

Review Article

Caffeine and Adenosine

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Abstract. Caffeine causes most of its biological effects via antagonizing all types of adenosine receptors (ARs): A1, A2A, A3, and A2B and, as does adenosine, exerts effects on neurons and glial cells of all brain areas. In consequence, caffeine, when acting as an AR antagonist, is doing the opposite of activation of adenosine receptors due to removal of endogenous adenosinergic tonus. Besides AR antagonism, xanthines, including caffeine, have other biological actions: they inhibit phosphodiesterases (PDEs) (e.g., PDE1, PDE4, PDE5), promote calcium release from intracellular stores, and interfere with GABA-A receptors. Caffeine, through antagonism of ARs, affects brain functions such as sleep, cognition, learning, and memory, and modifies brain dysfunctions and diseases: Alzheimer's disease, Parkinson's disease, Huntington's disease, Epilepsy, Pain/Migraine, Depression, Schizophrenia. In conclusion, targeting approaches that involve ARs will enhance the possibilities to correct brain dysfunctions, via the universally consumed substance that is caffeine.

Keywords: Adenosine, Alzheimer's disease, anxiety, caffeine, cognition, Huntington's disease, migraine, Parkinson's disease, schizophrenia, sleep

INTRODUCTION

Caffeine causes most of its biological effects via antagonizing all types of adenosine receptors (ARs). When acting as an AR antagonist, caffeine, used acutely, is doing the opposite of activation of adenosine receptors, due to removal of the adenosinergic tonus. The adenosine A1 and A2A receptors have high affinity for adenosine and are those responsible for tonic actions of endogenous adenosine. So, in the present review we will focus on A1 and A2A adenosine receptors and on the mechanisms they operate in order to infer how caffeine exerts most of its actions in the brain. There are many studies reporting actions of caffeine in humans where it is not completely clear if those actions are mediated by adenosine receptors. These studies, in spite of being relevant for caffeine research *per se*,

were considered out of the scope of the present work. For more detailed analysis of the actions of caffeine in humans, namely cognition, dementia, and Alzheimer's disease, the reader may refer to other papers published in the present issue.

The broad caffeine intake in common beverages, together with the impact of xanthines on biomedical research, prompted many studies that focus on specific caffeine effects rather than using it as a tool to antagonize adenosine receptors (ARs) [1–3]. Caffeine is mainly present in coffee, which also contains trace amounts of theophylline, but no theobromine. Tea is another common source of caffeine. As a pharmacological tool, caffeine is not very useful since its affinity for ARs is low and its selectivity towards the different ARs is also very poor. Caffeine is an antagonist of all subtypes of ARs, and chronic or acute intake of caffeine may affect ARs in different and even opposite ways. Having similar affinity for A1 and A2A Rs [1], acute caffeine actions at a given brain area will reflect the preponderant AR activation in that area, since most of the adenosinergic tonus are exerted through that receptor.

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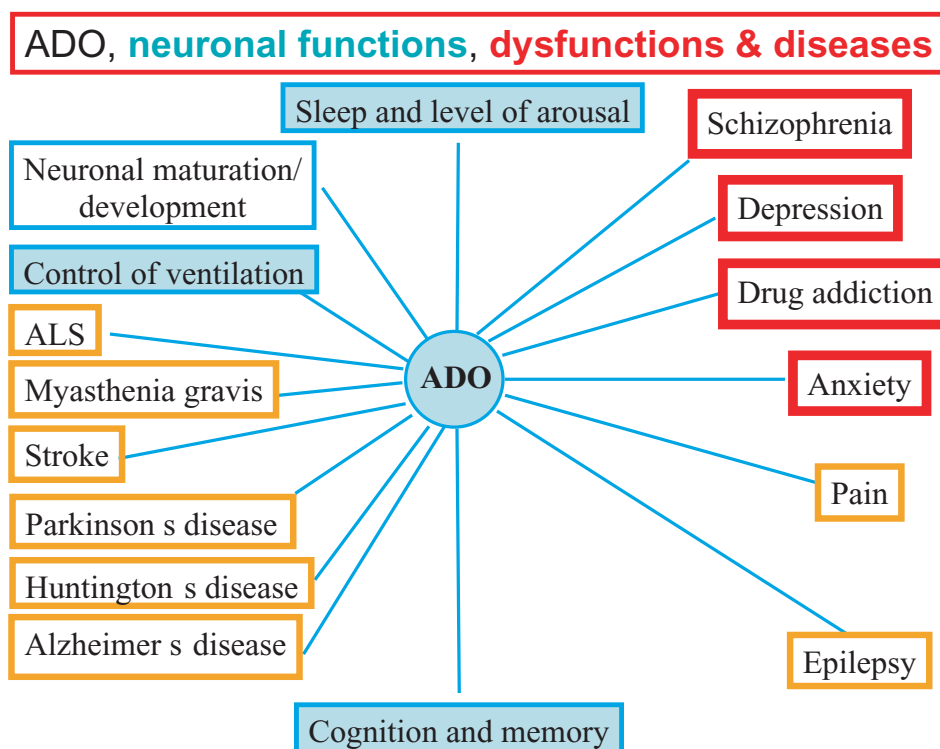


