the cerebral cortex, hippocampus and striatum, it was concluded that the most affected area was the cerebral cortex, followed by the hippocampus (Fig. 2), whereas the density of $A_1$ARs in the striatum was little affected by aging in rats (Cunha et al. 1995). $A_1$AR density in the cerebellum is also poorly affected by aging (Pagonopoulou and Angelatou 1992).

In contrast to $A_1$ARs, there is a significant increase in the density of $A_{2A}$ARs in the cortex (Cunha et al. 1995) and hippocampus (Diogenes et al. 2007) of aged rats, which correlates with their enhanced ability to facilitate glutamatergic synaptic transmission (Rebola et al. 2003) and acetylcholine release (Lopes et al. 1999b) in the hippocampus (Fig. 2). In the striatum there is a tendency for a decrease in $A_{2A}$AR density in aged rats (Cunha et al. 1995), and within the striatum, age may influence the $A_{2A}$ARs in glutamatergic, dopaminergic or GABAergic nerve terminals in different ways (Corsi et al. 1999, 2000). Taken together, these findings clearly show that there are age-related shifts in the $A_1$AR inhibitory/$A_{2A}$AR excitatory balance, and that this shift may be different in different areas of the brain, with the trend for the forebrain being towards an increase in $A_{2A}$AR-mediated influences and a decrease in $A_1$AR density. Due to the $A_{2A}/A_1$ AR interactions

(see Sect. 5.1.5), an increase in $A_{2A}$AR density may itself reduce $A_1$AR tonus. Due to the influence of $A_{2A}$ARs on other receptors (see Sect. 5), the change in the $A_{2A}$AR influence upon aging may markedly affect the action of other modulators. Indeed, the nonmonotonous age-related changes in the ability of brain-derived neurotrophic factor (BDNF) to influence synaptic transmission in the hippocampus are related to both a decrease in the density of tropomyosin-related kinase receptors (Trk) for BDNF (TrkB receptors) and an increase in the density of $A_{2A}$ARs, which allow TrkB receptor-mediated actions in the aged hippocampus (Diogenes et al. 2007).

## 5 Adenosine as a Modulator of Other Neuromodulators

Besides its direct pre- and postsynaptic actions on neurones, adenosine is rich in nuances of priming, triggering and braking the action of several neurotransmitters and neuromodulators. Because adenosine acts in such a subtle fashion, it was proposed as a fine tuner. In this way, adenosine is a partner in a very sophisticated interplay between its own receptors and receptors for other neurotransmitters and/or neuromodulators. Several possibilities exist for this interplay, either at the transducing system level (Sebastião and Ribeiro 2000) or as a consequence of receptor–receptor heteromerization (Ferré et al. 2007a), greatly expanding the number of possible receptor combinations to modulate cell signalling.

### *5.1 Interactions with G-Protein-Coupled Receptors*

Besides the well known $A_{2A}/D_2$ dopamine interaction in the stritaum (Ferré et al. 1991), which has been explored intensively due to the implication of this receptor interaction for Parkinson's disease and other basal ganglia dysfunctions (Fuxe et al. 2007; Morelli et al. 2007), adenosine, mostly through activation of $A_{2A}$ARs, is also able to influence the functioning of other GPCRs (Fig. 3a). A brief overview of the influence of adenosine on these receptors will follow.

#### 5.1.1 Dopamine Receptors

A first hint at the ability of $A_{2A}$ARs to interact with dopamine $D_2$ receptors came from binding studies showing that activation of $A_{2A}$ARs decreases the affinity of dopamine $D_2$ receptors in rat striatal membranes (Ferré et al. 1991). The possibility that this $A_{2A} - D_2$ receptor interaction is crucial to the behavioral effects of adenosine agonists and antagonists (like caffeine) was immediately highlighted (Ferré et al. 1991) and soon tested (Svenningsson et al. 1995). The functional consequences of $A_{2A}$AR and $D_2$ receptor agonists upon dopamine and GABA release (Ferré et al. 1994; Mayfield et al. 1996) in the basal ganglia became evident soon thereafter.

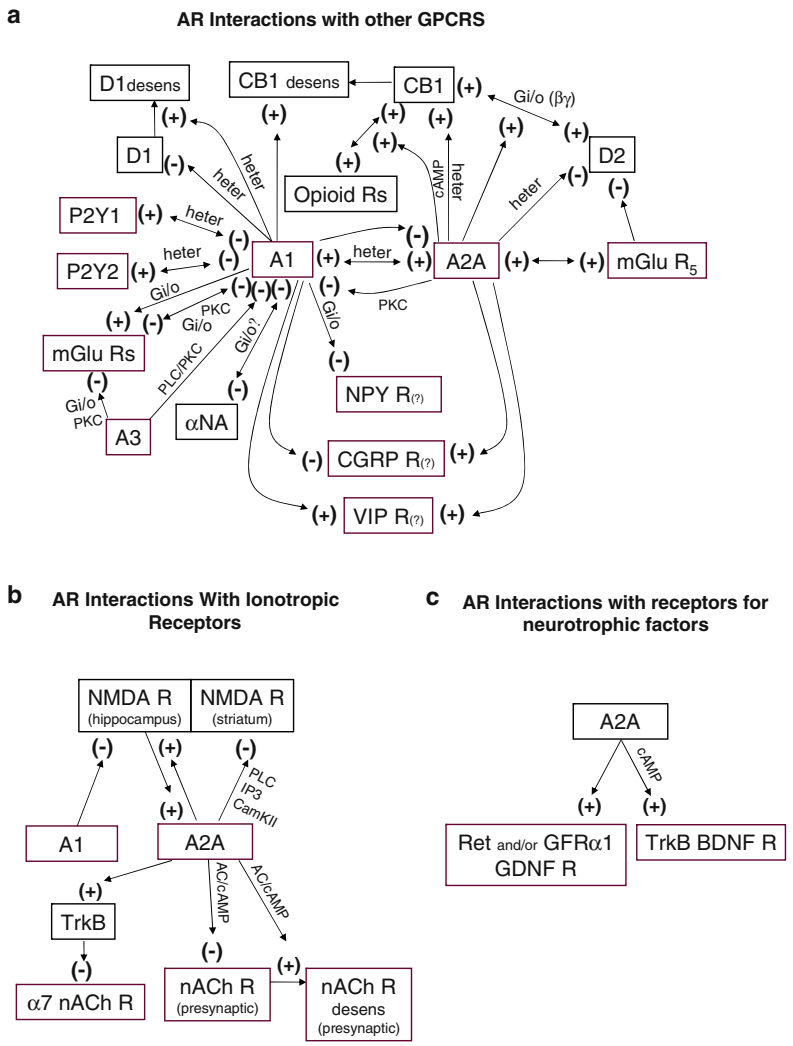


**Fig. 3** **a–c** Interactions between adenosine receptors and receptors for other neurotransmitters. The known interactions with other G-protein-coupled receptors (GPCRs) **a**, with ionotropic receptors **b**, and with receptors for neurotrophic factors **c** are illustrated, where a *plus symbol* represents a facilitation or triggering of the action, or synergy between receptors, or the facilitation of desensitization (*desens*), and a *minus symbol* represents an inhibition, or an occlusion of the action, or less than addictive effects. Whenever the mechanisms involved in the interaction have been evaluated, they are indicated close to the *arrow*. G-protein sharing is indicated by the name of the G protein close to the *arrow*. Whenever receptor heteromerization (*heter*) has been shown to occur, it is also indicated close to the *arrow*. An absence of knowledge about the receptor subtype is indicated by a *question mark* close to the receptor name. See text for references. Other abbreviations: $\alpha NA$, $\alpha$ receptor for noradrenaline; *BDNF*, brain-derived neurotrophic factor; *CB*, cannabinoid; $CB_1$: cannabinoid receptor type 1; *CGRP*, calcitonin gene-related peptide; *D*, dopamine receptor; *GFRα1 and Ret*: neurotrophic factors for GDNF; *GDNF*, glial cell line-derived neurotrophic factor; *mGluRs*, metabotropic glutamate receptor; *nAChR*: nicotinic acetylcholine receptor; *NMDAR*: *N*-methyl-D-aspartate receptor; *NPY*, neuropeptide Y; *VIP*, vasoactive intestinal peptide; *P2Y*, ATP receptor; *TrkB*, tropomyosin-related kinase receptor type B

Since this time, interest in the adenosine/$D_2$ interaction has continued to increase, extending to psychiatric and neurologic fields such as drug addiction, schizophrenia and Parkinson's disease, and has been the subject of many reviews by groups that have been involved in this subject since its origin (Ferré et al. 2007b). For more information on $A_{2A}$ARs and Parkinson's disease, please refer to Chap. 18, "Adenosine $A_{2A}$ Receptors and Parkinson's Disease" (by Morelli et al.), in this volume.

$A_1$ARs and $D_1$ receptors also interact in the basal ganglia (Ferré et al. 1996), an interaction that has implications for the control of GABA release at the substantia nigra (Florán et al. 2002) and nucleus accumbens (Mayfield et al. 1999), as well as dopamine release in the striatum (O'Neill et al. 2007). Furthermore, $A_1$AR activation has been shown to facilitate $D_1$ receptor desensitization (Le Crom et al. 2002). $D_1/A_1$ receptor heteromerization may play a role in $D_1$ receptor desensitization mechanisms and be a molecular basis for the antagonistic modulation of $A_1$AR over $D_1$ receptor signaling (Ginés et al. 2000).

### 5.1.2 Neuropeptides

By activating $A_{2A}$ARs, adenosine tonically potentiates a facilitatory action of the neuropeptide calcitonin gene-related peptide (CGRP) on neurotransmitter release from motor nerve terminals (Correia-de-Sá and Ribeiro 1994a). The ability of CGRP to facilitate synaptic transmission in the CA1 area of the hippocampus is also under tight control by adenosine, with tonic $A_1$AR activation by endogenous adenosine "braking" the action of CGRP, and the $A_{2A}$ARs triggering this action (Sebastião et al. 2000b). This $A_1$AR-mediated inhibition of the action of CGRP, together with the $A_1$AR-induced inhibition of CGRP release (Carruthers et al. 2001), can be related to pain inhibition by adenosine (see Sect. 7). Indeed, CGRP is a potent vasodilator released from activated trigeminal sensory nerves that dilates intracranial blood vessels and transmits vascular nociception, and is implicated in the genesis of vascular pain such as migraine. Hence, inhibition of trigeminal CGRP release and CGRP receptor blockade have been proposed as promising antimigraine strategies (Goadsby 2008).

The facilitatory action of vasoactive intestinal peptide (VIP) on ACh release from motor nerve endings is prevented by $A_{2A}$AR blockade or by the removal of extracellular adenosine with adenosine deaminase, indicating that the activation of these $A_{2A}$ARs, attained with high-frequency motor neuron firing, is necessary to trigger the facilitatory action of VIP (Correia-de-Sá et al. 2001). VIP enhances synaptic transmission at the CA1 area of the hippocampus by enhancing GABA release from GABAergic neurones that make synapses with other interneurones, therefore reducing GABAergic inhibition into pyramidal glutamatergic neurones (Cunha-Reis et al. 2004, 2005). This action of VIP is dependent on both $A_1$ and $A_{2A}$ AR activation by endogenous adenosine (Cunha-Reis et al. 2007, 2008). Interestingly, the finding that VIP-induced modulation of GABA release from hippocampal nerve terminals is under the control of adenosine $A_1$ARs constituted the first evidence of a role of $A_1$ receptors in hippocampal GABAergic terminals. This is an example of

a situation where $A_1$ARs per se may not affect neurotransmitter release, just like GABA in the hippocampus (Lambert and Teyler 1991; Yoon and Rothman 1991), but instead influence the actions of other modulators of GABA release.

Neuropeptide Y (NPY) agonists inhibit presynaptic calcium influx through N- and P/Q-type calcium channels and inhibit glutamate release at the CA3–CA1 synapses of rat hippocampus, an action that is fully occluded by coactivation of adenosine $A_1$ARs (Qian et al. 1997). Interestingly, the inhibitory action of the $GABA_B$ agonist baclofen was not fully occluded by AR activation, indicating partially shared pathways between G-protein-coupled NPY, adenosine and GABA receptors. In PC12 cells, exocytosis of NPY-containing vesicles is facilitated by $A_{2A}$AR activation (Mori et al. 2004), but this does not occur in nerve endings from the rat mesenteric artery, where ARs affect noradrenaline but not NPY release (Donoso et al. 2006).