Fig. 1. Actions proposed for adenosine on the central nervous system, including on brain functions, dysfunctions and diseases. As caffeine is a non-selective adenosine antagonist and crosses easily the blood brain barrier, it is likely that the caffeine effects on these adenosine receptors mirror those caused by adenosine actions. For further details see Sebastião and Ribeiro, 2009 – Handbook of Experimental Pharmacology, **193**, 471–534.

Besides the high affinity A1 and A2A receptors, the cloned adenosine receptors also include the high affinity A3 receptor, and the low affinity A2B receptor. Other entities have been proposed based on functional and/or binding studies (e.g., an atypical A2A receptor in the rat hippocampus [4]; the A3 receptor in the frog motor nerve endings [5]). The first proposal for the existence of an A3 AR was based upon pharmacological characteristics, namely high affinity for agonists and xanthine sensitivity [5]. Cloning and cellular expression of the rat A3 AR [6] challenged these criteria since the rat A3 receptor is xanthine-insensitive and has low agonist affinity. Cloning and expression of the human A3 AR [7] reversed the situation again since the human A3 AR is xanthine sensitive and is a high affinity receptor for A3 AR ligands. Although research on the relevance of the A3 AR under pathological conditions is gaining progressive interest, these receptors are poorly expressed in the brain and studies involving them on the actions of caffeine are scarce. Therefore, we decided not to discuss this aspect in the present review.

Adenosine is ubiquitously present in all cells, with receptors distributed in all brain cells; any imbalance of

such a widespread system is expected to lead to neurological dysfunctions/diseases (see Fig. 1). When acting as an AR antagonist, caffeine is doing the opposite of adenosine receptors activation, whenever the levels of endogenous adenosine are tonically activating receptors. So caffeine, like adenosine, can potentially exert effects on all brain areas, providing that endogenous adenosine is tonically activating its receptors. As a result of its psychoactive effects, caffeine is considered by some religions (e.g., Mormons, Adventists, Hindus), along with alcohol, nicotine, and other drugs, to cloud the mind and over-stimulate the senses.

In 1819 the German chemist Friedrich Ferdinand Runge isolated caffeine at the behest of Johann Wolfgang von Goethe. As in the work 'Faust' by Goethe, the soul of Faust has been sold to the devil in exchange for 'jeunesse', it appears that Goethe was anticipating, in almost 200 years, the use of caffeine to treat diseases that predominate during aging, such as neurodegenerative diseases.

The structure of caffeine was elucidated near the end of the 19th century by Hermann Fischer, and it is similar to that of adenosine. Caffeine is metabolized in

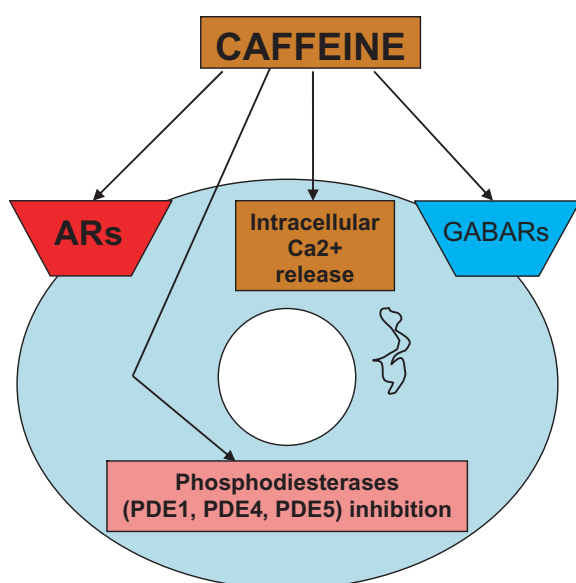


Fig. 2. Sites/mechanisms of action of caffeine. ARs: adenosine receptors. GABARs: GABA receptors.

the liver by the cytochrome P450 oxidase enzyme system into three dimethylxanthines: paraxanthine, which increases lipolysis, leading to elevated glycerol and free fatty acid levels in the blood plasma; theobromine, which dilates blood vessels and increases urine volume; and theophylline, which relaxes smooth muscles of the bronchi, and is used to treat asthma. The therapeutic dose of theophylline, however, is many times greater than the amount resulting from caffeine metabolism taken in non-toxic amounts. Each of those xanthines is further metabolized and then excreted into the urine. For an extensive review including consumption and metabolism of caffeine, see [2].

Adenosine is able to regulate synapses through *tuning* and *fine-tuning*. *Tuning* synapses occurs when adenosine, by activating its receptors, is controlling, e.g. the release of neurotransmitters, by interfering with Ca^{2+} or other mechanisms directly related to neurotransmitter release [8]. In the case of *fine-tuning*, adenosine is interfering with receptors for other neuromodulators [9]. Besides AR antagonism, xanthines, including caffeine, have other biological actions (see Fig. 2), such as 1) inhibition of phosphodiesterases (PDEs) (e.g., PDE1, PDE4, PDE5). These effects (up to 40 % inhibition of phosphodiesterases), according to Daly (2007) [1], are observed in concentrations well below those that cause toxic effects. In relation to PDE inhibition, it is interesting to note that caffeine, being a PDE5 inhibitor, operates through a mechanism also used by sildenafil, which is a vasodilator, via selective

PDE5 inhibition. So, the potential effects related to these actions need to be investigated to see whether consequent vasodilation might contribute to net caffeine effects. 2) Promotion of calcium release from intracellular stores. Application of caffeine-halothane contracture test in the diagnosis of malignant hyperthermia is an example of application of this effect. 3) Interfering with GABA-A receptors [1]. According to Daly [1], caffeine analogues can be developed to target any of these mechanisms rather than ARs, and this may be explored therapeutically [1]. However, in the case of caffeine, the effects seen at very low doses, achieved during normal human consumption, are mostly due to AR antagonism [2]. Because of its safety, its ability to antagonize ARs and to readily cross the blood brain barrier, caffeine has therapeutic potential in central nervous system dysfunctions (see below and Fig. 1). Adverse effects of caffeine may include anxiety, hypertension, drug interactions, and withdrawal symptoms [1]. Caffeine improves cognition [1]; however, it also affects sleep [3]. Moreover, a relationship between adenosine A2A ARs and genetic variability in caffeine metabolism associated with habitual caffeine consumption, has been proposed [10], which provides a biological basis for caffeine consumption. In that 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 considerably different, depending on whether it is administered chronically or acutely. For example, chronic caffeine intake, which increases plasma concentrations of adenosine [11], may be neuroprotective. This is in contrast with the consequences of acutely antagonizing A1 ARs [12]. Chronic AR antagonism with caffeine may also influence cognition and motor activity in a way that resembles the acute effects of AR agonists [13]. Such opposed actions of chronic versus acute treatment not only have important implications in the development of xanthine-based compounds as therapeutic agents, but also constitute a frequently confounding parameter for research. Up-regulation of A1 ARs after chronic AR antagonism with xanthines occurs, but A2A 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 [13]. The increased expression of A1 ARs in response to chronic antagonism of ARs by caffeine, as compared with A2A ARs, may lead to a shift in the A1/A2A AR balance

after prolonged caffeine intake [3]. Moreover, chronic caffeine treatment may lead to modifications in the function of the A1R–A2AR heteromer and this may, in part, be the scientific basis for the strong tolerance to the psychomotor effects of chronic caffeine [14]. Alteration of astrocytogenesis via A2A AR blockade during brain development has been reported [15], raising the possibility that postnatal caffeine treatment could have long-term consequences on brain function, and therefore care should be taken during breast feeding.