In cultured primary hippocampal neurones, agonists of delta-opioid receptors and of cannabinoid (CB) receptors of the $CB_1$ subtype act synergistically to activate protein kinase A (PKA) signaling through Gi-$\beta/\gamma$ dimers, and this synergy requires $A_{2A}$AR activation (Yao et al. 2003). CB1 agonists also act synergistically with $\mu$ opiod receptors in primary nucleus accumbens/striatal neurones, and again this synergy requires adenosine $A_{2A}$ARs (Yao et al. 2006). Interestingly, $A_{2A}$AR blockade eliminates heroin-seeking behavior in addicted rats (Yao et al. 2006), suggesting that $A_{2A}$AR antagonists may be effective therapeutic agents in the management of abstinent heroin addicts (see Sect. 9).

### 5.1.3 Metabotropic Glutamate Receptors

Activation of metabotropic glutamate receptors (mGluR) with 1*S*, 3*R*-ACPD potentiates cAMP responses mediated by several receptors that are positively coupled to adenylate cyclase, namely $A_2$ARs and VIP and $\beta$-adrenergic receptors (Alexander et al. 1992; Winder and Conn 1993). mGluRs also influence $A_1$AR functioning in neurones, and this seems to involve PKC activity. In fact, PKC activity is required for the attenuation of the inhibitory effect of $A_1$AR activation on synaptic transmission at the hippocampus by agonists of group I mGluRs (mGlu1, mGlu5) which are coupled to phospholipase C, as well as by agonists of group III mGluRs (mGlu4, mGlu6, mGlu7, mGlu8), which are usually negatively coupled to cAMP (de Mendonça and Ribeiro 1997a). Agonists of group I mGluRs also attenuate $GABA_B$-mediated inhibition of synaptic transmission, a process that involves PKC activity (Shahraki and Stone 2003). In addition, activation of PKC by phorbol esters or activation of PKC-coupled mGluRs suppresses the inhibitory action of $A_1$AR agonists on glutamate release from cerebrocortical synaptosomes (Budd and Nichols 1995).

The inhibitory effects of an $A_1$AR agonist and of an agonist of group II mGluRs (mGlu2, mGlu 3) on glutamate release or cAMP formation was less than additive (Di Iorio et al. 1996), suggesting that the presynaptic $A_1$ and group II mGluRs are reciprocally occlusive, probably by sharing a pertussis toxin (PTX)-sensitive, PKC-regulated G protein (Zhang and Schmidt 1999).

Activation of $A_3$AR leads, through a PKC-dependent process, to a marked attenuation of the presynaptic inhibitory functions of cAMP-coupled mGluRs (groups II and III) at the CA1 area of the hippocampus (Macek et al. 1998). Again, the action of PKC and probably also that of $A_3$ARs on mGluRs might result from an inhibition of the coupling of mGluRs with G proteins, because PKC activation inhibits the increased [$^{35}$S]GTPγS binding induced by mGluR agonists (Macek et al. 1998). Thus, the actions of $A_1$ or $A_3$ ARs and those of mGluRs in neurones are mutually occlusive, through a process probably involving the crosstalk of transducing systems or the sharing of G proteins, as proposed several years ago to explain the mutual occlusion between presynaptic adenosine $A_1$ and $\alpha_2$-adrenergic receptors (Limberger et al. 1988).

In contrast, in astrocytes, activation of $A_1$AR enhances the intracellular calcium response induced by mGluRs (Ogata et al. 1994), a process that involves a PTX-sensitive G protein (Cormier et al. 2001; Tom and Roberts 1999). Adenosine-induced calcium response in astrocytes requires $A_1/A_2$ AR cooperation, and is synergistic with mGluR response, leading to enhancement of cAMP levels (Ogata et al. 1996).

With respect to the interaction between $A_{2A}$AR and mGluR, $A_{2A}$AR agonists act synergistically with group I mGluR agonists to modulate dopamine $D_2$ receptors in the rat striatum, decreasing the affinity state of these receptors (Ferré et al. 1999). Furthermore, $A_{2A}$ARs act synergistically with mGlu5 receptors to increase dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) phosphorylation, so that blockade of one of the receptors is enough to prevent phosphorylation induced by activation of the other receptor (Nishi et al. 2003). $A_{2A}$ARs and mGlu5 receptors are also co-localized presynaptically, namely at striatal glutamatergic terminals, where they facilitate glutamate release in a synergistic manner (Pintor et al. 2000; Rodrigues et al. 2005). Prevention of mGlu5 receptors and $A_{2A}$AR synergy at the pre- and the postsynaptic level will, therefore, eventually lead to decreased glutamate release with consequent reduced excitotoxicity, together with a facilitation of $D_2$ dopaminergic receptor functioning, and this is the rational for the use of antagonists of these receptors as antiparkinsonian drugs (see also Chap. 18, "Adenosine $A_{2A}$ Receptors and Parkinson's Disease," by Morelli et al., in this volume). Indeed simultaneous blockade of $A_{2A}$ and mGlu5 receptors showed high efficacy in reversing parkinsonian deficits in rodents (Coccurello et al. 2004; Kachroo et al. 2005). Combined antagonism of mGlu5 receptors and $A_{2A}$ARs also efficiently reduced alcohol self-administration and alcohol-seeking in rats (Adams et al. 2008), further reinforcing the importance of the mGlu5 and $A_{2A}$AR interaction in the mesolimbic and basal ganglia areas.

### 5.1.4 Cannabinoid Receptors

The high density of adenosine $A_{2A}$ARs in the basal ganglia, together with the profound motor-depressant effects of cannabinoids (CBs), prompted interest in investigating a putative crosstalk between $A_{2A}$ARs and $CB_1$ receptors in this brain

area. $CB_1$ receptor signaling in a human neuroblastoma cell line is dependent on $A_{2A}$AR activation, and blockade of $A_{2A}$ARs counteracts the motor-depressant effects produced by $CB_1$ receptor activation in vivo (Carriba et al. 2007). Interestingly, the motor-depressant effects produced by $CB_1$ receptor activation are attenuated by genetic inactivation of the DARPP-32 (Andersson et al. 2005), which is abundantly expressed in the medium spiny neurons of the striatum and is crucially involved in the striatal actions of cAMP-coupled receptors (Greengard 2001), as in the case of $A_{2A}$ARs. Molecular interactions between striatal $A_{2A}$ARs and $CB_1$ receptors at the striatum may also exist since $CB_1$ and $A_{2A}$ARs form heteromeric complexes once transfected to human embryonic kidney cells, HEK-293T (Carriba et al. 2007).

$A_{2A}$AR activation is required for the synergistic actions between $CB_1$ receptors and μ opioid receptors in nucleus accumbens (NAc)/striatal neurons (Yao et al. 2006), as well as for the synergistic actions that occur between $CB_1$ agonists and $D_2$ agonists (Yao et al. 2003). Synergy between $G_i$-coupled receptors, such as $CB_1$ and $D_2$ receptors, with respect to the facilitation of cAMP-mediated signaling involves $\beta-\gamma$ dimers of $G_i$ proteins, and these are also required for the interplay with $A_2$ARs (Yao et al. 2003). The implications of these multiple interactions as they pertain to therapies for drug addiction will be discussed below (see Sect. 9.4).

$A_1$ARs also appear to be involved in motor impairment by CBs, and this may occur at the cerebellum, since the incoordination induced by $CB_1$ agonists is attenuated by intracerebellar injection of an $A_1$AR-selective antagonist (DeSanty and Dar 2001). A reciprocal ability to heterologously desensitize $CB_1$ and $A_1$AR responses through prolonged agonist exposure has also been reported (Kouznetsova et al. 2002; Selley et al. 2004).

### 5.1.5 $A_1$, $A_{2A}$ and $A_3$ARs

The existence of $A_1 - A_{2A}$ AR heteromers has been demonstrated and complicates the overall picture for adenosine as a neuromodulator and the role of ARs in neurotransmission. Co-immunoprecipitation and bioluminescence resonance energy transfer (BRET) techniques have shown the existence of $A_1 - A_{2A}$ AR heteromers in co-transfected HEK cells, as well as the existence of an intermolecular crosstalk, and radioligand-binding techniques have allowed the identification of an intramembrane receptor–receptor interaction in the $A_1 - A_{2A}$ receptor heteromer (Ciruela et al. 2006). According to Ferré et al. (2007a), the $A_1$–$A_{2A}$ receptor heteromer may provide a "concentration-dependent switch" mechanism by which low and high concentrations of synaptic adenosine produce the opposite effects on glutamate release. Thus, a weak input might cause stimulation of the receptor with the highest affinity in the $A_1/A_{2A}$ heteromer, while a strong input might cause additional stimulation of the other receptor, with crosstalk between both receptors that may allow a response that is different from the summation of both of them. However, as discussed in point 3 above, other factors such as the topographical arrangement of ectoenzymes, transporters and receptors as well as the neuronal firing frequency may also influence the $A_1$ versus $A_{2A}$AR-mediated actions at each synapse where both receptors co-localize.

With respect to crosstalk between $A_1$ and $A_{2A}$ ARs, this was clearly documented with data obtained from experiments at the hippocampus, where activation of $A_{2A}$ARs attenuates the ability of $A_1$ AR agonists to inhibit excitability and synaptic transmission (Cunha et al. 1994a; O'Kane and Stone 1998). An $A_{2A}$AR-mediated decrease in $A_1$AR binding was also shown to occur in hippocampal (Lopes et al. 1999a) and striatal (Dixon et al. 1997) synaptosomes. $A_{2A}$AR-induced inhibition of $A_1$AR binding does not occur in membrane fragments, which indicates that the cross talk between $A_1$ and $A_{2A}$ receptors involves a diffusible second messenger. The $A_{2A}/A_1$AR crosstalk might be related to PKC, rather than to the classical $A_{2A}$AR second messenger, the adenylate cyclase-cAMP–PKA pathway, because the interactions between $A_{2A}$ and $A_1$ARs are prevented by PKC inhibitors but not by PKA inhibitors (Dixon et al. 1997; Lopes et al. 1999a). PKC activators, such as phorbol esters, mimic the ability of $A_{2A}$ receptor agonists to decrease $A_1$AR binding (Lopes et al. 1999a). Thus, with respect to their ability to inhibit $A_1$AR-mediated responses, $A_{2A}$ARs appear to behave similarly to the phospholipase C-coupled metabotropic glutamate and muscarinic receptors (Worley et al. 1987); i.e., they operate through a phosphoinositide-PKC-dependent pathway. Activation of PKC inhibits presynaptic $A_1$ARs on motor nerve terminals without affecting the affinity of competitive receptor antagonists (Sebastião and Ribeiro 1990), suggesting that the target of PKC is not the receptor ligand-binding domain, but probably a locus related to G-protein coupling, the G protein itself, or both.

Besides the $A_{2A}/A_1$ AR interaction, which can be observed using either BRET, radioligand binding, or functional studies with selective agonists for both receptors, there are other ways through which $A_{2A}$AR activation can also induce a decrease in $A_1$AR tone. $A_{2A}$ARs enhance adenosine transport through equilibrative nucleoside transporter (ENT)s, with a consequent reduction in the availability of endogenous adenosine to tonically activate $A_1$ARs (Pinto-Duarte et al. 2005). As occurs with $A_{2A}$AR-mediated inhibition of $A_1$AR binding (Lopes et al. 1999a), the $A_{2A}$AR-induced enhancement of ENT activity is lost upon the inhibition of PKC but not PKA activity, suggesting the involvement of the phospholipase C (PLC) pathway rather the adenylate cyclase/cAMP one (Pinto-Duarte et al. 2005).

While evaluating the evoked release of acetylcholine at different frequencies of stimulation from hippocampal slices, it becomes clear that the $A_{2A}$AR-mediated enhancement of ENT activity plays a pivotal role in adjusting adenosine neuromodulation to different physiological needs (Pinto-Duarte et al. 2005). Thus, at high-frequency neuronal firing, there is a predominant release of ATP and a predominant formation of adenosine from released ATP (Cunha et al. 1996b). Therefore, the extracelluar adenosine concentration exceeds the intracellular one and the gradient of adenosine concentration across the plasma membrane will direct ENT to take up adenosine. Since $A_{2A}$ARs are concomitantly activated, the $A_{2A}$AR-induced enhancement of ENT activity leads to an enhancement of the removal of adenosine from the synaptic cleft, leading to a reduced tonic $A_1$ − AR-mediated inhibition of hippocampal acetylcholine release at high-frequency firing rates (Pinto-Duarte et al. 2005). This $A_{2A}$AR-mediated inhibition of tonic inhibitory adenosinergic tone may add to the $A_{2A}$AR inhibition of $A_1$AR activation (see above), thus efficiently

reinforcing the enhanced firing rate of cholinergic afferents into the hippocampus, which are known to play a key role in the control of cognitive processes such as attention and memory (Hasselmo and Giocomo 2006).