Tolerance/Withdrawal

Tolerance develops very quickly, after heavy doses, e.g. tolerance to sleep disruption (400 mg of caffeine 3 times a day for 7 days), tolerance to subjective effects of caffeine (300 mg 3 times per day for 18 days), and withdrawal symptoms, including inability to concentrate, headache, irritability, drowsiness, insomnia, and pain in the stomach, upper body, and joints (within 12 to 24 hours after discontinuation of caffeine intake, peak being at roughly 48 hours, and usually lasting from one to five days, see Fredholm et al. [2]). This is the time required for the number of adenosine receptors in the brain to revert to “normal” levels. Analgesics, such as aspirin, can relieve the pain withdrawal symptoms, as can a small dose of caffeine [1]. Overuse and dependency occurs after consumption of caffeine in large amounts, and in particular over extended periods of time, inducing caffeinism. Caffeinism combines caffeine dependency with a wide range of unpleasant physical and mental conditions including nervousness, irritability, anxiety, tremulousness, muscle twitching, hyperreflexia, insomnia, headaches, respiratory alkalosis, and heart palpitations. Caffeine increases production of stomach acid; high usage over time can lead to peptic ulcers, erosive esophagitis, and gastroesophageal reflux disease.

The influence of caffeine-adenosine receptor interactions upon *brain functions and dysfunctions* will be discussed below.

ANXIETY

Caffeine is well known to promote anxious behaviour in humans and animal models, and can precipitate panic attacks [16]. 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 [17]. It is, however, worthwhile to note that

chronic and acute caffeine consumption may lead to quite different consequences with respect to the function of ARs [18,19]. Short-term anxiety-like effect of caffeine in mice might not be related solely to the blockade of A1 and A2A ARs, since it is not shared by selective antagonists of each receptor [20]. In contrast, anxiolytic effects of xanthine derivatives containing an arylpiperazine moiety have been reported, but this is most probably related to agonist activity at serotonin receptors rather than antagonism of adenosine receptors [1].

The possibility that drugs which facilitate A1 AR-mediated actions could be effective for anxiety was supported by the observations that A1 AR agonists have anxiolytic actions in rodents [20,21]. The inhibitory action of A1 ARs on the nervous system, together with the identification of cross-talk mechanisms between benzodiazepines and ARs [22] and transporters [23], soon suggested that adenosine could mediate the anxiolytic action of several centrally active drugs [24]. Accordingly, A1 AR KO mice showed increased anxiety-related behaviour [25], but this also holds true for A2A AR KO mice [26]. A1 and A2A ARs are involved in benzodiazepine withdrawal signs. In mice, these signs of withdrawal are manifested by increased seizure susceptibility, and agonists of A1 ARs [27] or A2A ARs [28] attenuate them. The potential of A1 AR agonists to reduce the anxiogenic effects during ethanol withdrawal have also been suggested [29]. The caffeine-induced anxiety disorder, which can result from long-term excessive caffeine intake, can mimic organic mental disorders, such as panic disorder, generalized anxiety disorder, bipolar disorder, or even schizophrenia. Caffeine-intoxicated people might be misdiagnosed and unnecessarily medicated when the treatment for caffeine-induced psychosis would simply be to stop further caffeine intake. Other adverse effects of caffeine besides anxiety, sleep disorders, withdrawal symptoms and hypertension, include drug interactions [1].

A significant association between self-reported anxiety after caffeine administration and two linked polymorphisms of the A2A AR gene has been reported [30]. Furthermore, evidence for a susceptibility locus for panic disorder, either within the A2A AR gene or in a nearby region of chromosome 22, was reported [31,32]. This positive association between A2A AR gene polymorphism and panic disorder may, however, not occur in the Asian population [33] suggesting an ethnicity-dependent association.