Other interactions of $A_1$ and $A_2$ ARs include the influence of $A_1$ARs on $A_{2A}$AR activity, where desensitization of striatal $A_1$ARs is accompanied by a time-dependent amplification of $A_2-$ AR-mediated stimulation of adenylate cyclase (Abbracchio et al. 1992). Moreover, presynaptic interactions between $A_1$ and $A_{2A}$ ARs were clearly observed at motor nerve terminals where $A_1$AR inhibitory responses are enhanced in the presence of $A_2$AR antagonists, and $A_{2A}$AR excitatory responses are increased in the presence of $A_1$AR antagonists (Correia de Sá et al. 1996). However, in contrast to what occurs in neurones, positive cooperation between $A_1$ and $A_2$ ARs, which also requires concomitant activation of metabotropic glutamate receptors (groups I and II), was observed in cultured astrocytes (Ogata et al. 1996).

With respect to $A_3$ARs and the interaction of $A_3$ARs with other ARs, $A_3$AR activation attenuates the synaptic inhibitory actions of adenosine in the CA1 area of the hippocampus (Dunwiddie et al. 1997). Because adenosine $A_3$ARs might couple to phospholipase C, and phospholipase C-coupled receptors are able to inhibit $A_1-$ AR-mediated responses (see above), it is possible that this $A_3-A_1$ AR-mediated interaction involves this transduction pathway, in a similar manner to that described in relation to the $A_3-$AR-mediated inhibition of metabotropic receptor functioning (Macek et al. 1998).

### 5.1.6 P2 Purinoceptors

Although ATP and adenosine operate distinct families of receptors and although they may play very distinct roles in the CNS—adenosine being exclusively a neuromodulator and ATP behaving as a neurotransmitter, neuromodulator, or comodulator—interactions between receptors for these two "family related" molecules have been reported. $P2Y_1$ receptors and $A_1$ARs can form heteromeric complexes and display a high degree of colocalization in the brain (Yoshioka et al. 2002). $P2Y_1$ receptors and $A_1$ARs are colocalized at glutamatergic synapses and surrounding astrocytes, and $P2Y_1$ receptor stimulation impairs the $A_1$AR coupling to the G protein probably by inducing heterologous desensitization (Tonazzini et al. 2008), whereas the stimulation of $A_1$ARs increases the functional responsiveness of $P2Y_1$ receptors (Tonazzini et al. 2007). Similar findings were found in relation to the crosstalk between $A_1$ARs and $P2Y_2$ receptors, where oligomerization of $A_1$ARs and $P2Y_2$ receptors generates a complex in which the simultaneous activation of the two receptors induces a structural alteration that interferes with signaling via $G_{i/o}$ but enhances signaling via $G_{q/11}$ (Suzuki et al. 2006).

The presynaptic facilitatory dinucleotide receptor is also under the control of ARs colocalized at the same nerve terminals. Thus, the apparent affinity of diadenosine pentaphosphate (Ap5A) for its receptor in hippocampal nerve terminals is increased up to the low nanomolar range by coactivation of $A_1$ or $A_{2A}$ ARs, whereas it is

decreased towards the high micromolar range when $A_3$ARs are coactivated (Díaz-Hernández et al. 2002). P2 purinoceptor activation by endogenous ATP may also inhibit dinucleotide receptor functioning (Díaz-Hernández et al. 2000).

## *5.2 Interaction with Ionotropic Receptors*

ARs can interact with ionotropic receptors (Fig. 3b), with putative implications for neuroprotection, plasticity and learning, as it is the case for AMPA and NMDA glutamate receptors as well as nicotinic acetylcholine receptors (nAChRs). A brief overview of the published data follows.

### 5.2.1 Modulation of NMDA and AMPA Receptors by $A_1$ and $A_2$ ARs

In isolated rat hippocampal neurones (de Mendonça et al. 1995), as well as in bipolar retinal cells (Costenla et al. 1999), $A_1$AR activation inhibits *N*-methyl-D-aspartate (NMDA) receptor-mediated currents. Interestingly, the inhibitory postsynaptic action of $A_1$AR agonists is observed at very low concentrations, compatible with a tonic inhibitory action of adenosine. Accordingly, selective $A_1$AR antagonism enhances the NMDA component of excitatory postsynaptic currents in CA1 hippocampal neurones, probably due to the recruitment of previously silent NMDA receptors at synapses (Klishin et al. 1995). Through a postsynaptic action, endogenous adenosine also inhibits voltage- and NMDA receptor-sensitive dendritic spikes in the CA1 area of the hippocampus (Li and Henry 2000). Because of the important role played by NMDA receptors in synaptic plasticity phenomena, as well as in neuronal injury after prolonged stimulation or depolarizing conditions, it is conceivable that the ability of $A_1$ARs to inhibit NMDA receptor-mediated currents together with the well-known $A_1$AR-mediated inhibition of glutamate release are the basis for the A1–AR-mediated inhibition of synaptic plasticity phenomena such as long-term potentiation (LTP) and long-term depression (LTD) at CA3/CA1 excitatory synapses of the hippocampus (de Mendonça and Ribeiro 1997b). These two $A_1$AR-mediated actions also contribute to $A_1$–AR-mediated neuroprotective actions during hypoxia (Sebastião et al. 2001) and to stopping epileptiform firing in CA1 pyramidal cells (Li and Henry 2000).

On medium spiny neurones at the striatum, $A_{2A}$AR activation inhibits (rather than facilitates) the conductance of NMDA receptor channels by a mechanism involving the phospholipase C/inositol (1,4,5)-triphosphate/calmodulin and calmodulin kinase II pathway (Wirkner et al. 2000). In $Mg^{2+}$-free conditions, and therefore in conditions where NMDA receptors are not blocked, $A_{2A}$AR activation postsynaptically inhibits the NMDA receptors in a subpopulation of striatal neurones; however, if the NMDA receptors are blocked by $Mg^{2+}$, the predominant $A_{2A}$AR-mediated action is a presynaptic inhibition of GABA release (Wirkner et al. 2004). Whether the $A_{2A}$AR-mediated inhibition of NMDA receptors in the

striatum explains the unexpected protective influence of $A_{2A}$AR agonists towards NMDA-induced excitotoxicity (Popoli et al. 2004; Tebano et al. 2004) remains to be evaluated.

Interactions between $A_{2A}$ARs and ionotropic glutamate receptors with implications for synaptic plasticity have been reported. LTP of synaptic transmission between CA3 and CA1 hippocampal areas of the hippocampus involves a postsynaptic facilitation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) currents, a well-known process that requires previous activation of NMDA receptors and involves both pre- (enhanced glutamate release) and post- (depolarization-induced relief of NMDA receptor blockade by $Mg^{2+}$) synaptic mechanisms. Interestingly, $A_2$AR activation induces a form of LTP in the CA1 area that is NMDA receptor independent (Kessey and Mogul 1997). In contrast, $A_{2A}$ARs localized postsynaptically at synapses between mossy fibers and CA3 pyramidal cells are essential for a form of LTP of NMDA currents, sparing AMPA currents (Rebola et al. 2008). Considering that CA3/CA1 LTP is predominantly NMDA receptor dependent, and that LTP at mossy fibers/CA3 synapses is predominantly presynaptic and NMDA receptor independent, it appears that $A_{2A}$ARs are particularly devoted to unmasking nonpredominant forms of plasticity and therefore fine-tuning the networking and information flow within the hippocampus.

NMDA receptor activation suppresses neuronal sensitivity to adenosine in the hippocampus, and this interaction appears to result from an increase in the excitatory action of adenosine $A_{2A}$ARs rather than a depression of $A_1$AR function (Nikbakht and Stone 2001).

Direct actions of purines upon NMDA receptor subunits (NR) may also occur. Thus, ATP, probably by directly binding to the glutamate-binding pocket of the NR2B subunit and not to ARs or ATP purinoceptors, can inhibit NMDA receptors and attenuate NMDA-mediated neurotoxicity (Ortinau et al. 2003).

### 5.2.2 Nicotinic Acetylcholine Receptors

Endogenous adenosine, by activating $A_{2A}$ARs coupled to the adenylate cyclase/cAMP transduction pathway, tonically downregulates nAChR-mediated control of [3H]-ACh release at either the skeletal neuromuscular junction (Correia-de-Sá and Ribeiro 1994b) or myenteric plexus (Duarte-Araújo et al. 2004). Furthermore, at the skeletal neuromuscular junction, $A_{2A}$ ARs enhance nicotinic receptor desensitization due to prolonged agonist exposure (Correia-de-Sá and Ribeiro 1994b).

The homopentameric α-7 subtype of nAChR is particularly relevant to brain functioning due to its high calcium permeability. By supplying calcium signals, these receptors influence several calcium-dependent events, including transmitter release and plasticity (Gray et al. 1996; Ji et al. 2001), and so several pathways must converge on their regulation. Adenosine, through $A_{2A}$AR and BDNF, through TrkB receptors, exert double control over α-7-nicotinic currents at GABAergic interneurons in the hippocampus, since blockade of $A_{2A}$ARs abolishes the BDNF-induced

current inhibition (Fernandes et al. 2008). Since postsynaptic α7 nAChR-mediated inputs to GABAergic interneurons regulate inhibition within the hippocampus, $A_{2A}$AR, by allowing the inhibition of cholinergic currents by BDNF, may temporarily relieve GABAergic inhibition and therefore facilitate plasticity phenomena.

### *5.3 Interaction with Receptors for Neurotrophic Factors*

Trk receptors belong to a third class of membrane receptors which, by themselves, possess catalytic activity involving autophosphorylation in tyrosine residues as a consequence of ligand binding, triggering a subsequent chain of phosphorylations that leads to the activation of several casacades involved in the regulation of cell death, survival and differentiation. Examples of this class of receptors are the receptors for neurotrophins, such as TrkA for nerve growth factor (NGF), TrkB for BDNF, TrkC for neurotrophin 3 (NT-3), and receptors for other neurotrophic factors, such as GFRα1 and Ret for GDNF. In spite of the structural differences between the GPCRs and receptor kinases, ARs, in particular $A_{2A}$AR, can tightly interact with receptors for neurotrophic factors, namely with receptors for BDNF and GDNF (Fig. 3c), which may have several implications for neurodegenerative diseases, as discussed below.

It has been known for several years that presynaptic depolarization (Boulanger and Poo 1999a)—which is known to increase extracellular adenosine levels, as well as enhancement of intracellular cAMP (Boulanger and Poo 1999b)—the most frequent $A_{2A}$AR transducing pathway, trigger synaptic actions of BDNF. On the other hand, $A_{2A}$ARs are known to transactivate TrkB receptors in the absence of the neurotrophin (Lee and Chao 2001). This transactivation requires long-term incubation with GPCR agonists and receptor internalization (Rajagopal et al. 2004), and it is not yet clear whether it operates the same mechanism as the more recently identified ability of $A_{2A}$ARs to trigger synaptic and promote survival actions of neurotrophic factors. Indeed, it has recently been recognized that adenosine $A_{2A}$AR activation is a crucial prerequisite for the functioning of neurotrophic receptors at synapses. This has been shown for the facilitatory actions of BDNF on synaptic transmission (Diógenes et al. 2004; Tebano et al. 2008) and on LTP (Fontinha et al. 2008) at the CA1 area of the hippocampus, as well as for the action of GDNF at striatal dopaminergic nerve endings (Gomes et al. 2006). $A_{2A}$ARs and TrkB BDNF receptors can coexist in the same nerve ending since the facilitatory action of $A_{2A}$ARs upon TrkB-mediated BDNF action is also visible at the neuromuscular junction (Pousinha et al. 2006), a single nerve ending synapse model.

The ability of BDNF to facilitate synaptic transmission is dependent on the age of the animals (Diógenes et al. 2007), and this may be related to the degree of activation of $A_{2A}$ARs by endogenous adenosine at different ages. Thus, in infant animals (i.e., immediately after weaning), in order to trigger a BDNF facilitatory action it is necessary to increase the extracellular levels of adenosine, either by inhibiting AK, through a brief depolarization (Diógenes et al. 2004; Pousinha et al. 2006), or by inducing high-frequency neuronal firing, such as those inducing LTP

(Fontinha et al. 2008); in all cases the actions of BDNF are lost by blocking $A_{2A}$ARs with selective antagonists. In adult animals, BDNF per se can facilitate synaptic transmission through TrkB receptor activation, but this effect is also fully lost with blockade of $A_{2A}$ARs (Diógenes et al. 2007) or in $A_{2A}$AR knockout (KO) mice (Tebano et al. 2008). Nicotinic α7 cholinergic currents in GABAergic hippocampal neurons are inhibited by BDNF, and this also requires coactivation of adenosine $A_{2A}$ARs (Fernandes et al. 2008). Inhibition of GABA transporters (GAT) of the predominant neural subtype, GAT1, by BDNF does not fully depend upon coactivation of $A_{2A}$ARs since it is not abolished by $A_{2A}$AR blockade; however, $A_{2A}$AR activation can facilitate this BDNF action (Vaz et al. 2008).