SLEEP

Most studies on ARs and sleep regulation in humans rely upon consequences of caffeine ingestion by human volunteers, and it is now widely accepted that caffeine prolongs wakefulness by interfering with the key role of adenosine upon sleep homeostasis [34]. In a review on the role of adenosine upon sleep regulation, Porkka-Heiskanen et al. [35] proposed adenosine as a sleeping factor and hypothesized that adenosine works as a neuroprotector 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 occurring after prolonged wakefulness [36]. In contrast, AR antagonists, like caffeine, increase wakefulness. Prolonged wakefulness induces signs of energy depletion in the brain, which causes facilitation of sleep [37]. Molecular imaging showed that there is A1 receptor upregulation in cortical and subcortical brain regions after prolonged wakefulness in humans [38]. Adenosinergic mechanisms contribute to individual differences associated with sleep deprivation sensitivity in humans [39]. Furthermore, a genetic variation in the adenosine A2A AR gene may contribute to individual sensitivity to the effects of caffeine on sleep [40].

It is well documented that A₁ ARs are involved in sleep regulation by inhibiting ascending cholinergic neurons of the basal forebrain [41]. However, more recent studies, which include experiments with A2A and A1 AR KO mice, indicate that A2A 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 [42]. These studies suggest that A2A AR antagonists may represent a novel approach as potential treatments for narcolepsy and other sleep-related disorders [43]. Adenosine A2A 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 A2A AR-induced enhancement of GABAergic inhibition of arousal promoting neurons [44]. In addition to its action in the basal forebrain, adeno-

sine exerts its sleep-promoting effect in the lateral hypothalamus by A1 AR-mediated inhibition of hypocretin/orexin neurons [45,46]. According to the American Psychiatric Association (APA), the caffeine-induced sleep disorder is sufficiently severe to warrant clinical attention.

In summary, the two high affinity ARs, the A1 and the A2A ARs affect multiple mechanisms in several brain areas involved in regulation of sleep and arousal. Therefore, the influences of caffeine upon sleep felt by many humans, and as mentioned above, also documented in controlled studies in healthy volunteers, can be attributed to both A1 and A2A AR blockade. Chronic caffeine consumption may alter AR function and the A1/A2A AR balance, and as a consequence influences the involvement of both ARs upon sleep.

COGNITION, LEARNING, AND MEMORY

Endogenous adenosine, through A1 ARs, inhibits long-term synaptic plasticity phenomena, such as long term potentiation (LTP) [47], long term depression (LTD), and depotentiation [48]. In accordance, A1 AR antagonists have for a long time been proposed to treat memory disorders [49]. Cognitive effects of caffeine are mostly due to its ability to antagonise adenosine A1 ARs in the hippocampus and cortex, the brain areas mostly involved in cognition, but as discussed in detail [2], positive actions of caffeine on information processing and performance might also be attributed to improvement of behavioural routines, arousal enhancement and sensorimotor gating, and these actions may be not solely related to A1 receptor function (see below). Theophylline enhances spatial memory performance only during the light period, which is the time of sleepiness in rats [50]. Independently of the processes used by caffeine or theophylline to improve cognition, there is a consensus 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 A1 receptors improves cognition came from a study using a mixed A1/A2A receptor antagonist, ASP5854 [51]. This orally active drug could reverse scopolamine-induced memory deficits in rats, whereas a specific adenosine A2A AR antagonist, KW-6002, did not. Reduced A2A AR activation may also be relevant for cognitive improvements since A2A AR KO mice have improved spatial recognition memory [52]. Accordingly, over-expression of A2A ARs leads to memory deficits [53].

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 have better performances on memory and other cognitive tests than non-drinkers [54]. A case – control study was specifically designed to evaluate if chronic intake of caffeine might be related to a lower risk of Alzheimer's disease [55], 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 [55]. 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 [56].