Whether the ability of $A_{2A}$ARs to protect retinal neurones against glutamate-induced excitotoxicity (Ferreira and Paes-de-Carvalho 2001) is due to its ability to facilitate actions of neurotrophic factors, as has been shown to occur in relation to $A_{2A}$AR-mediated neuroprotection of motor neurones (Wiese et al. 2007), requires further investigation. It is worth noting that while Wiese et al. (2007) reported a TrkB-mediated enhancement in the survival of injured facial motor neurons in vivo, TrkB receptor activation by BDNF may render spinal cord-cultured motor neurons more vulnerable to insult (Mojsilovic-Petrovic et al. 2006). Interestingly enough, in both cases, activation of $A_{2A}$ARs by endogenous adenosine was required, since $A_{2A}$AR antagonism prevented both the favorable (Wiese et al. 2007) and the deleterious (Mojsilovic-Petrovic et al. 2006) TrkB-mediated actions.

Activation of $A_{2A}$ARs enhances NGF-induced neurite outgrowth in PC12 cells and rescues NGF-induced neurite outgrowth impaired by blockade of the mitogen-activated protein kinase (MAPK) cascade, an action that requires PKA activation (Cheng et al. 2002). Furthermore, activation of $A_{2A}$ARs through Trk-dependent and phosphatidylinositol 3-kinase/Akt mechanisms promoted PC12 cell survival after NGF withdrawal (Lee and Chao 2001). A similar $A_{2A}$AR-mediated neuroprotection mechanism has been shown to occur in hippocampal neurones after BDNF withdrawal (Lee and Chao 2001). In contrast to $A_{2A}$ receptors, which usually promote the actions of neurotrophic factors, $A_{2A}$ARs inhibit neurite outgrowth of cultured dorsal root ganglion neurons in both the absence and the presence of NGF (Thevananther et al. 2001).

Besides interactions at the neurotrophin receptor level, AR activation may also induce the release of neurotrophic factors. Thus, the expression and/or release of NGF are enhanced by the activation of $A_{2A}$ARs in microglia (Heese et al. 1997) and by the activation of $A_1$ARs in astrocytes (Ciccarelli et al. 1999). $A_{2B}$ARs in astrocytes are also able to enhance GDNF expression (Yamagata et al. 2007). $A_{2A}$ARs are required for normal BDNF levels in the whole hippocampus (Tebano et al. 2008).

Interactions among purinergic, growth factor and cytokine signaling are also highly relevant in nonpathologic brain functioning, namely in the regulation of neuronal and glial maturation as well as development. In neuronal-dependent glial maturation, both ATP purinoceptors and adenosine ARs are involved (Fields and Burnstock 2006). The extracellular adenosine levels attained during high-frequency neuronal firing are sufficient to stimulate ARs in oligodendrocyte ancestor cells,

inhibiting their proliferation and stimulating their differentiation into myelinating oligodendrocytes (Stevens et al. 2002), but unfortunately the nature of the AR involved was not identified in this work. In premyelinating Schwann cells, $A_{2A}$ARs activate phosphorylation of extracellular signal-regulated kinases (ERKs), namely ERK1/2, and inhibit Schwann cell proliferation without arresting differentiation (Stevens et al. 2004).

Decreases in the levels and/or actions of neurotrophic factors have been implicated in the pathophysiological mechanisms of many diseases of the nervous system, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, diabetic neuropathies, amyotrophic lateral sclerosis, and even depression, making the use of naturally occurring neurotrophic factors a very promising approach to the treatment of these disorders (Schulte-Herbrüggen et al. 2007). However, the pharmacological administration of neurotrophic factors in vivo has not been easy so far because these molecules are unable to cross the blood–brain barrier, making invasive application strategies like intracerebroventricular infusion necessary. The evidence that $A_{2A}$ARs trigger or facilitate actions of neurotrophins upon synaptic strength and neuronal survival has led to a new therapeutic strategy: the use of adenosine $A_{2A}$AR agonists that cross the blood–brain barrier to potentiate neurotrophic actions in the brain.

However, we should particularly mention epilepsy, where neurotrophic factors have been considered both harmful, being causal mediators in the development of acquired epileptic syndromes, and also eventually useful in treating epilepsy-associated damage (Scharfman and Hen 2007; Simonato et al. 2006). On top of this controversy, we can add discrepant findings of both anticonvulsive (Huber et al. 2002) and proconvulsive (Zeraati et al. 2006) $A_{2A}$AR-mediated actions, with the proconvulsive actions being the more expected due to the usually excitatory nature of these receptors.

Finally, the crosstalk between $A_{2A}$ARs and receptors for neurotrophins also points to the need for caution about therapies with $A_{2A}$AR antagonists in neurodegenerative diseases, as has been proposed for Parkinson's disease to ameliorate L-DOPA-induced dyskinesias. For more information on $A_{2A}$ARs and Parkinson's disease, please refer to Chap. 18, "$A_{2A}$ Adenosine Receptors and Parkinson's Disease" (by Morelli et al.), in this volume. Indeed, the identification of postsynaptic $A_{2A}/D_2$ receptor interactions in the striatum, together with the findings that $A_{2A}$AR antagonists are neuroprotective in Parkinson's disease models (Chase et al. 2003) and increase dopamine synthesis from L-DOPA (Golembiowska and Dziubina 2004), led to the proposed use of $A_{2A}$AR antagonists in Parkinson's disease. On the other hand, neurotrophic factors, in particular GDNF, may be a potential therapeutic approach in the management of Parkinson's disease (Love et al. 2005; Patel et al. 2005). Enhancing GDNF actions via $A_{2A}$AR agonists (Gomes et al. 2006) may also be of high therapeutic interest. In any case, the finding that the actions of GDNF on dopamine release in the striatum are prevented by $A_{2A}$AR antagonism (Gomes et al. 2006) points to the need for further studies on the consequences of long-term therapy with $A_{2A}$ receptor blockers in neurodegenerative diseases where

neurotrophic factors may play a beneficial role. One issue that should be explored in the future is the optimal time window for combined beneficial effects of neurotrophic factors and $A_{2A}$AR agonists/antagonists. Perhaps $A_{2A}$AR antagonists may be advantageous in the late stages of neurodegenerative diseases; however, in the early stages, where an enhancement of neurotrophic factors is highly desirable, $A_{2A}$AR antagonists should be avoided and $A_2$AR agonists should perhaps be considered, in order to allow neurotrophic influences. A schematic representation of what has been reported so far on the interactions of ARs and neurotrophin receptors and on neurotrophin release, as well as the implications of these interactions at the hippocampus and striatum in relation to Alzheimer's and Parkinson's diseases, is illustrated in Fig. 4.

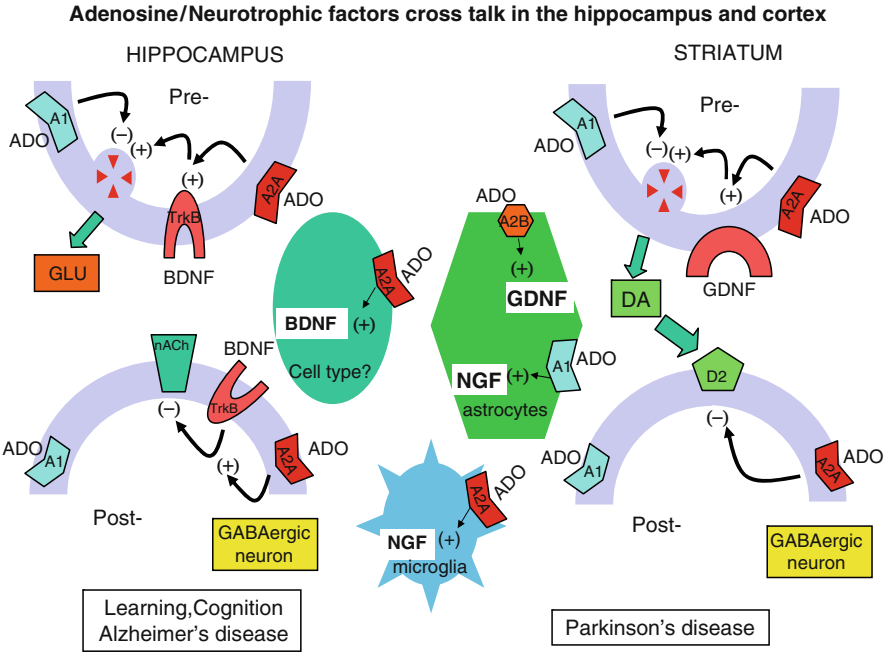


**Fig. 4** Schematic representation of what has been reported regarding the interaction between adenosine receptors (*ARs*) and neurotrophic factor receptors, as well as the influence of *ARs* on neurotrophic factor synthesis or release, focusing on brain areas with implications for learning, cognition and Alzheimer's disease (hippocampus) or Parkinson's disease (striatum). A *plus symbol* denotes facilitation and a *minus symbol* denotes inhibition of receptor functioning or neurotrophic factor synthesis or release. The positive influence of $A_{2A}$ARs upon brain-derived neurotrophic factor (*BDNF*) levels was studied in slices, so the cell type cannot be identified. See text for references. Other abbreviations: *ADO*, adenosine; *D*, dopamine receptor; *DA*, dopamine; *GDNF*, glial cell line-derived neurotrophic factor; *GLU*, glutamate; *nAChR*, nicotinic acetylcholine receptor; *NGF*, nerve growth factor; *TrkB*, tropomyosin-related kinase receptor type B

# 6 Hypoxia and Ischemia

## *6.1 Adenosine and Control of Synaptic Transmission During Hypoxia*

A very intimate relationship between hypoxia/ischemia and adenosine is well established. This relationship has been the subject of extensive reviews (de Mendonça et al. 2000), and the implications of this relationship for neuroprotection are discussed in Chap. 17, "Adenosine Receptors and Neurological Disease: Neuroprotection and Neurodegeneration" (by Stone et al.), in this volume. Thus, we will focus on hypoxia and the synaptic actions of adenosine.

High amounts of adenosine, and perhaps surprisingly, of ATP are released into the synaptic cleft during a hypoxic/ischemic insult (Frenguelli et al. 2007), leading to $A_1$AR activation and profound inhibition of synaptic transmission (Fowler 1993). This $A_1$AR-mediated inhibition promotes recovery after the insult, since blockade of $A_1$ARs reduces inhibition of synaptic transmission but also impairs recovery after reoxygenation (Sebastião et al. 2001). Similar observations have been made using $A_1$AR KO mice (Johansson et al. 2001) or through focal deletion of the presynaptic $A_1$ARs (Arrigoni et al. 2005). The facilitation of recovery of synaptic transmission after a hypoxic insult involves both presynaptic inhibition of glutamate release and subsequent reduction of NMDA receptor activation during the hypoxic episode (Sebastião et al. 2001). In other words, the neuroprotection induced by adenosine operates two well-known synaptic actions of $A_1$ARs that also occur under normoxic conditions and are of particular relevance in the case of hypoxia: a decrease of neurotransmitter release via inhibition of presynaptic calcium entry through the blocking of calcium channels (Ribeiro et al. 1979), and postsynaptically inhibiting calcium entry via inhibition of NMDA receptors (de Mendonça et al. 1995).

The mammalian brain can adapt to injurious insults such as cerebral ischemia to promote cell survival in the face of subsequent injury, a phenomenon known as ischemic preconditioning (Gidday 2006). Adenosine, through $A_1$AR, is responsible for the protective actions of ischemic preconditioning in the hippocampus; $A_{2A}$ARs are not involved in this process, whereas $A_3$AR activation is harmful to ischemic preconditioning, impairing recovery (Pugliese et al. 2003). However, hypoxia leads to a rapid (<90 min) homologous desensitization of $A_1$AR-mediated inhibition of synaptic transmission that is likely due to an internalization of $A_1$ARs in nerve terminals (Coelho et al. 2006). This may alleviate $A_1$AR-mediated functional disconnection of GABAergic neurones (Congar et al. 1995; Lucchi et al. 1996), allowing sequential time windows for a protective role of adenosine and GABA during hypoxia (Sebastião et al. 1996). Changes in the activity of adenosine-producing enzymes also occur during hypoxic/ischemic episodes. This is the case for AK, which is downregulated (Pignataro et al. 2008), and for the enzyme chain-hydrolyzing extracellular ATP, which is upregulated (Braun et al. 1998), with both processes leading to more intense extracellular adenosine production and contributing to its neuroprotection.

## 6.2 Adenosine and Control of Ventilation

The partial pressure of oxygen in the blood is sensed by the carotid body located at the bifurcation of the carotid artery. Low levels of oxygen in the arterial blood activate the carotid body and ventilation is subsequently enhanced. Since the early 1980s, when the first description of the excitatory effects of adenosine on carotid body chemoreceptor activity appeared (McQueen and Ribeiro 1981), this nucleoside has emerged as a key molecule in the regulation of chemosensory activity and ventilation (Lahiri et al. 2007). Adenosine enhances carotid body chemosensory activity either in vivo (McQueen and Ribeiro 1986) or in vitro (Runold et al. 1990), as well as ventilation (Monteiro and Ribeiro 1987). This action of adenosine is mediated by $A_{2B}$ARs in sensory terminals and $A_{2A}$ARs at carotid body cells, which are activated by its endogenous release as a consequence of a decrease in the partial pressure of oxygen around those cells (Conde et al. 2006; McQueen and Ribeiro 1986). $A_{2A}$AR mRNA, but not $A_1$AR mRNA, is expressed in type I carotid body cells, and these receptors modulate $Ca^{2+}$ homeostasis during hypoxia (Kobayashi et al. 2000a).

Upon denervation of the carotid bodies, AR agonists depress ventilation by activating $A_{2A}$ARs in the CNS (Koos and Chau 1998). Adenosine also modulates cardiorespiratory control through presynaptic actions in the nucleus tractus solitarius, where it modulates transmitter release (Spyer and Thomas 2000).

In humans, intravenous (i.v.) injection of adenosine produces hyperventilation and dyspnea resulting from direct activation of the carotid body (Watt et al. 1987). However, some secondary effects, including heat sensation, flushed face, dyspnea and chest discomfort in humans, have been reported after i.v. adenosine infusion (Uematsu et al. 2000). Adenosine enhances the ventilatory response to hypoxia but not to hypercapnia (Maxwell et al. 1986), which argues against a major contribution from the central chemosensory centers, where adenosine increases the sensitivity to hypercapnia (Phillis 2004), suggesting a major role for peripheral sensors in the ventilatory response to adenosine in humans (Lahiri et al. 2007).

The usefulness of the carotid body in maintaining oxygen homeostasis is magnified by its plasticity, which to a large extent is due to changes in gene expression (Lahiri et al. 2007). The contribution of purines to the control of carotid body activity may also be developmentally regulated. For example, the $A_{2A}$AR and $D_2$ dopaminergic receptors are differentially expressed in glomus cells during development, with greater relative expression of mRNA message for the $A_{2A}$AR found in earlier stages, and for the $D_2$ receptors in the adult animal (Gauda et al. 2000). $A_{2B}$ARs in the carotid body are slightly downregulated within 24 h exposure to moderate (10% $O_2$) hypoxia (Ganfornina et al. 2005), whereas $A_{2A}$ARs are upregulated by chronic hypoxia, at least in PC12 cells (Seta and Millhorn 2004). As occurs in brief hypoxic/ischemic episodes (see Sect. 6.1), chronic hypoxia decreases the expression of AK, adenosine deaminase and the adenosine transporter, while it increases the expression of ecto-5′-nucleotidase (Kobayashi et al. 2000b). All of these hypoxia-induced changes in the expression of ARs and the enzymes involved in the control of extracellular adenosine levels may contribute to a protective adaptation to hypoxia.

### 6.2.1 Adenosine and Respiration in the Newborn

The inhibitory effect of CNS $A_{2A}$AR activation on respiratory drive is more evident early in life, and is mediated via GABAergic inputs to the inspiratory timing neural circuitry (Mayer et al. 2006). Blockade of these receptors is probably the mechanism by which xanthine therapy alleviates apnea in prematures (Aranda and Turmen 1979; Bairam et al. 1987; Uauy et al. 1975). Indeed, blockade of $A_{2A}$ARs blunts the respiratory roll-off response to hypoxia in newborn lambs (Koos et al. 2005). Xanthine therapy in the newborn may, however, increase the risk of seizures (see Table 1).

## 7 Role of ARs in Pain

Pain can have multiple causes and origins, and therefore the ability of adenosine to influence pain also has multiple sites of action and diverse mechanisms. Activation of $A_1$AR in the spinal cord produces antinociceptive properties in acute nociceptive, inflammatory and neuropathic pain tests (Sawynok 2007; Sawynok and Liu 2003). In humans, the first evidence for antinociceptive actions of adenosine was detected during adenosine infusion (i.v.), which had beneficial effects in two patients with neuropathic pain (Sollevi et al. 1995). A few years later, the same group showed that adenosine can also reduce secondary hyperalgesia in two human models of cutaneous inflammatory pain (Sjölund et al. 1999). Although peripheral $A_2$AR activation can exacerbate pain responses (Sawynok 1998), its anti-inflammatory action may also contribute to decreasing inflammatory pain. As a consequence, $A_1$AR agonists have entered clinical trials for neuropathic pain, whereas $A_{2A}$AR agonists are entering clinical trials as anti-inflammatory agents (Gao and Jacobson 2007). There is also growing interest in the use of allosteric enhancers of $A_1$AR activation due to the putative tissue selectivity of $A_1$ARs. Allosteric modulation of adenosine $A_1$ARs reduces allodynia, and this has been shown to occur not only after intrathecal injection but also after systemic administration (Pan et al. 2001).

The pain-relieving effect of activating $A_1$ARs at the level of the spinal cord is related to their ability to presynaptically inhibit excitatory transmission to neurons of the substantia gelatinosa (Lao et al. 2001). The inhibition of NMDA receptors by adenosine (see Sect. 5.2.1) probably also occurs at the level of the spinal cord (DeLander and Wahl 1988) and contributes to a reduction of central sensitization and plasticity mechanisms involved in chronic pain. In contrast, adenosine $A_{2A}$AR activation sensitizes peripheral afferent fibers that project to the spinal cord, enhancing nociception (Hussey et al. 2007). Accordingly, mice lacking the $A_{2A}$AR have reduced responses to thermal nociceptive stimuli (Ledent et al. 1997), whereas mice lacking the $A_1$AR show increased nociceptive response (Wu et al. 2005)

The peripheral administration of adenosine in humans produces pain responses resembling those generated under ischemic conditions (Sawynok 1998). This pain-initiating effects of adenosine are augmented by substance P (Gaspardone et al.

1994) and nicotine (Sylvén et al. 1990), and are usually a limiting factor in the use of adenosine-related compounds for the control of chronic pain. Activation of $A_3$ARs produces pain due to the release of histamine and 5-hydroxytryptamine from mast cells and subsequent actions on the sensory nerve terminal (Sawynok 1998). However, in spite of these algogenic consequences of peripheral administration of adenosine, its net action on pain processing is inhibitory, since enhancers of extracellular adenosine levels have antinociceptive action (see below).

Due to the simultaneous $A_1$AR-mediated antinociceptive and $A_{2A}$AR-mediated anti-inflammatory actions of adenosine, there has been increasing interest in the development of drugs that, by influencing extracellular adenosine levels, could have analgesic actions. Successful examples include inhibitors of AK (see Sect. 3), whose spinally-mediated antinociceptive properties were noted over a decade ago (Keil and DeLander 1992). Most likely due to the anti-inflammatory actions of adenosine, AK inhibitors administered orally are more effective at reducing inflammatory pain than neuropathic or acute pain (Jarvis et al. 2002). By comparing the antinociceptive and anti-inflammatory properties of AK inhibitors administered at the ipsilateral or contralateral sides of the injury, it was concluded that much of the anti-inflammatory action is locally mediated, whereas the antinociceptive action is systemically mediated, exerted predominantly at the level of the spinal dorsal horn (Poon and Sawynok 1999). Indeed, AK inhibitors are able to reduce the increased *c-Fos* expression in the spinal dorsal horn induced by peripheral injection of an inflammatory (carrageenan) substance (Poon and Sawynok 1999).

Antidepressants are widely used in the treatment of neuropathic pain, but their analgesic efficacy seems to occur irrespective of mood-altering effects, and may involve an increase in extracellular adenosine levels. This has been shown after either acute (Esser and Sawynok 2000) or chronic (Esser et al. 2001) amitriptyline administration in rat models of neuropathic pain. Similarly, endogenous adenosine seems to be involved in the antiallodynic action of amitriptyline in a rat model of painful diabetic neuropathy (Ulugol et al. 2002). As pointed out by Esser and Sawynok (2000), the manipulation of endogenous adenosine by amitriptyline, while important, is unlikely to be the sole mechanism underlying its ability to reduce pain, but the attenuation of its effect by modest doses of caffeine (within those levels easily attained in humans after two cups of strong coffee) raise the possibility that dietary caffeine consumption may influence the efficacy of amitriptyline in alleviating neuropathic pain in humans.

Increases in adenosine levels may contribute to the analgesic action of opioids. An increase in adenosine levels in the cerebrospinal fluid has been detected in humans following intrathecal administration of morphine (Eisenach et al. 2004). It is of interest that in neuropathic rats the release of adenosine induced by morphine is reduced (Sandner-Kiesling et al. 2001), which may explain a decreased efficacy and potency of opioids in the treatment of neuropathic pain. Moreover, modifications in the expression of several types of opioid receptors were recently detected in mice lacking the $A_{2A}$AR gene (Bailey et al. 2002), suggestive of a functional interplay between $A_{2A}$AR and opioid receptors with respect to pain modulation.

A critical review of the applications of adenosine and ATP in pain control, summarizing most of the human studies, suggests a high potential for adenosine compounds to alleviate pain (Hayashida et al. 2005). This review suggests that the doses, the routes and the timing of administration together with the tissue penetration of the drugs must be taken into consideration, and that there is a need for more basic research to clarify several points. Caffeine, via its antagonistic actions on ARs, can modulate pain; however, as recently discussed (Shapiro 2007), the type of effect (e.g., generation or alleviation of headache) depends on the site of action as well as the dosage and timing of exposure. Both $A_{2A}$ and $A_{2B}$ ARs are probably involved in the interaction between paracetamol and caffeine in pain control. Blockade of $A_{2B}$ARs causes an enhancement of the action of paracetamol in tail immersion and hot-plate tests in mice, and blockade of $A_{2A}$ARs produces an antinociceptive effect, even in the absence of paracetamol (Godfrey et al. 2006). Moreover, theophylline ameliorates chest pain in patients with a hypersensitive esophagus, possibly by altering adenosine-mediated nociception (Rao et al. 2007).

As a potent vasodilator, CGRP, which is released by the trigeminocerebrovascular system, plays a key role in the pathophysiology of migraine headache; antagonism of CGRP has been suggested as a promising new approach for the treatment of this condition (Goadsby 2008). Another approach to blocking the trigeminovascular system and CGRP to treat migraine headache may include the use of $A_1$AR agonists. Activation of $A_1$ARs inhibits trigeminovascular activation by acting on the trigeminal nucleus and by inhibiting the release of CGRP in the cranial circulation, with this second action being attributable to activation of $A_1$ARs on peripheral terminals of the trigeminal nerve (Goadsby et al. 2002). Tonic activation of $A_1$ARs may also prevent the facilitatory actions of CGRP, as has been shown to occur in the hippocampus (Sebastião et al. 2000b). Interestingly, $A_{2A}$AR activation facilitates the actions of CGRP (Correia-de-Sá and Ribeiro 1994a; Sebastião et al. 2000a, b), but the relevance of these observations to an approach for the treatment of migraine headache (i.e., with $A_2$AR antagonists) remains to be established.

## 8 Caffeine and ARs

Ever since the delights of tea were first discovered by Emperor Shen Nung in 2737 BC, methylxanthines, including caffeine, have been widely consumed by humans all over the world. The broad caffeine intake associated with common beverages, together with the impact of xanthines on biomedical research, prompted many studies that have focused on specific caffeine effects rather than using it as a tool to antagonize ARs (Daly 2007; Ferré 2008). Indeed, as a pharmacological tool, caffeine is no longer very useful, because its affinity for ARs is low and its selectivity towards the different ARs is also very poor. It is interesting to note that the first proposal for the existence of an $A_3$AR was based upon pharmacological characteristics, namely high affinity for agonists and xanthine sensitivity (Ribeiro and Sebastião 1986). Cloning and cellular expression of the rat $A_3$AR (Zhou et al. 1992) challenged these criteria,

since the rat $A_3$ receptor is xanthine insensitive and has low agonist affinity. Cloning and expression of the human $A_3$AR (Salvatore et al. 1993) reversed the situation again, since the human $A_3$AR is xanthine sensitive and a high-affinity receptor for $A_3$AR ligands. For more information on the affinity of the human $A_3$AR for $A_3$AR ligands, the reader is referred to Chap. 5, "Medicinal Chemistry of the $A_3$ Adenosine Receptor: Agonists, Antagonists, and Receptor Engineering" (Jacobson et al.), in this volume.