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 4 and 9 months of age, with behavioural testing done during the final 6 weeks of treatment; the results revealed that moderate daily intake of caffeine may delay or reduce the risk of cognitive impairment in these mice [57]. Amnesia can be induced experimentally in mice by central administration of beta-amyloid peptides, a process that involves cholinergic dysfunction [58]. Acute intravenous administration of caffeine or A2A AR antagonists affords protection against beta amyloid-induced amnesia [59]. These acute effects of A2A AR blockade are somehow unexpected because A2A ARs are known to facilitate cholinergic function mainly in the hippocampus [60], and therefore, either adenosine A2A AR agonists or A1 AR antagonists, which prevent A1 AR-mediated inhibition of acetylcholine release, were more likely 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 [61]. These apparent discrepancies point toward the need of more basic research to understand the biological basis and the potential benefit for the emerging adenosine-based therapies for Alzheimer's disease. It is interesting to note the very recent reports by Arendash's group on caffeine protec-

tion in Alzheimer's disease transgenic mice [62,63]. In a very recent study [64] it has been shown that human coffee drinking at midlife is associated with a decreased risk of dementia/AD later in life. This finding further supports possibilities for prevention of dementia/AD.

PARKINSON'S DISEASE

A significant association between higher caffeine intake and lower incidence of Parkinson's disease was reported some years ago [65]. Moreover, the beneficial effects of caffeine in Parkinson's disease patients was also reported [66]. Furthermore, caffeine administered before levodopa may improve its pharmacokinetics in some patients with Parkinson's disease [67].

Caffeine has well-known stimulatory actions upon locomotion due to the antagonism of A2A and A1 ARs in the striatum [3], and in most animal models of Parkinson's disease, antagonizing A2A ARs attenuates some disease symptoms, which has been matter of several reviews published as proceedings of a meeting on the topic [68–71]. So, we will highlight a point that is less focused, which concerns interactions between adenosine and neurotrophic factors. The putative role of the neurotrophic factor, GDNF, in slowing or halting disease progression through facilitation of neuronal survival [72], and the facilitatory action of A2A ARs upon GDNF actions in striatal dopaminergic nerve endings [73], raise the need of great caution when blocking A2A ARs in the early phases of Parkinson's disease. If trophic GDNF actions on dopaminergic neurons will also prove to be dependent upon co-activation of A2A ARs, as it has been observed in relation to fast synaptic actions of this neurotrophic factor [73], it is possible that blockade of A2A ARs will be deleterious during a window of time when it is possible to rescue neurons with trophic support.

Another relevant consideration is related to the recent finding [74] 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 A1 ARs by adenosine during this procedure is an essential step to reduce tremor and control spread of excitability, thereby reducing the side effects of deep brain stimulation. However, since A2A ARs are expressed in thalamic areas, it may be expected that A2A ARs are also activated during deep brain stimulation. A2A receptors attenuate A1 receptor functioning [75]. Furthermore, they attenuate D2

dopaminergic responses [3]. Thus, in late stages of the disease, where it is desirable to prevent A2A AR-mediated inhibition of dopamine D2 receptor function, the use of an A2A AR antagonist in combination with deep brain stimulation may be beneficial.

HUNTINGTON'S DISEASE

The role played by ARs in Huntington's disease was recently reviewed and discussed [76]. The complexity inherent to a genetically – based, slowly progressing neurodegenerative disease, the different experimental models which are very frequently non-chronic or sub-chronic models, as well as changes in receptor levels due to cell loss or to prolonged drug administration, give an apparent contradictory picture on the AR involvement in this disease. The pre – versus post-synaptic localization of ARs, in particular of A2A 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 [77]. Indeed, A1 AR agonists [78], A2A AR agonists [79], as well as A2A AR antagonists [79], are all able to influence diverse symptoms in experimental models of Huntington's disease. For a detailed discussion of the causes for this conflicting evidence see [76].

Another aspect that applies to all neurodegenerative diseases, and that may be particularly relevant in the case of Huntington's disease, is related to 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 up-regulates transcription of BDNF [80], and decreased BDNF levels may be an initial cause of neuronal death in this disease. A2A AR activation can facilitate or even trigger BDNF actions in the brain [76, 81–83], pointing toward the possibility that A2A 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 A2A ARs have a dual action in Huntington's disease [76]. The ability of A2A ARs to facilitate actions of BDNF, which is clearly deficient in this neurodegenerative disease [84], is most likely part of the positive influences of A2A ARs against the disease.

EPILEPSY

There are several clinical reports on caffeine or theophylline intake and seizure susceptibility [86,87], but surprisingly, no mention is made of the main cause of seizure induction by these drugs, i.e., AR antagonism.

Indeed, after the initial observation that adenosine has anticonvulsant actions [87], the therapeutic potential of adenosine related compounds in epilepsy was immediately pointed out [88], and it is now widely accepted that adenosine is an endogenous anticonvulsant, an action mediated by inhibitory A1 ARs that restrain excessive neuronal activity. Other ARs are, however, involved in seizure control, though their role is most frequently related to exacerbation of seizures. The influence of A3 and A2 ARs on GABAA receptor stability has been recently suggested [89], based on the observation that A3 or A2B AR antagonists, acutely applied to oocytes transfected with human GABAA receptors, reduce rundown of GABAA currents. A2A ARs, by promoting neuronal excitability, may also increase seizure susceptibility. Indeed, A2A ARs KO mice are less sensitive to pentylenetetrazol-induced seizures [90].

It has been shown that A1 AR activation by locally released adenosine is an efficient way to keep an epileptic focus localized [91]. Therefore, attention is now focused on the development of biocompatible materials for adenosine-releasing intrahippocampal implants [92]. In line with the evidence for the anti-epileptic role of A1 ARs, A1 AR KO mice are more susceptible to seizures and develop lethal status epilepticus after experimental traumatic brain injury [93]. There are, however, limitations in the use of A1 AR agonists as anticonvulsant drugs due to their pronounced peripheral side effects like cardiac asystole, as well as central side effects like sedation [94]. A possibility would be the use of partial agonists, which are more likely to display tissue selectivity. A N6,C8-disubstituted adenosine derivative with low efficacy towards A1 AR activation in whole brain membranes but with high efficacy as an inhibitor of hippocampal synaptic transmission was identified [95]. Another approach that has been more intensely explored is the use of compounds that increase the extracellular concentrations of adenosine. This has been attempted with adenosine kinase (AK) inhibitors, which showed beneficial effects in animal models of epilepsy, and an improved preclinical therapeutic index over direct acting AR agonists [96]. An even more refined approach was the 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 [97]. The reverse also holds true, since transgenic mice over-expressing AK in the brain have increased seizure susceptibility [91]. Furthermore, intrahippocampal implants of AK-deficient stem cell-derived neural precursors suppress kindling epileptogenesis [98]. The above evidence suggests that adenosine-augmenting cell and gene therapies may lead to improved treatment options for patients suffering from intractable epilepsy [99].

AK is mostly expressed in astrocytes [100], and over-expression of AK after seizures, with consequent reduced adenosine inhibitory tone, contributes to seizure aggravation [91]. However, release of interleukin-6 (IL-6) from astrocytes induces an upregulation of A1 ARs both in astrocytes [101] and neurons [102]. This leads to an amplification of A1 AR function, enhances the response to readily released adenosine, enables neuronal rescue from glutamate-induced death, and protects animals from chemically induced convulsing seizures [102]. Indeed, IL-6 KO mice are more susceptible to seizures and lack the well known seizure-induced up-regulation of A1 ARs [102].

Seizure-induced release of neurotrophic factors, such as BDNF, may have beneficial and aggravating actions upon epilepsy, the beneficial ones being mostly related to promotion of cell survival, the deleterious ones being related to excessive cell proliferation and neuronal sprouting [103]. Adenosine, through A2A AR activation, triggers and facilitates BDNF actions in neurons [82,83], but the relevance of this interplay for epilepsy remains to be explored.

PAIN/MIGRAINE

Caffeine makes pain relievers 40% more effective in alleviating headaches and helps the body to absorb headache medications more quickly, bringing faster relief. Many headache drugs include caffeine in their formula. It is also used with the vasoconstrictor ergotamine in the treatment of migraine and cluster headaches as well as to overcome the drowsiness caused by antihistaminics. It is well established that A2A receptors are potent vasodilators, and therefore the influence of caffeine in migraine probably occurs through A2A receptor antagonism [2]. However, other mechanisms might also coexist. Calcitonin Gene Related Peptide – Receptors (CGRP-R) antagonists are useful in the treatment of migraine, and as CGRP effects on synaptic transmission are greatly enhanced by A2A receptors activation [104], one is tempted to speculate

that A2A receptor blockade and consequent attenuation of CGRP-R activation might also contribute to the ability of caffeine to alleviate migraine. It is likely that its association with CGRP receptor antagonists, useful to treat migraine, will substantially increase the efficacy of these drugs in the treatment of headache/migraine.