However, xanthines such as caffeine have other biological actions besides AR antagonism. They inhibit PDEs (PDE4, PDE1, PDE5), promote calcium release from intracellular stores, and interfere with $GABA_A$ receptors (Daly 2007). Caffeine analogs can be developed to target any of these mechanisms rather than ARs, and this may be explored therapeutically (Daly 2007), but in the case of caffeine, the effects seen at the low doses taken in during normal human consumption are mostly due to AR antagonism (Fredholm et al. 1999). Due to its ability to antagonize ARs, to cross the blood–brain barrier, and also due to the low risk of intake, caffeine has therapeutic potential in central nervous system dysfunctions (e.g., Alzheimer's disease and Parkinson's disease). Adverse effects of caffeine may include anxiety, hypertension, drug interactions, and withdrawal symptoms (Daly 2007). In human volunteers, caffeine improves cognition; however, it also affects sleep (see Table 1). Moreover, a relationship between adenosine $A_{2A}$ARs and genetic variability in caffeine metabolism associated with habitual caffeine consumption has been proposed (Cornelis et al. 2007), which provides a biological basis for caffeine consumption. In this study, persons with the *ADORA2A TT* genotype were significantly more likely to consume less caffeine than carriers of the *C* allele.

The therapeutic or adverse effects of caffeine are quite different depending on whether it is administered chronically or acutely. For example, chronic caffeine intake, which increases plasma concentrations of adenosine (Conlay et al. 1997), may be neuroprotective. This contrasts with the consequences of acutely antagonizing $A_1$ARs (de Mendonça et al. 2000). Chronic AR antagonism with caffeine may also influence cognition and motor activity in a way that resembles the acute effects of AR agonists (Jacobson et al. 1996). Such opposite actions of chronic versus acute treatment not only have important implications for the development of xanthine-based compounds as therapeutic agents but also constitute a frequent confounding parameter in research. Upregulation of $A_1$ARs after chronic AR antagonism with xanthines does occur, but $A_{2A}$AR levels apparently do not change; in addition, there are changes in the levels of receptors for neurotransmitters with chronic administration of xanthines, namely a marked decrease in β-adrenergic receptors and an increase in 5-HT and $GABA_A$ receptors (Jacobson et al. 1996). The increased expression of $A_1$ARs in response to chronic antagonism of ARs by caffeine, as compared with $A_{2A}$ARs, may lead to a shift in the $A_1/A_{2A}$ AR balance after prolonged caffeine intake (Ferré 2008). Moreover, chronic caffeine treatment leads to modifications in the function of the $A_1$R–$A_{2A}$R heteromer and this may, in part, be the scientific basis for the strong tolerance to the psychomotor effects of chronic caffeine (Ciruela et al. 2006).

Furthermore, alteration of astrocytogenesis via $A_{2A}$AR blockade during brain development raises the possibility that postnatal caffeine treatment could have long-term negative consequences on brain function, and should perhaps be avoided in breast-feeding mothers (Desfrere et al. 2007)

## *8.1 Influence on Brain Function and Dysfunction*

### 8.1.1 Sleep

One of the main reasons for drinking a cup of strong coffee is to repel sleep. Most studies on ARs and sleep regulation in humans rely upon consequences of caffeine ingestion by human volunteers (see Table 1), and it is now widely accepted that caffeine prolongs wakefulness by interfering with the key role of adenosine upon sleep homeostasis (Landolt 2008). In an innovative review of the role of adenosine upon sleep regulation, Porkka-Heiskanen et al. (2002) proposed adenosine as a sleeping factor and hypothesized that adenosine functions in a similar way to neuroprotection against energy depletion. In the critical arousal area (basal forebrain), extracellular adenosine levels start to rise in response to prolonged neuronal activity during wakeful periods. This increase leads to a decrease in neuronal activity, and sleep is induced before the energy balance in the whole brain is affected. Microdialysis measurements performed in freely moving cats showed an increase in the concentrations of adenosine during spontaneous wakefulness, and adenosine transport inhibitors mimicked the sleep-wakefulness profile that occurs after prolonged wakefulness (Porkka-Heiskanen et al. 1997). In contrast, AR antagonists like caffeine increase wakefulness (see Table 1). Prolonged wakefulness induces signs of energy depletion in the brain, which induces an increase in sleep (Benington and Heller 1995). Molecular imaging provided evidence for an $A_1$ receptor upregulation in cortical and subcortical brain regions after prolonged wakefulness in humans (Elmenhorst et al. 2007). Adenosinergic mechanisms contribute to individual differences associated with sleep deprivation sensitivity in humans (Rétey et al. 2006); furthermore, a genetic variation in the adenosine $A_{2A}$AR gene may contribute to individual sensitivity to the effects of caffeine on sleep (Rétey et al. 2007, see Table 1).

It is well documented that $A_1$ARs are involved in sleep regulation through the inhibition of ascending cholinergic neurons of the basal forebrain (Basheer et al. 2004). However, more recent studies, which include experiments with $A_{2A}$ and $A_1$ AR KO mice, indicate that $A_{2A}$ARs (most probably localized in the ventrolateral preoptic area of the hypothalamus) also play a crucial role in the sleep-promoting effects of adenosine and the arousal-enhancing effects of caffeine (Huang et al. 2005). These studies suggest that $A_{2A}$AR antagonists may represent a novel approach as potential treatments for narcolepsy and other sleep-related disorders (Ferré et al. 2007b). Adenosine $A_{2A}$ARs in the pontine reticular formation promote acetylcholine release, rapid eye movement (REM) and non-REM sleep in mice. This effect on non-REM sleep is probably due to $A_{2A}$AR-induced enhancement of GABAergic

inhibition of arousal promoting neurons (Coleman et al. 2006). In addition to its effect in the basal forebrain, adenosine exerts its sleep-promoting effect in the lateral hypothalamus by $A_1$AR-mediated inhibition of hypocretin/orexin neurons (Liu and Gao 2007; Thakkar et al. 2008).

In conclusion, the two high-affinity ARs, the $A_1$ and the $A_{2A}$ ARs, affect multiple mechanisms in several brain areas involved in the regulation of sleep and arousal. Therefore, the influences of caffeine upon sleep, felt by many humans and recently also documented in controlled studies in healthy volunteers (see Table 1), can be attributed to both $A_1$ and $A_{2A}$ AR blockade. As discussed above (see Sect. 8), chronic caffeine consumption may alter AR function and the $A_1/A_{2A}$ AR balance and consequently the influence of both ARs upon sleep.

### 8.1.2 Epilepsy

There are several clinical reports on caffeine or theophylline intake and seizure susceptibility (Kaufman and Sachdeo 2003; Mortelmans et al. 2008), but surprisingly, no mention is made of the main cause of seizure induction by these drugs, AR antagonism.

Indeed, after the initial observation that adenosine has an anticonvulsant action (Barraco et al. 1984), the therapeutic potential of adenosine-related compounds in epilepsy was immediately pointed out (Dragunow et al. 1985), and it is now widely accepted that adenosine is an endogenous anticonvulsant, an action mediated by inhibitory $A_1$ARs that restrain excessive neuronal activity. Other ARs are, however, involved in seizure control, though their roles are most frequently related to exacerbating seizures. An influence of $A_3$ and $A_2$ ARs in $GABA_A$ receptor stability has been suggested recently (Roseti et al. 2008), based on the observation that $A_3$ or $A_{2B}$ AR antagonists reduce rundown of $GABA_A$ currents. $A_{2A}$ARs, by promoting neuronal excitability, may also increase seizure susceptibility. Indeed, $A_{2A}$AR KO mice are less sensitive to pentylenetetrazol-induced seizures (El Yacoubi et al. 2008).

It has been shown that $A_1$AR activation by locally released adenosine is an efficient way to keep an epileptic focus localized (Fedele et al. 2006). Therefore, attention is now focused on the development of biocompatible materials for adenosine-releasing intrahippocampal implants (Wilz et al. 2008). In line with the evidence for the antiepileptic role of $A_1$ARs, $A_1$AR KO mice are more susceptible to seizures and develop lethal status epilepticus after experimental traumatic brain injury (Kochanek et al. 2006). There are, however, limitations on the use of $A_1$AR agonists as anticonvulsant drugs due to their pronounced peripheral side effects, like cardiac asystole as well as central side effects like sedation (Dunwiddie 1999). One possibility would be the use of partial agonists, which are more likely to display tissue selectivity. An $N^6$,C8-disubstituted adenosine derivative with low efficacy towards $A_1$AR activation in whole brain membranes but with high efficacy as an inhibitor of hippocampal synaptic transmission was identified (Lorenzen et al. 1997). Another approach that has been more intensely explored is with the use of compounds that increase the extracellular concentrations of adenosine. This has been

attempted with AK inhibitors, which showed beneficial effects in animal models of epilepsy and an improved preclinical therapeutic index over direct-acting AR agonists (McGaraughty et al. 2005). An even more refined approach would be local reconstitution of the inhibitory adenosinergic tone by intracerebral implantation of cells engineered to release adenosine, and this has been done using AK-deficient cells (Güttinger et al. 2005). The reverse also holds true, since transgenic mice overexpressing AK in the brain have increased seizure susceptibility (Fedele et al. 2005). Furthermore, intrahippocampal implants of AK-deficient stem cell-derived neural precursors suppress kindling epileptogenesis (Li et al. 2007). The above evidence suggests that adenosine-augmenting cell and gene therapies may lead to improved treatment options for patients suffering from intractable epilepsy (Boison 2007).

AK is mostly expressed in astrocytes (Studer et al. 2006), and overexpression of AK after seizures, with consequent reduced adenosine inhibitory tone, contributes to seizure aggravation (Fedele et al. 2005). However, release of interleukin-6 (IL-6) from astrocytes induces an upregulation of $A_1$ARs in both astrocytes (Biber et al. 2001) and neurons (Biber et al. 2007). This leads to an amplification of $A_1$AR function, enhances the responses to readily released adenosine, enables neuronal rescue from glutamate-induced death, and protects animals from chemically induced convulsing seizures (Biber et al. 2007). Indeed, IL-6 KO mice are more susceptible to seizures and lack the well-known seizure-induced upregulation of $A_1$ARs (Biber et al. 2007).

Seizure-induced release of neurotrophic factors, such as BDNF, may have beneficial and aggravating actions upon epilepsy, with the beneficial ones being mostly related to promotion of cell survival and the deleterious ones being related to excessive cell proliferation and neuronal sprouting (Simonato et al. 2006). Adenosine, through $A_{2A}$AR activation, triggers and facilitates BDNF actions in neurons (Diógenes et al. 2004; Fontinha et al. 2008, see Sect. 5.3 above), but the relevance of this interplay for epilepsy remains to be explored. This may be of particular relevance whenever designing therapies that lead to enhanced extracellular adenosine levels, since besides $A_1$ARs, $A_{2A}$ARs can also be activated.

### 8.1.3 Cognition, Learning, and Memory

Endogenous adenosine, through $A_1$ARs, inhibits long-term synaptic plasticity phenomena such as LTP (de Mendonça and Ribeiro 1994), LTD, and depotentiation (de Mendonça et al. 1997c). In accordance, $A_1$AR antagonists have been proposed for the treatment of memory disorders (Stone et al. 1995). Cognitive effects of caffeine are mostly due to its ability to antagonize adenosine $A_1$ARs in the hippocampus and cortex, the brain areas mostly involved in cognition, but as already discussed in detail (see Fredholm et al. 1999), positive actions of caffeine on information processing and performance may also be attributed to improvements in behavioral routines, arousal enhancement and sensorimotor gating. This interpretation was supported by the observation that the AR antagonist theophylline enhances spatial memory performance only during the light period, which is the time of

sleepiness in rats (Hauber and Bareiss 2001). Independently of the processes caffeine or theophylline use to improve cognition, there is little doubt that the beneficial effects most of us feel after a few cups of coffee or tea are due to the actions of these psychoactive substances upon ARs. Recent evidence that blockade of $A_1$ receptors improves cognition came from a study using a mixed $A_1/A_{2A}$ receptor antagonist, ASP5854 (Mihara et al. 2007). This orally active drug could reverse scopolamine-induced memory deficits in rats, whereas a specific adenosine $A_{2A}$AR antagonist, KW-6002, did not. Reduced $A_{2A}$AR activation may also be relevant for cognitive improvements, since $A_{2A}$AR KO mice have improved spatial recognition memory (Wang et al. 2006). Accordingly, overexpression of $A_{2A}$ARs leads to memory deficits (Giménez-Llort et al. 2007).

### 8.1.4 Alzheimer's Disease

There is the possibility that chronic intake of caffeine during one's lifetime might protect from cognitive decline associated with aging. Elderly women who drank relatively large amounts of coffee over their lifetimes gave better performances in memory and other cognitive tests than nondrinkers (Johnson-Kozlow et al. 2002). A case–control study was specifically designed to evaluate whether chronic intake of caffeine might be related to a lower risk of Alzheimer's disease (Maia and de Mendonça 2002), the most common form of dementia. Levels of caffeine consumption in the 20 years that preceded the diagnosis in patients were compared with those taken by age- and sex-matched controls with no signs of cognitive impairment. Data analysis showed that caffeine intake was inversely associated with the risk of Alzheimer's disease and that this association was not explained by several possible confounding variables related to habits and medical disorders (Maia and de Mendonça 2002). This was confirmed in a larger-scale study (4,197 women and 2,820 men) with similar objectives, showing that the psychostimulant properties of caffeine appear to reduce cognitive decline in aged women without dementia (Ritchie et al. 2007).

Long-term protective effects of dietary caffeine intake were also shown in a controlled longitudinal study involving a transgenic murine model of Alzheimer's disease. Caffeine was added to the drinking water of mice between four and nine months of age, with behavioral testing done during the final six weeks of treatment; the results revealed that moderate daily intake of caffeine may delay or reduce the risk of cognitive impairment in these mice (Arendash et al. 2006). Amnesia can be induced experimentally in mice by central administration of β-amyloid peptides, a process that involves cholinergic dysfunction (Maurice et al. 1996). Acute i.v. administration of caffeine or $A_{2A}$AR antagonists afforded protection against β-amyloid-induced amnesia (Dall'Igna et al. 2007). These acute effects of $A_{2A}$AR blockade are somewhat unexpected, because $A_{2A}$ARs are known to facilitate cholinergic function (namely in the hippocampus; Cunha et al. 1994b), and therefore either adenosine $A_{2A}$AR agonists or $A_1$AR antagonists (to prevent $A_1$AR-mediated inhibition of acetylcholine release) were expected to be cognitive enhancers. Indeed, the

most widely used drugs in Alzheimer's disease are directed towards an increase in cholinergic function by inhibiting acetylcholinesterase (Doody et al. 2001). These apparent discrepancies point towards the need for more basic research to understand the biological basis and the potential benefits of the emerging adenosine-based therapies for Alzheimer's disease.

### 8.1.5 Anxiety

The inhibitory action of $A_1$ARs on the nervous system, together with the identification of crosstalk mechanisms between benzodiazepines and ARs (Boulenger et al. 1982) and transporters (Bender et al. 1980), soon suggested that adenosine could mediate the anxiolytic action of several centrally active drugs (Phillis and Wu 1982). The possibility that drugs that facilitate $A_1$AR-mediated actions could be effective for anxiety was supported by the observations that $A_1$AR agonists have anxiolytic actions in rodents (Florio et al. 1998; Jain et al. 1995). Accordingly, $A_1$AR KO mice showed increased anxiety-related behavior (Johansson et al. 2001), but this also holds true for $A_{2A}$AR KO mice (Ledent et al. 1997). $A_1$ and $A_{2A}$ARs are involved in benzodiazepine withdrawal signs. In mice, these signs of withdrawal are manifested by increased seizure susceptibility, and agonists of $A_1$ARs (Listos et al. 2005) or $A_{2A}$ARs (Listos et al. 2008) attenuate them. The potential of $A_1$AR agonists to reduce the anxiogenic effects during ethanol withdrawal has also been suggested (Prediger et al. 2006).

It is of interest that patients suffering from panic disorder, a serious form of anxiety disorder, appear to be particularly sensitive to small amounts of caffeine (Boulenger et al. 1984). Caffeine is well known to promote anxious behavior in humans and animal models, and can precipitate panic attacks (Klein et al. 1991). It is, however, worth noting that chronic and acute caffeine consumption may lead to quite different consequences with respect to the function of ARs (see above; Boulenger et al. 1983; Jacobson et al. 1996). The short-term anxiety-like effect of caffeine in mice may not be related solely to the blockade of $A_1$ and $A_{2A}$ARs, since it is not shared by selective antagonists of each receptor (El Yacoubi et al. 2000). In contrast, anxiolytic effects of a xanthine derivative have been reported, but this is most probably related to agonist activity at serotonin receptors (Daly 2007).

A significant association between self-reported anxiety after caffeine administration and two linked polymorphisms of the $A_{2A}$AR gene has been reported (Alsene et al. 2003). Furthermore, evidence for a susceptibility locus for panic disorder, either within the $A_{2A}$AR gene or in a nearby region of chromosome 22, was reported (Deckert et al. 1998, Hamilton et al. 2004). However, this positive association between $A_{2A}$AR gene polymorphism and panic disorder may not occur in the Asian population (Lam et al. 2005), suggesting an ethnicity-dependent association.

### 8.1.6 Depression

$A_{2A}$AR KO mice and wild-type mice injected with $A_{2A}$AR antagonists were found to be less sensitive to "depressant" challenges than controls (El Yacoubi et al. 2001),

suggesting that blockade of adenosine $A_{2A}$ARs might be an interesting target for the development of antidepressant agents. This antidepressant-like effect of selective $A_{2A}$AR antagonists is probably linked to an interaction with dopaminergic transmission, possibly in the frontal cortex, since administration of the dopamine $D_2$ receptor antagonist haloperidol prevented antidepressant-like effects elicited by selective $A_{2A}$AR antagonists in the forced swim test (putatively involving cortex), whereas it had no effect on stimulant motor effects of selective $A_{2A}$AR antagonists (putatively linked to ventral striatum) (El Yacoubi et al. 2003). Depression is frequently associated with loss of motivation and psychomotor slowing. In this context, it is interesting to note that $A_{2A}$ARs in the nucleus accumbens appear to regulate effort-related processes and action that could be related to modulation of the ventral striatopallidal pathway (Mingote et al. 2008).

Besides $A_{2A}$ARs, $A_1$ARs are also probably involved in the antidepressant-like effect of adenosine (Kaster et al. 2004), which may be of consequence for interactions with the opioid system (Kaster et al. 2007).

It is worth noting that that deep brain stimulation, now widely used by neurosurgeons to treat tremor and other movement disorders, as well as in a number of psychiatric diseases, including obsessive–compulsive disorders and depression, produces its effects by inducing the release of ATP, which is subsequently converted extracellularly to adenosine (Bekar et al. 2008).

Results from clinical and basic studies have demonstrated that stress and depression decrease BDNF expression and neurogenesis, leading to the neurotrophic hypothesis of depression (Castrén et al. 2007; Kozisek et al. 2008). How adenosine $A_{2A}$AR-dependent facilitation of BDNF actions on hippocampal synapses (see Sect. 5.3), namely enhancement of synaptic transmission (Diógenes et al. 2004) and enhancement of synaptic plasticity (Fontinha et al. 2008), may contribute to these antidepressive actions of adenosine remains to be established.

### 8.1.7 Schizophrenia

No study, so far, has directly evaluated the influence of caffeine in schizophrenia, but there is growing evidence that adenosine dysfunction may contribute to the neurobiological and clinical features of schizophrenia (Lara et al. 2006). Indeed, adenosine, via activation of $A_1$ and $A_{2A}$ARs, is uniquely positioned to influence glutamatergic and dopaminergic neurotransmission, the two neurotransmitter systems that are most affected by the disease. It is possible that an adenosine inhibitory deficit may emerge, resulting in reduced control of dopamine activity and increased vulnerability to excitotoxic glutamate action in the mature brain. Interactions between $A_{2A}$ARs and $D_2$ receptors allow further opportunity for mutual modulation between the adenosine and dopamine systems (Fuxe et al. 2007). These mechanisms could provide a rationale for an antipsychotic-like profile for AR agonists, in particular $A_{2A}$AR agonists to promote a reduction in $D_2$ receptor signaling (Fuxe et al. 2007) and $A_1$AR agonists to promote a reduction in dopamine release (Lara et al.

2006). Indeed, dypiridamole, a well-known inhibitor of adenosine transporters and therefore an enhancer of extracellular adenosine levels, may be of some therapeutic interest in schizophrenic patients (Akhondzadeh et al. 2000).

Reduced NMDA receptor function may contribute to the cognitive and negative symptoms of schizophrenia (Ross et al. 2006). The relationships between adenosine and NMDA receptor function are complex and may operate in opposite ways. Thus, NMDA receptor activation induces adenosine release (Hoehn and White 1989; Schotanus et al. 2006), and therefore NMDA receptor hypofunction may induce a decrease in adenosine-mediated actions. On the other hand, NMDA receptor activation suppresses neuronal sensitivity to adenosine (Nikbakht and Stone 2001). In addition, both $A_1$ and $A_{2A}$ARs can influence NMDA receptor functioning, with both receptors being able to inhibit NMDA currents in different brain areas (see Sect. 5.2.1 above).

### 8.1.8 Huntington's Disease

The role played by ARs in Hungtington's disease was recently reviewed and discussed (Popoli et al. 2007) and is a topic in another chapter in this volume, Chap. 17, "Adenosine Receptors and Neurological Disease: Neuroprotection and Neurodegeneration" (by Stone et al.). Therefore, only a few considerations will be mentioned in this section. The complexity inherent to a genetically based, slowly progressing neurodegenerative disease; the different experimental models, which are very frequently nonchronic or subchronic models; as well as changes in receptor levels due to cell loss or to prolonged drug administration give an apparent contradictory picture of the AR involvement in this disease. The pre- versus postsynaptic localization of ARs, in particular $A_{2A}$ARs, which have highly distinct roles in striatal function according to their synaptic localization, may also contribute to conflicting neuroprotective/neurotoxic consequences of AR manipulation (Blum et al. 2003). Indeed, $A_1$AR agonists (Blum et al. 2002), $A_{2A}$AR agonists (Popoli et al. 2007), as well as $A_{2A}$AR antagonists (Domenici et al. 2007) are all able to influence diverse symptoms in experimental models of Huntington's disease.

Another aspect that applies to all neurodegenerative diseases, and that may be particularly relevant in the case of Huntington's disease, is related to the loss of neurotrophic support. Huntington's disease is caused by a mutation in a protein named huntingtin that, in its mutated form, is neurotoxic. It happens that wild-type huntingtin upregulates transcription of BDNF (Zuccato et al. 2001), and decreased BDNF levels may be an initial cause of neuronal death in this disease. $A_{2A}$AR activation can facilitate or even trigger BDNF actions in the brain (Diógenes et al. 2004, 2007; Fontinha et al. 2008), pointing to the possibility that $A_{2A}$AR activation, at least in the early stages of the disease, may rescue striatal neurons from death due to diminished trophic support by BDNF. It is worth noting that $A_{2A}$ARs have a dual action in Huntington's disease (Popoli et al. 2007). The ability of $A_{2A}$ARs to facilitate the actions of BDNF, which is clearly deficient in this neurodegenerative disease (Zuccato and Cattaneo 2007), is most probably some of the positive influence that $A_{2A}$ARs have on the disease.

### 8.1.9 Parkinson's Disease

A significant association between higher caffeine intake and lower incidence of Parkinson's disease was reported some years ago (Ross et al. 2000). Moreover, the beneficial effects of caffeine in Parkinson's disease patients have also been reported (Kitagawa et al. 2007, see Table 1). Furthermore, caffeine administered before levodopa may improve its pharmacokinetics in some patients with Parkinson's disease (Deleu et al. 2006).

Caffeine has well known stimulatory actions upon locomotion due to the antagonism of $A_{2A}$ and $A_1$ARs in the striatum (Ferré 2008), and in most animal models of Parkinson's disease, antagonizing $A_{2A}$ARs attenuates some disease symptoms. Since a full chapter in this volume is devoted to ARs and Parkinson's disease, Chap. 18, "Adenosine $A_{2A}$ Receptors and Parkinson's Disease" (by Morelli et al.), and since a recent sequence of reviews were published as proceedings of a meeting on the topic (Chen et al. 2007; Fredholm et al. 2007; Morelli et al. 2007; Schiffmann et al. 2007), we will only highlight a point that is focused upon less, which concerns interactions between adenosine and neurotrophic factors. The putative role of the neurotrophic factor GDNF in slowing or halting disease progression through the facilitation of neuronal survival (Peterson and Nutt 2008) and the facilitatory action of $A_{2A}$ARs upon the actions of GDNF in striatal dopaminergic nerve endings (Gomes et al. 2006) indicate the need for great caution when blocking $A_{2A}$ARs in the early phases of Parkinson's disease. Indeed, if the actions of GDNF in dopaminergic neurons depend upon coactivation of $A_{2A}$ARs (Gomes et al. 2006), it is highly probable that blockade of $A_{2A}$ARs will be deleterious during the time window when it is possible to rescue neurons with trophic support.