DEPRESSION

A2A AR KO mice and wild-type mice injected with A2A AR antagonists were found to be less sensitive to ‘depressant’ challenges than controls [105], suggesting that blockade of adenosine A2A ARs might be an interesting target for the development of antidepressant agents. This antidepressant-like effect of selective A2A AR antagonists is probably linked to an interaction with dopaminergic transmission, possibly in the frontal cortex, since administration of the dopamine D2 receptor antagonist, haloperidol, prevents antidepressant-like effects elicited by selective A2A AR antagonists in the forced swim test (putatively involving cortex), whereas it had no effect on stimulant motor effects of selective A2A AR antagonists (e.g. caffeine, putatively linked to ventral striatum) [106]. Depression is frequently associated to loss of motivation and psychomotor slowing. In this context, it is interesting to note that A2A AR in the nucleus accumbens appear to regulate effort-related processes, an action that could be related to modulation of the ventral striatopallidal pathway [107].

Besides A2A ARs, A1 ARs are also probably involved in the antidepressant-like effect of adenosine [108], which may be consequence of interactions with the opioid system [109].

It is worthwhile to note that deep brain stimulation, now widely used by neurosurgeons to treat tremor and other movement disorders, as well as 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 [74,110].

Results from clinical and basic studies have demonstrated that stress and depression decrease BDNF expression and neurogenesis, leading to the neurotrophic hypothesis of depression [111,112]. How adenosine A2A AR-dependent facilitation of BDNF actions on hippocampal synapses, namely enhancement of synaptic transmission [81] and enhancement of synaptic plasticity [83], may contribute to some antidepressive actions of adenosine remains to be established.

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 [113]. Indeed, adenosine, via activation of A1 and A2A ARs, is uniquely positioned to influence glutamatergic and dopaminergic neurotransmission, two neurotransmitter systems that are mostly 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 A2A ARs and D2 receptors allow further opportunity for mutual modulation between the adenosine and dopamine systems [114]. These mechanisms could provide a rationale for an antipsychotic-like profile of AR agonists, in particular A2A AR agonists, to promote a reduction in D2 receptor signaling [114], and A1 AR agonists to promote a reduction in dopamine release [113]. Indeed, dipyridamole, a well known inhibitor of adenosine transporters, and therefore an enhancer of extracellular adenosine levels, may be of some therapeutic interest in schizophrenic patients [115].

Reduced NMDA receptor function may contribute to the cognitive and negative symptoms of schizophrenia [116]. The relationships between adenosine and NMDA receptor function are complex and may operate in opposite ways. Thus, NMDA receptor activation induces adenosine release [117,118], 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 [119]. In addition, both A1 [120] and A2A ARs [121] can influence NMDA receptor functioning, both receptors being able to inhibit NMDA currents in different brain areas

CONCLUSION

Adenosine builds its influence on neuronal communication via *fine-tuning*, ‘synchronizing’ or ‘desynchronizing’ receptor activation [9]. On the other hand, abnormal neural synchronization is considered to be central to and the underlying basis for several neurological diseases such as epilepsy, schizophrenia, autism, Alzheimer’s disease, and Parkinson’s disease [122]. It is well established that adenosine is involved in brain

homeostasis, and recently proposed to be crucial to the effects of deep brain stimulation [74], which aims to affect neuronal ‘synchronization’ and, therefore, influence several psychiatric and neurodegenerative diseases. One is, therefore, tempted to propose that adenosine works as a sort of “universal modulator” or a “maestro”, being the main molecule involved in coordinating and controlling the synchronization of the release and actions of many synaptic mediators. These actions of adenosine are operated by high affinity A1 and A2A receptors, and caffeine affects both. Chronic caffeine may up-regulate adenosine receptors and exacerbate adenosine levels and some adenosine actions in the brain.

In conclusion, targeting approaches that involve abnormal synchronization, namely ARs, will enhance our possibilities to interfere in and/or correct brain dysfunctions. An efficient way may be through the use of the universally consumed substance caffeine.

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