Another relevant consideration is related to the recent finding (Bekar et al. 2008) that deep brain stimulation, a procedure now used to reduce tremor in Parkinson's disease patients, involves the release of considerable amounts of ATP, with its subsequent extracellular metabolism to adenosine. Activation of $A_1$ARs by adenosine during this procedure is an essential step in reducing tremor and controlling spread of excitability, thereby reducing the side effects of deep brain stimulation. However, since $A_{2A}$ARs are highly expressed in thalamic areas, it could be expected that $A_{2A}$ARs are also activated during deep brain stimulation. Thus, in the late stages of the disease, where it is desirable to prevent $A_{2A}$AR-mediated inhibition of dopamine $D_2$ receptor function, the use of an $A_{2A}$AR antagonist in combination with deep brain stimulation may be beneficial.

## 9 Drug Addiction and Substances of Abuse

It is currently believed that molecular adaptations of the corticoaccumbens glutamatergic synapses are involved in compulsive drug seeking and relapse. The high density of $A_{2A}$ARs that pre- and postsynaptically regulate glutamatergic transmission in this brain area lead to the proposal that $A_{2A}$AR-related compounds could

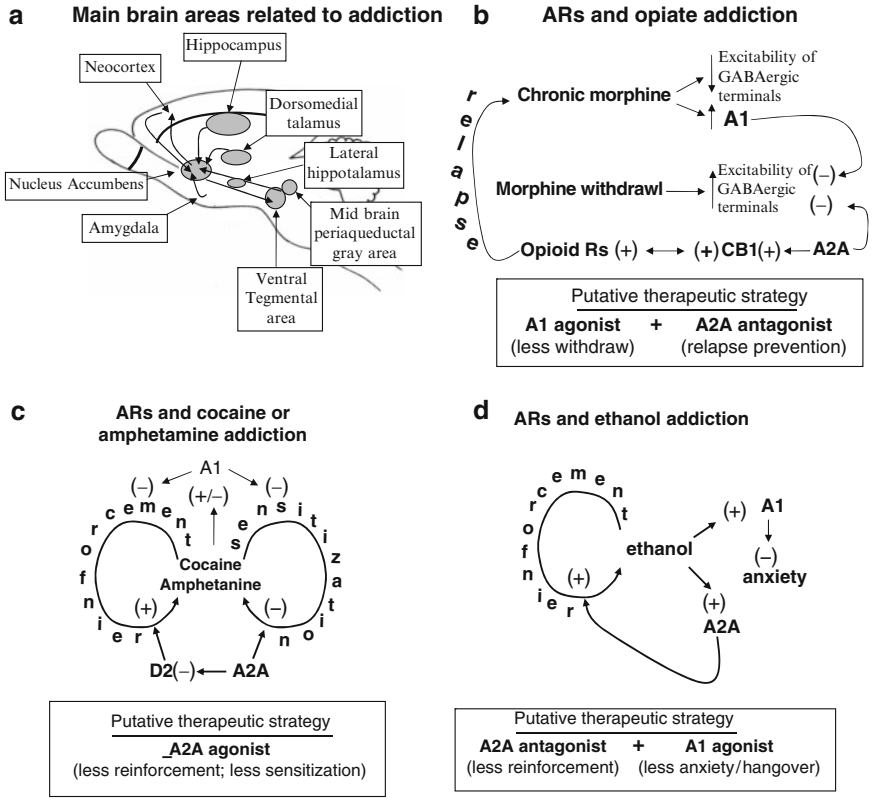


**Fig. 5** **a–d** Brain areas mostly involved in addiction **a**, and the role of $A_1$ and $A_{2A}$ adenosine receptors (ARs) in addictive behavior **b–d**. The putative therapeutic stratagy based on the ARs is indicated *below each panel*. A *plus symbol* denotes facilitation and a *minus symbol* denotes inhibition. See text for references

become new therapeutic agents for drug addiction (Ferré et al. 2007b). Other brain areas involved in reinforcement, motivational and withdrawal consequences of drug use and abuse are the limbic areas, such as the hippocampus and amygdala (Fig. 5a). Accordingly, there is a growing body of evidence suggesting that adenosine is involved in drug addiction and withdrawal, that both $A_1$ and $A_2$ ARs may be involved (Hack and Christie 2003), and that a considerable degree of compensation may occur.

## *9.1 Opioids*

Caffeine combined with the opioid antagonist naloxone produces a characteristic quasi-morphine withdrawal syndrome in opiate-naive animals that is almost completely abolished in $A_{2A}$ AR KO mice and has intermediate intensity in heterozygous

animals, suggesting an involvement of $A_{2A}$ARs in the withdrawal syndrome (Bilbao et al. 2006). These observations are in agreement with previous data that adenosine reduces morphine withdrawal in an acute model, while caffeine aggravates it (Capasso and Loizzo 2001).

Chronic treatment with opioids induces adaptations in neurons that lead to tolerance and dependence. Endogenous adenosine, through $A_1$AR activation, reduces the hyperexcitability of GABAergic terminals of the midbrain periaqueductal gray area (Fig. 5b) that occurs during withdrawal from chronic morphine treatment (Hack et al. 2003). Chronic morphine treatment significantly increased the number of $A_1$ARs (Kaplan et al. 1994) and adenosine transporters (Kaplan and Leite-Morris 1997) as well as the adenosine sensitivity in the nucleus accumbens (Brundege and Williams 2002). Surprisingly, chronic blockade of opioid receptors also causes upregulation of $A_1$ARs (Bailey et al. 2003), suggesting an adaptative mechanism in the purinergic system with chronic opioid receptor manipulation. Interestingly, $A_{2A}$AR levels in the striatum appear to be unaffected by chronic morphine (Kaplan et al. 1994) or chronic opioid antagonism (Bailey et al., 2003).

Both $A_1$ and $A_{2A}$AR agonists attenuate opiate withdrawal symptoms (Fig. 5b), but the specific symptoms affected by each AR are different, and the corresponding AR antagonists exacerbate those symptoms (Kaplan and Sears 1996), suggesting that AR agonists rather than AR antagonists may be useful as therapeutics for opioid withdrawal. In line with this idea is the observation that AK inhibitors attenuate opiate withdrawal symptoms (Kaplan and Coyle 1998). Adenosine also seems to act as a regulator of regional cerebral blood flow in both morphine-dependent rats and morphine withdrawal in rats (Khorasani et al., 2006).

Relapse is the most serious limitation of effective medical treatment of opiate addiction. In this respect, $A_{2A}$AR antagonists may prove useful since $A_{2A}$AR antagonists administered either directly into the nucleus accumbens or indirectly by intraperitoneal injection eliminate heroin-induced reinstatement in rats that are trained to self-administer heroin, a model of human craving and relapse (Yao et al. 2006). The mechanism wherein $A_{2A}$AR antagonists block heroin reinstatement most likely involves opiate receptors and their synergy with other GPCRs, namely crosstalk between $CB_1$ receptors and $A_{2A}$AR signaling, as well as $\beta-\gamma$ dimers (see Sect. 5.1.4 and Fig. 5b).

## *9.2 Cocaine*

Activation of $A_{2A}$ARs is required to develop the addictive effects to cocaine, since the lack of $A_{2A}$ARs diminishes the reinforcing efficacy of cocaine (Soria et al. 2006). On the other hand, $A_{2A}$AR activation protects against cocaine sensitization (Filip et al. 2006), which suggests a therapeutic potential of $A_{2A}$AR agonists in the treatment of cocaine dependence (Fig. 5c). This is not unexpected, since $A_{2A}$ARs inhibit $D_2$ receptor functioning, and these receptors are highly involved in brain-reinforcing circuits. In line with this idea are the observations that $A_{2A}$AR

agonists inhibit cocaine self-administration in rats (Knapp et al. 2001), and that a nonselective AR antagonist reinstates cocaine-seeking behavior and maintains self-administration in baboons (Weerts and Griffiths 2003). Interestingly, in high-risk situations, prophylactic activation of $A_{2A}$AR activation may prove beneficial, since $A_{2A}$AR agonists inhibit the initiation of cocaine self-administration in rats (Knapp et al. 2001). However, the ability of caffeine to prevent the extinction of cocaine-seeking behavior (Kuzmin et al. 1999) or even to reinstate extinguished cocaine self-administration (Green and Schenk 2002) may be related to its blocking effects on $A_1$ARs, rather than $A_{2A}$ARs. Moreover, in the nucleus accumbens, sorting and recycling of $A_1$ARs is dysregulated as a consequence of repeated cocaine administration, so that the amount of $A_1$AR protein and mRNA is upregulated but the number of membrane receptors, their coupling to G proteins, and their ability to form dimers with $D_1$ receptors is downregulated (Toda et al. 2003). Furthermore, adenosine uptake in the nucleus accumbens seems to be augmented after cocaine withdrawal (Manzoni et al. 1998)

## *9.3 Amphetamine*

Daily treatment with amphetamine markedly enhances locomotor responses, and this enhancement remains after washout, a process known as sensitization. No sensitization to amphethamines occurs either in conditional $A_{2A}$AR KO mice or in the presence of $A_{2A}$AR activation (Bastia et al. 2005), indicating that $A_{2A}$ receptors reduce sensitization (Fig. 5c). Also, selective $A_1$AR agonists may have some attenuating influence on the development of amphetamine dependence (Poleszak and Malec 2003).

## *9.4 Cannabinoids*

Several studies have reported crosstalk between ARs and CB receptors, as mentioned above (see Sect. 5.1.4). In this section, only the studies specifically addressing the influence of ARs upon CB addiction or tolerance will be mentioned. Crosstolerance between $A_1$AR and $CB_1$ receptor agonists has been reported in motor incoordination induced by CBs (DeSanty and Dar 2001). A significant reduction in tetrahydrocannabinol-induced rewarding and aversive effects was found in mice lacking $A_{2A}$ARs, indicating a specific involvement of $A_{2A}$ARs in the addiction-related properties of CBs (Soria et al. 2004). Somatic manifestations of tetrahydrocannabinol withdrawal were also significantly attenuated in $A_{2A}$AR KO mice; however, antinociception, hypolocomotion and hypothermia induced by acute tetrahydrocannabinol administration were not affected (Soria et al. 2004).

### *9.5 Ethanol*

The anxiolytic properties of ethanol are generally accepted to be an important motivational factor in its consumption and the development of alcohol dependence. The anxiolytic-like effect induced by ethanol in mice involves the activation of $A_1$ARs but not $A_{2A}$ARs (Prediger et al. 2004). The anxiety-like behavior observed during acute ethanol withdrawal (hangover) in mice is attenuated by nonanxiolytic doses of $A_1$AR agonists (Prediger et al. 2006). Tolerance to ethanol-induced motor incoordination is prevented by $A_1$AR and dopamine $D_1$ receptor antagonists, but not by $A_{2A}$AR antagonists (Batista et al. 2005). However, the reinforcing properties of ethanol are partially mediated via an $A_2$AR activation of cAMP/PKA signaling in the nucleus accumbens, indicating that administration of an $A_{2A}$AR antagonist may decrease ethanol reward and consumption (Fig. 5d). Indeed, $A_{2A}$AR antagonism produces a robust and behaviorally selective reduction of ethanol reinforcement (Thorsell et al. 2007).

## 10 Concluding Remarks

Several years ago, we (Sebastião and Ribeiro 2000) pointed out that "*In addition to its direct pre- and post-synaptic actions on neurones, adenosine is rich in nuances of priming, triggering and inhibiting the action of several neurotransmitters and neuromodulators* (. . .) *The harmonic way adenosine builds its influence at synapses to control neuronal communication is operated through fine-tuning, 'synchronizing' or 'desynchronizing' receptor activation*. . .". In a recent review, Uhlhaas and Singer (2006) considered that abnormal neural synchronization is central to and the underlying basis for several neurological diseases such as epilepsy, schizophrenia, autism, Alzheimer's disease, and Parkinson's disease. These authors highlighted the role of GABAergic neurons and their pivotal role in the primary generation of high-frequency oscillations and local synchronization, the role of glutamatergic connections in controlling their strength, duration, and long-range synchronization, and the role of cholinergic modulation in the fast state-dependent facilitation of high-frequency oscillations and the associated response synchronization. As reviewed in the present work, adenosine is a molecule involved in brain homeostasis that has recently been proposed to be crucial to the effects of deep brain stimulation (Bekar et al. 2008), which mainly aims to affect neuronal synchronization and therefore influence several psychiatric and neurodegenerative diseases. This review suggests that adenosine is a sort of "universal modulator" or a "maestro;" the main molecule involved in coordinating and controlling the synchronization of the release and actions of many synaptic mediators. It also suggests that targeting approaches that increase adenosine levels to provide this synchronization, or targeting ARs with novel safe, selective, and effective therapeutics that are currently in (or are poised to enter) clinical trials, will enhance our understanding of the role of this important endogenous "universal modulator" signaling molecule and its receptors in cognition, neurodegenerative diseases, psychiatric diseases, and drug addiction.

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